Milk Hygiene and Consumption Practices in The Gambia

by
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B.S., University of Michigan, 2015

A thesis submitted to the
Faculty of the Graduate School of the
University of Colorado in partial fulfillment
of the requirement for the degree of Masters of Anthropology
Department of Anthropology
2017

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Milk Hygiene and Consumption Practices in The Gambia
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IRB Protocol # 16-0463

Abstract

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Thesis Directed by Associate Professor Dr. Robin Bernstein

Milk, a food known to contain a wealth of key nutrients that supplement a healthy diet, is consumed by mammalian species after birth. While most mammals cease milk consumption after the weaning period, humans continue drinking milk into later life. However, the milk integrated into human diets post-infancy comes from non-human sources, such as cows and goats. Livestock and their products have held great importance for the livelihoods and nourishment of populations in low income countries, and The Gambia, located in West Africa, is no exception to this. As agropastoralists, many people in The Gambia rely on milk consumption throughout their lifespans. It is especially integrated into early life diets, where it is commonly given to infants and children in unpasteurized fresh and/or sour form and mixed with cereals and gruels. During the weaning period, infants are especially vulnerable to illness in response to exposure to new environments and foods because of their immature immune systems. Diarrheal diseases are frequent amongst Gambian infants and have contributed greatly to infant and child mortality in the country. There has been little research examining livestock handling practices, including milking practices, milk storage, and milk sales in The Gambia, along with little knowledge of bacterial contamination of milk products in the country, which may influence food safety. This thesis provides a foundational characterization of milk consumption and handling practices in The Gambia, and discusses the potential role of bacterial contamination on consumer health. By investigating presence of potentially pathogenic organisms in local milk, and conducting surveys

and interviews with populations in The Gambia, this research concludes that the food safety of milk, especially as a weaning food, requires greater attention, as does the cultural history and role of milk to the Gambian people.

Acknowledgements

First, I would like to express my deep appreciation and gratitude to my advisor, Dr. Robin Bernstein, for the patient guidance and mentorship she has provided to me in all of the years that I have known her. Dr. Bernstein's intellectual heft and creativity are matched only by her genuinely good nature and down-to-earth humility, and I am truly fortunate to have had, and to continue to have, the opportunity to work with her.

I would also like to thank my committee members, Drs. Terry McCabe and Darna Dufour for the friendly guidance, valuable suggestions, and the general collegiality that each have offered to me throughout the past two years. Dr. McCabe has deepened my understanding of cultural anthropology and the complexity of the relationships between humans and the environment. In addition, he has helped me develop a greater appreciation of fieldwork and all of the unexpected challenges and opportunities that can come with it. Dr. Darna Dufour has provided me with the tool set to become a stronger researcher and instructor, and has engaged me in new scientific ideas which has stimulated intellectual freedom in my own work. Finally, I would like to thank all of my committee members for persistently demanding a high quality of work in all my endeavors.

I would like to also recognize Dr. Bert Covert for the contributions that he has made to my intellectual growth and development as a student in the Department of Anthropology at the University of Colorado Boulder.

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List of Acronyms and Abbreviations

CRRN – Central River Region North
CRRS – Central River Region South
EB – *Enterobacteriaceae*
GBA – Greater-Banjul Area
HTST – High Temperature Short Time
ITC – International Trypanotolerance Centre
LAB – Lactic Acid Bacteria
LPS – Lipopolysaccharide outer membrane
LRR – Lower River Region
MRC – Medical Research Council
MRCG – Medical Research Council Gambia Unit
NBR – North Bank Region
TMTC – Too Many to Count
TVC – Total Viable Cell Count
UCM – Unpasteurized Cow's Milk
UHT – Ultra High Temperature
URR – Upper River Region
VI – Visible Impurity
WCR – West Coast Region
WHO – World Health Organizatio

INTRODUCTION

Food plays a critical role in human health and survival. Milk, which is recognized for its great nutritional value, is no exception. In fact, milk is often referred to as a superfood and is promoted by numerous health organizations worldwide. Milk is the primary source of nutrition for mammalian offspring before they are able to digest other foods, and it is packed with the elements necessary for healthful immune system development and somatic growth. Post-weaning, most mammals cease milk consumption. Humans are unique in that we continue our milk consumption practices well beyond early-life; however, after infancy, the milk humans consume comes from non-human species.

Following the domestication of animals during the Neolithic Revolution around 7,000-11,500 years ago, humans began extracting milk from cows, goats, and sheep for their own consumption (Zeder, 2011). In general, the development of agriculture changed the means by which food was obtained, but it also introduced novel types of food, including those that are historically associated with infant feeding such as cereal-based foods and animal milks. Mammals are genetically coded to stop producing lactase, an enzyme that breaks down the sugar lactose in milk, around the average age of weaning for each species. The majority of early Neolithic populations did not carry the allele that we now know is associated with lactase persistence, which would have allowed for lactose digestion into later-life (Burger et al., 2007; Plantinga et al., 2012). Because of this, consumption of raw milk by older children and adults would have resulted in gastrointestinal symptoms like abdominal pain and diarrhea (Mattar et al., 2012). However, infants and young children would have likely been able to consume raw milk without health repercussions. Over time, habitual consumption of non-human milk must have conferred significant health benefits that improved survival, because genetic research shows that

lactase production began to persist in these groups after weaning ages, and even into adult life. Today, there are more than 6 billion consumers of milk and milk products worldwide, with the majority in low-income countries (FAO, 2010).

Fieldsite Background

The Gambia, a small country (population 2.1 million) in on the Atlantic Coast of West Africa, heavily relies on livestock and livestock products such as milk. Over 45% of the population live in rural, or non-urban areas, and more than 60% of the country's population depends on agriculture for their livelihood (Ignacio & Garcia, 2012; Rural Poverty, 2010). In addition, at least half of the poor population consists of farmers and agricultural workers (Abukari, 2016). The Gambia is one of the poorest countries in Africa, with close to half of the population living below the poverty line, which is defined by international standards as living on less than $1.90 per day (Abukari, 2016; World Bank, 2017).

The Gambia is regarded as an agropastoralist society, which means there is a reliance on the combination of agriculture and pastoralism (Banjul, 1990; Falvey, 1999; Rass, 2006). In a more quantitative example, Rass (2006) defines agropastoralist households as those that derive between a quarter to half of their income from livestock. Common livestock in the country include cattle, goats, sheep, and poultry (Olaniyan, 2016). Much of the agricultural work in The Gambia revolves around the rice crops grown along the River Gambia, which runs through the country (FAO, 2010). Other common crops in The Gambia include groundnuts, millet, maize, fruits, and vegetables (FAO, 2010).

While crop farming is quite important for both income and sustenance in The Gambia, livestock are also essential to the rural communities. Livestock are regarded as being central to both food security and rural development in West Africa in general, and it has been suggested

that livestock will help bring certain regions of Africa out of poverty in the future (Ejlertsen et al., 2013). Livestock production is the fastest growing sector in agriculture worldwide, and has been proposed as a catalyst to improve diets and increase revenue in low-income countries (Thornton, 2010).

Cattle contribute to communities through their multiple valuable resources, including meat, milk, manure, traction/animal power to grow crops, and transportation (Ignacio & Garcia, 2012). These products, either directly or indirectly, contribute calories, proteins, and fats in daily energy intake. Milk provides a direct and rich source of protein, energy, and micronutrients, which makes it a valuable source of nutrition in The Gambia; adults and children consume it in both fresh and sour form, and with or without other food (Erinoso et al., 1992; Hempen et al., 2004). For example, many urban Gambians eat 'Chura Gerrte' (rice and peanuts – boiled) or 'ruy' (pap), with added yogurt or milk (Prentice & Paul, 2000; Falola & Jean-Jacques, 2016). In a country characterized by low nutritional status, milk is an excellent dietary supplement.

Infant Mortality in Gambia

In The Gambia, 971,000 are children under age 18 (almost 50% of the population) and 339,000 are under 5 years of age (UNICEF, 2015). Although infant mortality rates have improved in recent years, they remain high: in 2013, of all deaths of children under five years, 39% occurred before one month of age, 29% occurred before one year of age, and 31% occurred between 1 and 4 years of life (Liu et al., 2015). Of the neonatal deaths (before 1 month of age), 30% were caused by birth asphyxia and birth trauma, 28% by prematurity, 19% by sepsis and other infectious conditions (WHO/UNICEF, 2014). Of the deaths occurring in infancy (ages 1-59 months), 33% were caused by malaria, 17% by pneumonia, 12% by diarrheal diseases, and 10% by non-communicable diseases (Liu et al., 2015). In addition, more than 25% of children under 5

years of age are affected by chronic malnutrition, or lack of proper nutrition, which can result in growth stunting amongst other complications (FAO, 2010). Its prevalence has increased in recent years, and these issues largely impact the rural areas of The Gambia (FAO, 2010).

Micronutrient Deficiencies & Animal Product Consumption

Because milk is designed to support growth and development of mammalian offspring, it is widely accepted that human consumption of cow's milk during childhood will promote somatic growth and overall wellbeing (Dror & Allen, 2011). In populations with chronic micronutrient deficiencies, such as The Gambia, milk consumption can effectively alleviate morbidities associated with said deficiencies (Dror & Allen, 2011). For example, ample evidence has shown that milk intake after at least one year of age can greatly reduce the prevalence of anemia and iron deficiency, which could strongly influence other aspects of health (Rivera et al., 2010).

Micronutrient deficiencies are one of the leading causes of death in developing countries. In 1999, a National Micronutrient Survey in The Gambia reported that pregnant and lactating women and children had a mild to severe prevalence of vitamin A deficiency, mild to moderate prevalence of iodine deficiency, and high prevalence of iron deficiency. Milk consumption, however, can reduce common biochemical and functional nutritional deficiencies (Dror & Allen, 2011). For example, iron deficiency, most commonly caused by low intake of animal-based products, is responsible for 50% of global anemia cases in regions endemic with malaria (de Benoist et al., 2008; Lynch, 2011). Milk also stimulates growth factor production in the consumer (Dror & Allen, 2011), and animal-source foods in general provide a rich source of iron, vitamin A, zinc, and iodine (Dror & Allen, 2011; WHO, 2009; Lynch, 2011).

Current Nutrition Improvement Efforts in The Gambia

There has been a strong push in recent decades to improve the nutritional status of people in The Gambia. The Medical Research Council (MRC) Unit, The Gambia has been conducting research in nutrition and nutrition related subjects in The Gambia for over 60 years. This work is aimed at reducing the burden of death and illness in low and middle income countries, and improving health practices and policies that maximize the health impact of their research.

In addition to this research, other efforts are in effect to improve nutritional conditions, including the National Nutrition Agency (NaNA). Established in 2000, NaNA is involved in the implementation and coordination of the National Nutrition Policy in The Gambia, which involves efforts such as increasing visibility, expanding the funding base, and implementing nutrition programs in various communities (NaNA, 2017). These programs aim for goals such as empowering communities to improve maternal, infant, and young child nutrition, reducing or eliminating micronutrient deficiencies, and promoting breastfeeding.

Thesis Outline & Aims

In this thesis, I concentrate on the traditional use of dairy in both rural and periurban settings, the production systems that uphold the milk and milk products, and the current hygienic status of unpasteurized milk consumed in the greater-Banjul area of The Gambia. A biocultural perspective is particularly relevant to analyses presented in this thesis; there is a tightly interwoven relationship between culture, environment, and biology involved in dairy-related practices in The Gambia. By blending an ethnographic and biological perspective, this work has application beyond food safety issues. In order to truly understand a cultural food system, it must be examined from several internal vantage points. The ultimate, long-term goal of this work is to locate the intersections between person, health, and milk.

In the following chapters, I provide a discussion of topics regarding dairy, spanning from milk production to consumption. First, I provide a review of literature on infant feeding and weaning practices, and food contamination and its impact on infant health and development. After this review, I introduce the motivations for my pilot work in The Gambia and discuss my preliminary assessments of dairy consumption in rural Gambia. Next, I analyze findings from my main research study on current dairying practices by herdsmen and market vendors, and the hygienic quality of milks in The Gambia. More specifically, my thesis will address the following aims:

Preliminary Study

Aim 1: The first aim of my preliminary study is to detail modern milk consumption practices of mothers and children in rural Gambia through a representative survey. More specifically, I am interested in investigating how milk use (consumption frequency and milk form) by mothers and children has changed in rural Gambia (Keneba) since its last examination twenty-five years ago, by Erinoso et al. (1992).

Aim 1, Hypothesis1 (A_1H_1): The frequency of milk (in both fresh and sour form) consumption by mothers and children in rural Gambia (Keneba) has not changed since 1992 when these practices were examined by Erinoso and colleagues.

Aim 2: The second aim of my preliminary study is to describe common use and timing of introduction of non-breast milk liquids and foods to infant diet in rural Gambia.

Aim 2, Hypothesis 1 (A_2H_1): Non-breast milk liquids and other foods are regularly introduced earlier than WHO recommendations (6 months of age), which could have implications for infant health.

Main Study

Aim 1: The first aim of my main study is to describe in detail milk consumption and milk hygiene practices of herdsmen and vendors who sell milk at informal markets in The Gambia, including Bakau, Brikama, Latri Kunda, Serekunda, and other markets.

Aim 2: The second aim of my main study is to determine (a) which forms of milk (fresh, sour, and/or powdered) are the least hygienic – as defined by milk temperature, presence of visible impurities, and concentrations of Enterobacteriaceae (EB) – and (b) the stage in the milk production chain (herdsmen versus vendor) that has higher concentrations of EB.

Aim 2, Hypothesis 1 (A_2H_1): (a) Fresh milk is the least hygienic form of milk compared to powdered or sour milk because it has not undergone fermentation, a process that increases acidity (reduced pH), which can lower concentrations of pathogenic bacteria. Additionally, (b) milk collected from herdsmen and fresh milk purchased from vendors will have higher concentrations of EB compared to that of sour milk.

Aim 3: The final aim of the main study is to measure how bacterial concentrations in milk change after 24 hours in ambient temperature. This is an important step in determining how consumer practices can affect the bacterial content of milk purchased for consumption, since consumers of milk purchased at market in Gambia seldom own refrigeration systems and instead store milk in the open.

Aim 3, Hypothesis 1 (A_3H_1): Freshly purchased milk will have greater concentrations of EB than milk sitting at ambient temperature for 24 hours, due to its higher pH value and water content, which facilitates bacterial growth.

CHAPTER I: Agropastoralism & Cattle

1.1 Agropastoralism & Agriculture in The Gambia

The Gambia (Figure 1) in a tropical sub-humid environment, operates a mixed crop-livestock farming system (Jaiteh et al., 2010). The country includes a wealth of landscapes, including coastal, marine and wetland habitats, and is divided into 6 Regions: West Coast Region (WCR), Lower River Region (LRR), Central River Region North (CRRN), Central River Region South (CRRS), North Bank Region (NBR), and Upper River Region (URR); and the Greater Banjul Area (GBA) comprised of Kanifing and Banjul municipalities (Secka, 2016). My preliminary research discussed after Chapter 7 will focus on the LRR, WCR, and GBA, the latter two being considered peri-urban or a rural-urban transition zone where there is an interface between town and country.

In these different regions, the finer details of the agropastoralist systems vary. For example, there is a heavier reliance on rice in the LRR, whereas there is a greater reliance on livestock and fish in the WCR. Agriculture is a key sector of The Gambia's economy, and more than 75% of the population work in this sector (Secka, 2016). Within each of these regions, farmers report that livestock animals, specifically cattle, are raised to produce meat, milk, and draft, which provides both food and income (Secka, 2016). The agriculture sector employs 77% of the population and contributes 21% of the Gross Domestic Product (GDP); the livestock sub-sector contributes 46% of the agricultural contribution and 9.6% of the 2015 estimated GDP (Secka, 2016).

1.2 Gambia Climate & Environment

The Gambia has an environment conducive to this agropastoralist system. The climate is characterized by a long dry season (late October – early June), and a short wet season (mid-June

– early October) (NEA, 2010). Calving occurs during the wet season as there is ample plant life and abundant water from the strong rains, and in turn, there are many more cows to be milked at this time, thereby increasing overall workloads for those involved in cattle-related performances (Jeannin et al., 1988; Jaitner et al., 2003). Most crop growth also occurs during the wet season, and crops are harvested in the dry season.

1.3 Cattle in The Gambia

In all of Gambia, there are approximately 479,083 head of cattle (NASS Report, 2014). There are several breeds of cattle in the country, including: Gambian N'Dama, N'Dama from Bissau, European breeds crossed with N'Dama, Zebu crosses with N'Dama, and Zebu Gobra (Somda et al., 2003). Of these, the N'Dama, a *Bos taurus* breed, is the most populous, and is used in the traditional smallholder mixed farming production system for milk. A smallholder farming production system refers to rural producers or farmers, predominantly in low-income countries, who own small plots of land on which they grow crops and/or raise livestock, relying almost exclusively on family labor (Morton, 2007). The N'Dama are indigenous to the country, and are reputed to be trypanotolerant and resilient to a number of diseases (Jaitner et al., 2003). Trypanosomiasis is a vector-borne disease transmitted by the tsetse fly (Tano et al., 2002; Stein et al., 2009; Van den Bossche et al., 2010). Van den Bossche and colleagues (2010) report that of the estimated 165 million cattle in Africa, 50 million are kept in tsetse-infested areas. Trypanosomiasis is one of the main limitations for cattle production in the tropics, reducing milk and meat yields, and disrupting gestation (Rowlands et al., 1994; Naessens, 2002; Stein et al., 2009). Livestock, such as cattle, can host the human pathogen subspecies of the *Trypanosoma* genus, which can be transmitted to human hosts (WHO, 2017). These human hosts are oftentimes those living in rural areas and whose livelihoods involve work with livestock and/or

animal husbandry. The infection causes severe headaches, sustained fever, and neurological disorders (Simarro et al., 2008; WHO, 2017). Ultimately, trypanosomiasis affects overall human livelihoods as it poses a strong threat to both livestock and human health (Fall et al., 2016).

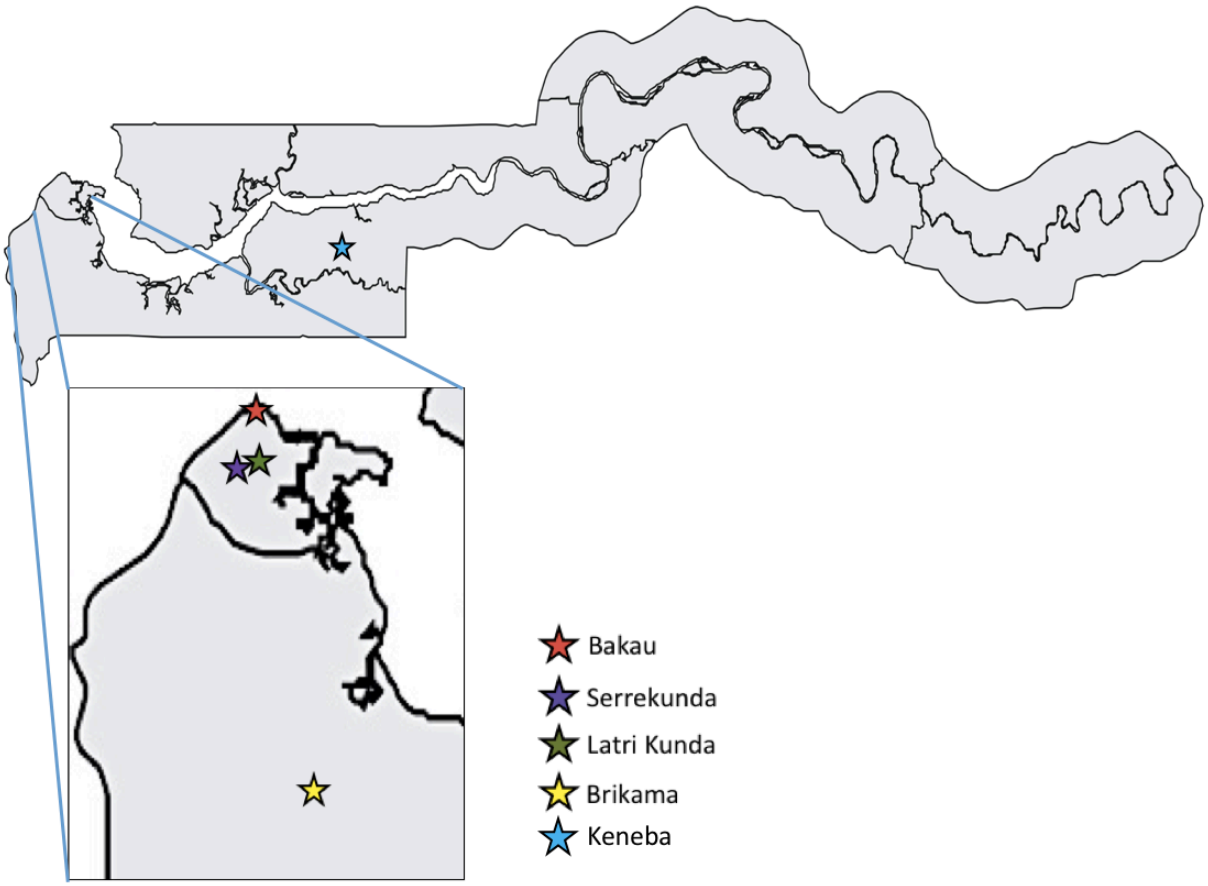
1.4 Breed Differences

Cattle breeds differ in a number of ways. Many breeds naturally developed within, and adapted to, local environmental conditions. They vary in milk output, tolerance to heat stress and disease, and resilience to climatic conditions (e.g., seasonality, feed and/or water shortages, wetness, etc.). The cattle in Gambia are bred to be better able to tolerate heat, certain diseases, and low intake of food due to seasonality. According to Jaitner et al. (2003), the average milk production for N'Dama cattle is 1.2 liters per day. Most cows are milked twice per day during the rainy season; once in the morning once in the evening. The average length of lactation for N'Dama cow is 375 days with an annual milk off-take for human consumption of 437 liters (Touray et al., 2010; Secka, 2016). Research on these topics is a specialty for the International Trypanotolerance Centre (ITC) in The Gambia. This organization focuses on cattle breeding strategies to improve health, disease resistance, and production in the country (Dempfle & Jaitner, 2000).

The F1 crossbreds and backcrosses (N'Dama x Holstein-Friesian or Jersey) bred by ITC and few purebreds of Holstein-Friesian or Jersey are used in the smallholder commercial dairy production system. However, the use of these cattle is limited in the country because the F1 and pure breed cattle bred by ITC are not available in the local market and are very costly to import from Senegal (Touray, 2016). The average lactation output of F1 cows managed at ITC is 1,400 liters over around a time period of one year, with an average daily production of 4.5 liters of milk

(Secka, 2016). For comparison, a Holstein dairy cow in the United States will produce around 20.8-31.3 liters of milk per day (Lehmann et al., 2014).

Figure 1. Field Site Locations in The Gambia (Bakau, Serrekunda, Latri Kunda, and Brikama are considered peri-urban, whereas Keneba is considered rural).



1.5 Herding Practices & Cattle Ownership

Cattle owners in The Gambia are almost entirely men (only 1% are women); however, women represent 67% of the small ruminant (goats and sheep) owners in the country (Jaitner et al., 2003). Gambian cattle are held in multi-owner herds, and owners are residents in the respective villages where the cattle graze (Itty, 1992; Jaitner et al., 2003). Herdsmen most often

receive payment in the form of milk off-take; only a small percentage of herdsmen receive cash only payments (Itty, 1992; Jaitner et al., 2003).

The Fulani are represented by both herd owners and herdsmen in dairying systems in The Gambia. The Fulani, an ethnic group that is widespread in West Africa – including the Senegambia area – represent a tribe that is highly dependent on agriculture and livestock for their livelihoods. The Fulani tribe is culturally linked to cattle. For example, Fulani traditions report that the group originated when both cattle and the first Fulani family emerged from a river, and from there they migrated across Africa (Reisman, 1977).

It is important to note that there are numerous ethnic groups in The Gambia, including (but not limited to) the Mandinka, Jola, Wolof and Serahuli people. Trail (1980) reports that the Fulani have managerial control over the majority of the cattle in the country, either through direct management and ownership of cattle, or in hired herding of cattle belonging to other ethnic groups. While the other ethnic groups own cattle for a variety of reasons, they are primarily sedentary agriculturalists not generally noted for their livestock-handling knowledge or abilities and therefore rely on hired herders (Trail, 1980). Because of this information, this thesis will concentrate on literature regarding the Fulani peoples specifically.

Value chains refer to the chain of activities that products pass through before reaching the consumer (TechnoServe, 2008; Karenzi et al., 2013). Figure 2 depicts the actors and steps involved in the milk production value chain in The Gambia as documented by Hempen et al. (2004). In this system, the starting point for the chain is the herds themselves (milk production) and the herdsmen. Next, the milk can be sold from the herdsmen to either consumers on the spot (sales in villages), or to collectors (professionals and others). After that, the milk can be sold from the collectors to milk pasteurization units (for milk processing), market vendors (wholesale

and retail), or it can be sold through urban sales (shop, market, town). Milk pasteurization units and market vendors, too, can sell the milk through urban sales.

Figure 2 depicts the actors and steps involved in the milk production value chain in The Gambia as documented by this thesis research. The system is as follows: herds/herdsmen sell milk to on the spot sales (villages) or milk vendors. The milk vendors can sell the milk through urban sale (shop, market, town). Based on observations and interviews, the intermediary steps noted in Hempen et al. (2004) have been merged with other positions over time. For example, market vendors purchase the milk directly from herdsmen instead of going through a collector. In addition, the milk processing unit segment of this chain has been removed since all of the pasteurization units are currently closed in the country.

Figure 2. Milk Production Chain: Created by Hempen et al. (2004)

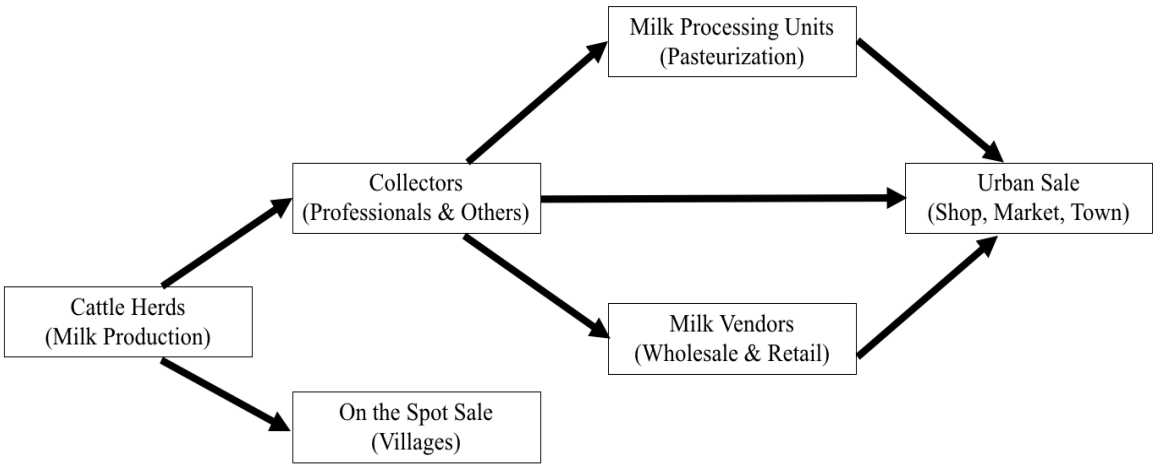
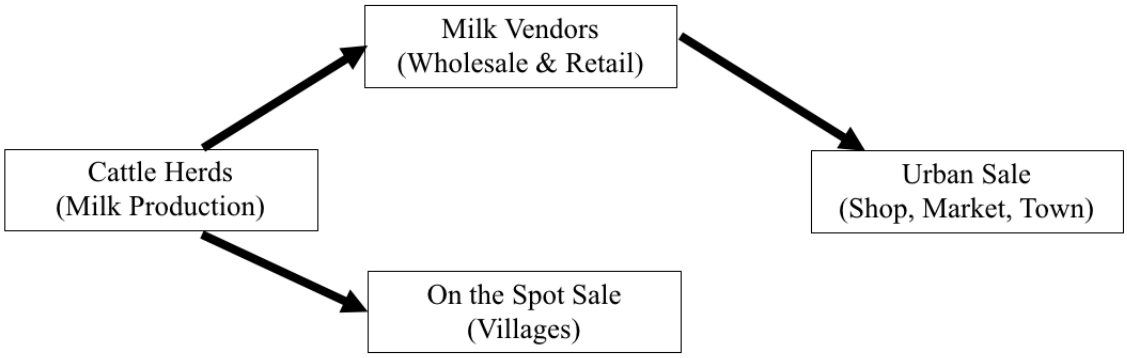


Figure 3. Milk Production Chain: Created by Washabaugh (2016)



CHAPTER II: Milk

2.1 Evolution of Milk

Because of a lack of fossil evidence, the evolution of the mammary gland and lactation is still somewhat unclear and controversial, however, there are widely accepted hypotheses for this development (Lefevre et al., 2010). The evolution of lactation has ancient origins that predate the development of the order Mammalia (McClellan et al., 2008; Lemay et al., 2009; Lefevre et al., 2010; Hinde & German, 2012; Oftedal, 2012). Recent explanations suggest that milk production began as a means to prevent parchment-shelled eggs from drying out and that it also may have played a role in development of the innate immune system (Oftedal, 2002, 2012; Vorbach et al., 2006; McClellan et al., 2008). From there, it is thought that the secretions from the mammary glands evolved to supply nutrients for offspring.

Today we understand breast milk to be an important vehicle of biological communication between mother and offspring, which plays a critical role in physiological development in infants. For example, milk provides infants with easily digestible forms of nutrients required to sustain growth (McClellan et al., 2008). Additionally, the elements composing breast milk are vital to the maturation of the infant immune system through the development and regulation of inflammatory response and intestinal inoculation, or introduction of bacteria to the gut.

Human breast milk is comprised of active ingredients, including immunoglobulins, immunocompetent cells, fatty acids, oligosaccharides, and glycoproteins (Saaverda, 2002; Quigley et al., 2013). This particular form of physiological communication based on maternal health experiences is an example of adaptive immunity. Memory cells are introduced from mother to infant, allowing immediate recognition and destruction of pathogens in the infant without prior exposure (Mor & Cardenas, 2010). Breast milk also provides bacteria that contribute to growth and development in the gut (Quigley et al., 2013). In addition, infants

receive a number of bioactive factors from breast milk, including cytokines and antibodies, which protect the infant against disease-causing agents such as pathogenic organisms (Robinson & Fall, 2012; Donovan 2006; Martin & Sela, 2013). As supportive evidence, breastfed infants experience lower rates of infection compared to infants consuming artificial milks (Robinson & Fall, 2012). Infants who are not breastfed have an increased risk of incidence of infectious morbidity, including otitis media, gasteroenteritis, and pneumonia, in addition to increased risk of childhood obesity, Type 1 and Type 2 diabetes, leukemia, and sudden infant death syndrome (Stuebe, 2009).

2.2 Probiotics & Prebiotics

Fermented dairy products are high in probiotics, which are living microorganisms that can confer health benefits to the host when consumed in adequate amounts (Quigley, 2010; Conlon & Bird, 2015). Prebiotics serve as food for probiotics, and they are selectively fermented by the beneficial bacteria that make up the probiotics. Mucosal barrier function can be reinforced by probiotics, and can increase mucosal antibody production (Conlon & Bird, 2015). Although most research on probiotics has been conducted using animal models, we also know that probiotics can produce neurotransmitters that can modify gut functions such as motility (Quigley, 2010). There is also evidence that when administered orally, probiotics can exert anti-inflammatory effects at body sites far away from the gut, such as an inflamed joint surface (Quigley, 2010). The oligosaccharides in human milk also produce prebiotic effects; they are metabolized by and so promote the growth of beneficial gut bacteria (Hunt et al., 2012). In addition, oligosaccharides can prevent pathogenic bacteria by inhibiting them from adhering to the surface of the intestinal walls (Bode, 2009; Martin & Sela, 2013).

2.3 Cow's Milk (and other animals)

Different mammalian species produce different milks, which are designed to best fit the needs of that particular species during early life development. Compared to human breast milk, cow's milk has more proteins, a higher casein/whey ratio, more fat, less lactose, less overall oligosaccharides, and around the same amount of energy (Bode, 2009; Claeys et al., 2014), It is likely that these compositional differences impact the human gastrointestinal tract and digestion processes, and thereby overall health of consumers.

Table 1 summarizes milk composition differences between humans, cows, sheep, goats, and camels, all of which are major milk producers for human populations consuming non-human milks today.

Table 1. Gross composition of mature milk from different mammals.

	Human	**Cow**	**Sheep**	**Goat**	**Camel**
Proteins (g/l)	9–19	30–39	45–70	30–52	24–42
Casein/whey ratio	0.4–0.5	4.7	3.1	3.5	2.7–3.2
Fat (g/l)	21–40	33–54	50–90	30–72	20–60
Lactose (g/l)	63–70	44–56	41–59	32–50	35–51
Energy (kJ/l)	2843	2709–2843	4038–4439	2802–2894	2410–3286

Milk digestibility can also differ based on milk form. For example, fermented or sour milk products have greater protein digestibility than that of fresh milk (Ahmed et al., 2014). In The Gambia, raw cow's milk is consumed in fresh, sour, and reconstituted powder forms (Hempen et al., 2004). For purposes of this thesis, 'fresh milk' refers to raw milk, which is defined as milk that has not been subject to any processing intended to alter its quality or composition. Food safety authorities and public health agencies alike have scrutinized the safety of consuming untreated raw milk, especially as raw milk consumption has been linked to numerous foodborne illnesses and outbreaks. 'Sour milk' refers to fresh milk that has been

fermented by sitting out in the open for at least 24 hours. Finally, 'powdered milk' refers to milk made by reconstituting milk powder (purchased from local stores) with locally produced sour milk and water.

2.4 Milk Consumption - Consumer Perspectives/Preferences

Beyond its nutritional value, milk holds a place of cultural significance in The Gambia. As described previously, milk represents a major part of livelihood for agropastoralist communities. Milk is a source of income and employment, and the positions within the milk production value chain are generally kept within a family (Mwakikagile, 2010). Within these populations and cultural systems, there exist various beliefs and taboos regarding milk (Perez & Garcia, 2013). The UNICEF Food-Care Health conceptual framework categorizes these taboos as a factor in the causes of malnutrition (Perez & Garcia, 2013). Both fresh and sour milk are avoided by the Fulani when suffering from malaria and pneumonia (Perez & Garcia, 2013), and milk consumption is taboo when symptoms including diarrhea, vomiting, and fever are present (Perez & Garcia, 2013). Finally, male and female circumcision is common in The Gambia, and consuming fresh or sour milk is considered taboo immediately following the circumcision ceremony (Perez & Garcia, 2013). Here, cultural beliefs and food taboos specifically linked to milk are intertwined in some significant traditional behaviors and practices.

CHAPTER III: Weaning Food & Culture

3.1 Infant Feeding Recommendations

The use of animal milks for infant feeding dates back to the ancient world. A survey of medical advice on infant feeding in Graeco-Roman, Byzantine, and Arabian populations dating back before the 1500s reveals that recommended weaning foods included animal milks (Soranus, 1956; Fildes, 1986; Bourbou, 2010). Animal milks also have historical medicinal applications aimed to improve human health. For example, in the 16th century, goat's milk was fed to infants whose mother/wet nurse had syphilis in order to protect the infant from acquiring the infection (Fildes, 1986). Use of non-human milks for infant feeding should be considered a revolutionary step in the evolution of infant feeding because of its great influence on infant health, growth, and survival (Howcroft, 2013).

The World Health Organization (WHO) global public health guidelines recommend that infants exclusively breastfeed until 6 months of age. After that time, WHO recommends that infants receive safe and nutritionally adequate weaning foods that meet their changing nutritional needs. Weaning is the period in which an infant begins to consume foods other than breast milk, which often includes introduction of non-human milks, most commonly cow's milk, used in populations from both low- and high-income countries (Gray, 1996; Garine, 2001; Sellen & Smay, 2001).

In urban and rural areas of The Gambia, less than 34% of mothers exclusively breastfeed their infants for 6 months (UNICEF, 2013; Eriksen et al., 2017). Weaning foods are commonly introduced from three months and onwards, which may put infants at greater risk for intestinal bleeding or other health complications (Whitehead et al., 1978; Barrell & Rowland, 1979). By 5 months of age, most infants (~75%) surveyed in rural Gambia were consuming non-breast milk foods (Whitehead, 1979; Eriksen et al., 2017). Prentice and Paul (2000) report that first weaning

foods include rice gruel with sugar, millet gruel with sugar, rice gruel with sour milk, rice porridge, and rice and groundnut porridge. These reports are supported by more recent work in the West Kiang region of The Gambia, where mothers reported that groundnuts were the most common weaning food ingredient, along with other foods such as corn porridge, mixture of groundnuts and rice, and animal milk (Xu et al., 2017).

Cow's milk should not be introduced to an infant's diet until after the first year of life because of potential adverse health effects or even life threatening responses to non-human milks as the gut is underdeveloped (Leung & Sauve, 2003). By triggering chronic inflammatory responses, unpasteurized cow's milk has the potential to produce morbidities in various areas of the infant body, including gastrointestinal distress and atopic dermatitis (skin rash). In addition, it is estimated that 40% of infants that consume cow's milk before 12 months of age suffer from occult intestinal blood loss (Ziegler, 2007). This intestinal bleeding may be related to the lack of immune tolerance to the foreign proteins (Sullivan, 1993). Our understanding of adverse reactions to cow's milk originates with Hippocrates (prior to 370BC), who described that skin and gastrointestinal symptoms were common after consumption (Chabot, 1951).

Ruminant milks have a high casein content (cow's milk contains twice as much casein as human milk), which causes a firm curd or clot to form in the stomach (McClellan et al., 2008). The curd traps fat globules within it, which leads to a slow release of amino acids, peptides, and fat to sustain offspring through an extended-release of protein between feedings (McClellan et al., 2008). This curd is difficult for human infants to digest, whereas the low casein:whey ratio of human milk protein produces a softer, more rapidly digestible curd (McClellan et al., 2008). These properties of cow's milk do not negatively affect calves, who have different digestive systems from human infants.

3.2 Infant Feeding Cultural Norms: Colostrum Taboos

Colostrum, or 'first milk,' is produced immediately postpartum. It is characterized by its thick off-white consistency, and is incredibly beneficial for infants as it is full of nutrients, proteins, and antibodies to aid the infant in its transition from the protective womb into an environment comprised of foreign substances and pathogens. The higher content of bioactive factors in colostrum compared to mature milk has been attributed to the need for protection and nutrients immediately after birth, since this is the most vulnerable period for the infant (Castellote et al., 2011).

Negative beliefs regarding colostrum have been documented in The Gambia, which largely stem from cultural and traditional beliefs, along with inadequate information regarding the benefits and importance of breastfeeding (Semega-Janneh et al., 2001; Njai & Dixey, 2013). Colostrum is seen by some Gambians as impure or unsafe, and in interviews by Semega-Janneh et al. (2001), some equated colostrum to pus. According to Semega-Janneh et al. (2001), colostrum was considered 'bad milk' and mothers were expected to discard it instead of feeding it to their infants. More specifically, these authors report that colostrum was considered 'hot milk,' which caused diarrhea and stomach pain if fed to the newborn. The respondents also said that newborn animals reacted in the same way if they consumed their mother's colostrum (Semega-Janneh et al., 2001).

While many Gambian mothers breastfeed their infants for 18-24 months, initiation is usually delayed at least one day after delivery (Njai & Dixey, 2013). In one study, around 8% of cases used wet nurses to feed the infant for the first few days of life as opposed to the infant's mother in order to avoid feeding the baby colostrum (Semega-Janneh et al., 2001). In other cases in The Gambia, babies who do not receive colostrum are instead given warm water with or

without sugar (and occasionally salt), cow's milk, or formula, until the mother begins to produce mature milk (Semega-Janneh et al., 2001; Perez & Garcia, 2013). By diluting milk with water, the newborn is unable to take in sufficient nutrients or meet daily nutritional requirements. In this way, consuming sugar water or diluted non-human milk instead of colostrum could compromise infant health. The water makes the neonate more susceptible to gastrointestinal infections (Prasad, 2015). More specifically, incorrect dilutions of formula or milk can result in reduced intake of the nutrients necessary to survive, which can leave an infant vulnerable to immune deficiencies and illnesses that can result in morbidities such as diarrhea. Additionally, using contaminated water can lead to diarrhea and other infections simply due to introduction of foreign molecules to the infant's gut (Greiner, 1991; WHO, 2015). Research in Botswana found that formula mixed with contaminated water increased a child's risk of death by 50-fold (Mead, 2008).

The WHO states that prelacteal feeds (artificial feeds or drinks given to an infant before breastfeeding is initiated) are dangerous for a number of reasons. To start, the prelacteal feeds replace colostrum as the infant's earliest food, which in turn makes the infant more likely to develop 1) infections such as diarrhea, septicemia, and meningitis, 2) intolerance to proteins in artificial feeds, and 3) allergies and atopies (WHO, 2006). A study in India found that infants who received prelacteal feeds were significantly more likely to be stunted and wasted compared to those that were exclusively breastfed (Meshram et al., 2012). In addition, the WHO reports that prelacteal feeds are linked to maternal complications such as engorgement, along with earlier breastfeeding cessation compared to if the infant was exclusively breastfed.

3.3 Weaning Foods, Timing, & Immune System

The human body invests heavily in cells designed to defend and protect against infections. Collectively, those cells form the immune system (Parham, 2014). The immune system develops and matures over the course of the human life, with infants and the elderly being the most vulnerable (Kennedy, 2005; Parham, 2014). The first line of defense against invaders is the innate immunity, which is comprised of both physical and chemical barriers to infection and is in place when we are born (Wilson & Hunt, 2002; Kennedy, 2005; Parham, 2014). While many infections are stopped by the nonspecific mechanisms formed by innate immunity, the defense system is not perfect. The adaptive immune system is the second line of defense if the innate immune system fails (Parham, 2014). The adaptive immune system is targeted towards specific antigens and molecules foreign to the host and is more complex than the innate (Wilson & Hunt, 2002; Alberts et al., 2002). It is composed of specialized cells and processes that are highly responsive to the particular pathogen that induced the immune response (Alberts et al., 2002). Adaptive immunity also has a 'memory' that makes future immune responses to the specific antigen more efficient.

When the infant is weaned, mother's milk as an exogenous source of immune molecules is lost (Kennedy, 2005). Following weaning, infant immune systems must become much more self-reliant instead of dependent on passive immunity from the mother (Robinson & Fall, 2012). Research in both low- and high-income countries has shown that introduction of weaning foods before 6 months of age increases infant morbidity and mortality (Dewey et al., 2001).

Introduction of non-breast milk foods begins to alter the gut microbiome, and provides a source of potential pathogens, such as diverse and complex bacteria. In addition, early introduction of cow's milk/non-breast milk weaning foods may reduce breast milk intake,

thereby contributing to the weanling's dilemma (Rowland, 1986; Downes et al., 1992). The weanling's dilemma refers to the transitional period in which an infant cannot rely on a mother's breast milk alone and must begin consuming non-breast milk foods. The dilemma is between the inevitable risk of growth faltering if the baby continues to only consume breast milk, and the infection risk from introducing weaning foods to supplement nutritional intake (Rowland et al., 1978).

3.4 Weaning Food Contamination in The Gambia

Contaminated weaning foods can serve as a major route of transmission of illness. It is a common practice in Gambia for infant foods to be prepared in large quantities in the morning, sufficient enough for several meals in the day (Barrel & Rowland, 1979; Washabaugh, pers. obs.). After preparation, these foods are stored at ambient temperatures, which allows the child to be fed on demand (Barrell & Rowland, 1979). In their research, Barrell and Rowland (1979) found that foods that were not consumed fresh were became bacterially contaminated after 8 hours in ambient temperature. In addition, foods prepared in the wet season contained higher levels of potential pathogens compared to foods from the dry season, presumably because the environmental conditions during the wet season are very conducive to bacterial growth (Barrell & Rowland, 1979). In The Gambia and other regions of the world, some gruels are prepared using contaminated water that contains potentially pathogenic coliform bacteria, from both animal and human sources (Whitehead, 1979). The health threats from contaminated weaning foods are compounded by the fact that the wet season is the time period when diarrheal diseases are at their highest.

3.5 Infant Feeding: Health, Gender, & Culture

Growth faltering, or stunting, is tightly linked with enteropathy, or chronic inflammation of the mucosa of the small intestine, which is the main site of digestion/nutrient absorption throughout the gastrointestinal tract (Lunn, 2000). Up to 25% of growth faltering in Gambian infants can be explained by the decreased ability to digest lactose, which is associated with mucosal enteropathy in the small intestine (Northrop-Clewes et al., 1997). Gastrointestinal enteropathy is thought to be associated with the introduction of unhygienically prepared weaning foods, and upon onset, it becomes a self-perpetuating system (Lunn, 2000; Campbell et al., 2003a). In addition, chronic inflammation has been shown to lead to growth stunting and poor defense to diarrheal diseases, which are prevalent health issues in The Gambia (Lunn et al., 1991; Campbell et al., 2003b; Prendergast et al., 2014). Exogenous factors such as seasonality and disease load likely contribute to growth stunting as well.

Gender roles and childcare practices may impact infant health as well. For example, women in The Gambia work long, hard hours in the agricultural fields especially during the wet season. While they sometimes take their babies with them, they may also leave their infants with family members – usually younger daughters – who are then responsible for feeding the infants during the day (Semega-Janneh et al., 2001). In this way, infant care (including infant feeding) can be shared by individuals other than the mother (Sellen & Smay, 2001; Sellen 2007). In general, women living in subsistence economies decrease breastfeeding frequency as subsistence activities increase (Nerlove, 1974; Piperata & Mattern, 2011).

Finally, the impact of women's work patterns on breastfeeding depends on overall workload, location of work/distance of work from home, ease of work interruption (being able to go home to feed infant), and the extent to which infant/child care can be shared with others (Huffman 1984, Piperata & Mattern, 2011). According to Fouts et al. (2005) and Ghosh et al.

(2006), women farmers report that the amount of physical effort required to carry a child to the fields is a reason to wean children at earlier ages. By placing infants and children under the care of elderly relatives or older siblings, an earlier introduction of weaning foods and possibly an earlier cessation of breastfeeding may be a necessary alteration to infant feeding (Howcroft, 2013).

CHAPTER IV: Milk Hygiene

4.1 Milk as a Medium for Bacterial Growth

Even though milk is a valuable nutritional resource, when collected, stored, and/or consumed under certain unhygienic conditions, it can increase risk for illnesses, thereby inadvertently negatively affecting consumer health. Milk is an especially sensitive medium for contamination as it provides the nutrients essential for bacterial growth, including sugar, protein, fats, and minerals (Quigley et al., 2013). In addition, proper acidity is important for optimum bacterial growth, and the acidity of fresh milk often hovers around pH 6.8, which is the point at which most microorganisms grow best (Quigley et al., 2013). Milk also has a high moisture content, which is essential for bacterial proliferation.

Milk can harbor numerous bacterial species, including pathogenic forms such as: *Brucella* spp., *Campylobacter jejuni*, Coliforms, *Coxiella burnetii*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Mycobacterium bovis*, *Mycobacterium paratuberculosis*, *Salmonella* spp., and *Yersinia enterocolitica*. These bacterial species are major milkborne pathogens, and their associated diseases are detailed in Table 2.

It is important to note that *E. coli* is specified as *E. coli* O157:H7 in the previous paragraph. This is because *E. coli* O157:H7 is a bacterium that produces a deadly toxin and is responsible for ~73,000 cases of foodborne illness each year in the United States (Grodner et al., 2016). It is a major food safety concern. It is a serotype (a group within a single species of microorganisms that share distinctive surface structures) of *E. coli*, and produces Shiga-toxin, which is one of the most potent bacterial toxins known (Karch et al., 2005). It is a common cause of foodborne illness, often originating from consumption of contaminated and raw foods, including raw milk (Karch et al., 2005). *E. coli* O157:H7 infection can lead to severe diarrhea, kidney failure, and death in children under five years of age and elderly patients, both of which

have weaker immune systems (Tamparo, 2016). It is transmitted via the fecal-oral route, and can be transmitted by contaminated food or water, or contact with contaminated surfaces. Many warm-blooded mammals have this particular *E. coli* serotype in their gastrointestinal tracts, and cattle lack the Shiga-toxin receptor needed to produce the Shiga-toxin, meaning that they can act as asymptomatic carriers (Priumboom-Brees et al., 2000). Throughout the remainder of this thesis, I will refer to *E. coli* O157:H7 as 'pathogenic *E. coli*.'

Table 2. Common milk bacteria and associated diseases

Organism	**Disease**	**Disease Symptoms**	**Source**
Campylobacter jejuni	Gastroenteritis	Diarrhea, abdominal pain, fever	Intestinal tract and feces
Coxiella burnetii	Q-fever	Chills, fever, weakness, headache, possible endocarditis	Infected cattle, sheep, and goats
Escherichia coli O157:H7* *pathogenic *E. coli*	Gastroenteritis Hemolytic uremic syndrome (HUS)	Diarrhea, abdominal pain, bloody diarrhea Kidney failure, possible death	Intestinal tract and feces
Listeria monocytogenes	Listeriosis	Flu-like symptoms, miscarriage, stillbirths, fetal death, and spontaneous abortion	Water, soil, environment
Mycobacterium bovis or *tuberculosis*	Tuberculosis	Lung disease	Infected animals
Mycobacterium paratuberculosis	Johne's (ruminants)	Unconfirmed link to Crohn's disease in humans	Infected animals
Salmonella spp.	Gastroenteritis Typhoid fever	Diarrhea, nausea, fever	Feces and environment
Staphylococcus spp.	Food poisoning	Fever, headache, chills, vomiting, abdominal pain, diarrhea	Meat, milk, eggs
Yersinia enterocolitica	Gastroenteritis	Diarrhea, appendicitis	Environment, water, infected animals

4.2 Food Safety and Foodborne Illness

Food safety focuses on the handling, preparation, and storage of food in ways that prevent foodborne illness. Foodborne illness refers to any illness caused by consuming contaminated food or drink. As Table 2 illustrates, the most common clinical symptoms of milk-based foodborne illnesses are gastrointestinal, but in general foodborne illnesses can also present in neurological or immunological disorders (Grace, 2015). Foodborne illness may contribute to both wasting and stunting in children through diarrhea, aflatoxins, or ingestion of fecal material on food or in the environment, which may contribute to enteropathy (Grace, 2015).

Research has demonstrated that informal markets in developing countries are major sources for foodborne illnesses (Grace, 2015). The foods that are particularly notorious sources of illness are also the foods that would otherwise provide high-quality nutrition to the consumer, and include leafy vegetables, fish, eggs, and dairy (Grace, 2015; Roesel & Grace, 2015). Here, 'informal markets' refers to (after Roesel and Grace, 2015): (1) markets where many vendors/salespeople are not licensed and do not pay tax, (2) markets where traditional processing, products, and retail prices predominate, and (3) markets which escape effective health and safety regulations.

Foodborne illness is not only a health concern, but can also determine export market access and can affect informal domestic markets (Grace, 2015). For example, certain pathogenic microorganisms can impact factors such as milk texture, flavor, scent, and shelf life of milk products (Quigley et al., 2013). Hempen and colleagues (2004) report that over 90% of Gambian milk samples analyzed in their study demonstrate bacterial contamination exceeding Kenyan Dairy Hygiene Standards. Using coliform bacteria as an indicator organism, Hempen et al. (2004) showed found that 88.6% of fresh and 54.9% of sour milk samples contained coliform bacteria in concentrations greater than $5x10^4$ colony forming units (CFU)/mL. Colony forming

units are used as a measure of the number of individual colonies of microorganisms present in or on a sample. Over 23% of fresh and sour milk samples contained concentrations of *E. coli* greater than $1x10^5$ CFU/mL. Additionally, 29.2% of fresh and 17% of sour milk contained concentrations of *Staphylococcus spp*. greater than $2x10^3$ CFU/mL. In total, 35.6% of fresh (84/236) and 8.5% of sour milk (12/142) contained visible impurities such as dirt or mold. In general, fresh milk was found to be more contaminated than sour milk; when comparing contamination across the different levels of the production chain, contamination is the highest at the level of collection (after milking but before transportation to markets) (Hempen et al., 2004).

Introducing measures to regulate food hygiene in local markets, such as requiring permits, may inadvertently reduce the availability of or access to these nutritious foods (Grace, 2015). Regulations have the potential to reduce household income if individuals are unable to sell their products at the markets. This may become an issue when the regulations require costly practices prior to sale. For example, dairy products are considered most safe for consumption after pasteurization. However, this technology is largely unavailable in many developing countries. Even if pasteurization units were commonly available, without reliable transport or monetary resources, sustaining the practice of pasteurizing milk could be impossible, thereby damaging a household's chance for steady income through dairy products.

4.3 Hygiene in the Production Chain: Introduction

There are many opportunities throughout the milk production chain, which encapsulates all processes from milk extraction to milk sale/consumption, for dairy and dairy products to become contaminated with unwanted bacteria. For example, harmful bacteria can contaminate milk through mastitis, fecal contamination pre- or post-milking, contaminated feed, from human skin following contact with udders during the milking process, and the environment (LeJuene &

Rajala-Shuzltz, 2009; Matua, 2013). While there are several obvious potential routes of bacterial contamination of milk, it is important to note that many human pathogens can also originate from clinically healthy animal hosts. Because of this, it will be important to standardize protocols for milk hygiene practices, and to emphasize the need for thorough hygiene policies on a global level (Matua, 2013).

Bacterial contamination can be minimized with properly designed milking systems and consistent hygienic practices, including cleanliness of facilities, supplies, and environmental conditions. In commercial dairies, there are active measures to maintain sterile work environments. The following sections will provide detail on the standard milking processes and hygienic practices used in commercialized dairy settings in the United States based on information gleaned from Jones (2006), in order to provide a basis of comparison for discussion of the hygiene practices employed in The Gambia.

4.4 Milking Hygiene Procedures in the Production Chain

In the U.S., dairy workers are required to wear sterile rubber gloves during all milking procedures (Gladis et al., 2014). Doing so can reduce new udder infections by 44%. Stripping, or removing, 4-5 squirts of milk before milk collection removes foremilk content, which may contain high bacteria counts (Wolf et al., 2016). Stripping should be done before the actual washing and drying of the udder; doing so reduces incidence of new udder infections by 18%, whereas stripping after udder preparation is less effective (Gladis et al., 2014).

If there is no sign of infection, teats are then scrubbed by hand with direct streams of sanitizing solution. After cleaning, the teats are dipped into germicidal ‘teat dip,’ which destroys microorganisms that can contaminate the teat skin before milking (Verhaeghe, 2015). This same

procedure is implemented post-milking as well. After drying, the teat cups on the milking equipment that extract milk from the udders are applied to the teats for milk collection.

4.5 Pasteurization and Other Practices to Inhibit Bacterial Growth

Pasteurization is a process that applies heat to destroy pathogens in food. In the dairy industry, this process heats every particle of the product to or above specific temperatures for a predetermined amount of time. This process was developed by Louis Pasteur in the 1800s after discovering that microbes caused alcohol to sour, and that by heating and cooling the beverages in a particular way, the bacteria were destroyed. Prior to the introduction of pasteurization, commercially-sold milk and milk from farms was a major contributor to severe human infections due to contamination (Robertson, 1919; Winslow, 1952; Holsinger et al., 1997; Kent et al., 2015). Today, the process of pasteurization is considered one of the greatest health achievements of the 20th century (Matua, 2013).

In the United States, the most commonly used method of pasteurization is High Temperature Short Time (HTST) pasteurization. In this process, metal plates and hot water heat milk to at least 161°F for at least 15 seconds (Stabel & Lambertz, 2004). HTST is the current primary method for heat treatment of all dairy products in processing plants (Stabel & Lambertz, 2004). In aseptic processing, or Ultra High Temperature (UHT) pasteurization, milk is heated using sterile equipment and filled under aseptic conditions into hermetically sealed packaging. Products handled under these conditions do not need refrigeration until opened (International Dairy Foods Association, 2016).

While pasteurization is not always possible, there are alternative methods of heat treatment that can still reduce bacterial contamination. For example, an alternative to pasteurizing is at-home boiling. However, Dhanashekar (2012) report that while boiling milk for

one hour lead to a decrease in general pathogenesis of the product, autoclaving at 15psi for 20 minutes was required to destroy the enterotoxin produced by *Staph.* spp.

Milk is not currently pasteurized in The Gambia, although it was previously in the early 2000s, and consumers in this area seldom boil milk on their own due to various beliefs and preferences (Touray, 2016). Milk is generally not refrigerated at any point throughout the production chain in The Gambia, as electricity and refrigerators are unreliable and/or unavailable. Because the average annual temperature is 90°F in The Gambia, unrefrigerated milk is at further risk of contamination and/or introduction of additional bacterial sources. Hempen et al. (2004) report that, under ambient temperatures in the tropics, a bacterial cell in milk will multiply to 2 million cells in a typical generation time of 20 minutes within 7 hours. In addition, keeping milk at ambient temperatures is associated with higher bacterial contamination of milk compared to milk kept in cooling tanks, which demonstrates the importance of temperature control during milk storage (Elmoslemany et al., 2010).

Some pathogenic organisms, such as *Listeria* spp., are able to grow at refrigeration temperatures (~2-4°C), and organisms such as *E. coli* and *Salmonella* spp. can multiply at temperatures of around 8°C (Matua, 2013). Nonetheless, reducing milk temperature after completion of milking and throughout the production chain may at least decrease proliferation rate of bacteria in the milk. In the United States, the Department of Health and Human Services specifies that milk must be cooled to 10ºC or less within four hours or less of the commencement of the first milking, and to 7°C (45ºF) or less within two hours after the completion of milking. Additionally, the temperature of milk in storage should not exceed 7ºC after that point (US Department of Health and Human Services, 2009).

CHAPTER V: Hygiene Indicator Organisms

5.1 Indicator Organisms

Indicator organisms are microorganisms whose presence is used to assess hygienic quality of products, liquids, and/or surfaces. Generally, presence of indicator organisms indicates probable presence of pathogens, which can be used to predict food safety (Jay, 2012). In large numbers, *Enterobacteriaceae* indicates fecal contamination of food and inadequate food processing and handling (Koneman et al., 1994). In the United Sates, coliforms have been the traditional indicator organisms, whereas Europe has shifted their testing standards to examine *Enterobacteriaceae*, which include more specific gram-negative bacterial species (Gilbert et al., 2000; Treyens, 2009). Measures of coliform bacteria are regularly used in the United States to determine hygiene status. Traditional microbial testing used coliform counts to determine food safety. Coliform bacteria originate in the environment and within the intestinal tracts of warm-blooded animals (Treyens, 2009). Because they are found in animal intestinal tracts, coliform bacteria are indicators of contamination with fecal material, and the possible presence of intestinal parasites and pathogens and contamination with fecal material (Treyens, 2009). Concentrations of these organisms reflect microbiological quality of products, including milk, and when detected, their presence suggests a need to further investigate milk hygiene, udder health, and safety of milk (Garnica et al., 2013).

The *Enterobacteriaceae* family may provide a more conclusive picture of potential contamination than detection of coliforms, and the genera are some of the biggest culprits of foodborne illness. *Enterobacteriaceae* may be superior to coliforms as indicators of sanitation because they have collectively greater resistance to heat treatment than the coliforms. This group is more widely used as indicators in Europe than in the United States. In the 2009 food hygiene guide published in the United Kingdom, *Enterobacteriaceae* levels greater than 10^4 CFU/mL are

considered unsatisfactory (HPA, 2009). Additionally, these guidelines specify that presence at these levels overall suggests poor hygiene status of a food product (HPA, 2009). The guidelines also state that likely causes of contamination occur from food handlers or food contact surfaces, as well as poor temperature control (HPA, 2009). In 2000, the FAO-WHO evaluated several hygiene indicator organisms, and concluded that *Enterobacteriaceae* are the "ideal tool to assess the effectiveness of preventative measures and detect the occurrence of contamination" (Buchanan & Oni, 2012).

Enterobacteriaceae are often used for assessing milk hygiene post-processing, as they are resistant to pasteurization (Quigley et al., 2013). While certain strains of some species are harmless commensals, such as certain strains of *E. coli*, others are serious human and animal pathogens (Baylis et al., 2011). The *Enterobacteriaceae* family includes the primary pathogens of concern with regard to food safety and hygiene, including pathogenic *E. coli, Salmonella,* and *Staph*. Other pathogens include*, Klebsiella, Citrobacter, Enterobacter, Serratia, Shigella,* and *Yersinia*.

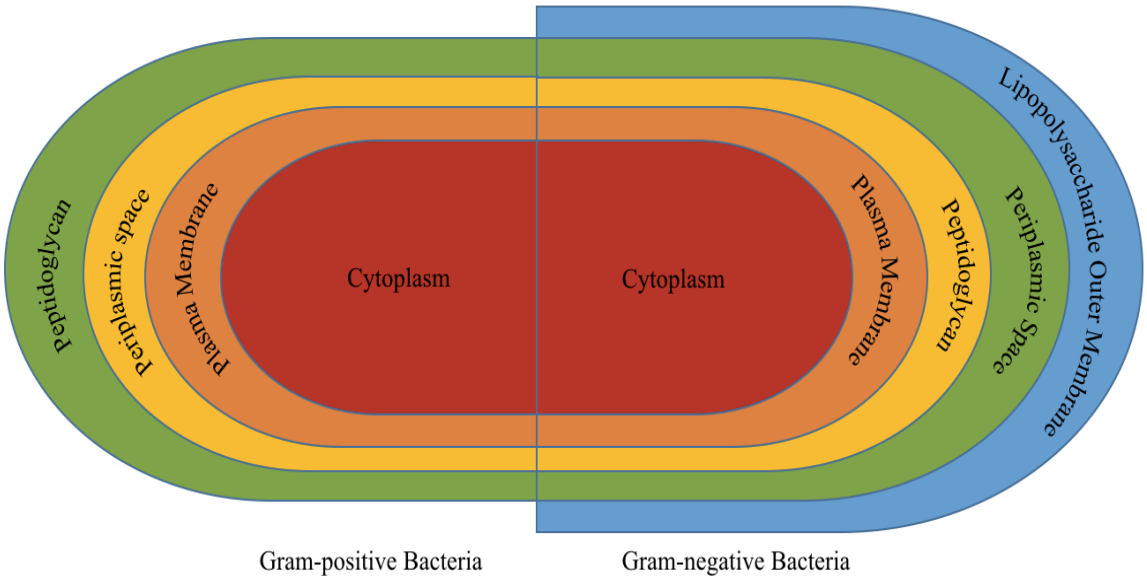
5.2 Gram-negative & Gram-positive Bacteria

Gram-negative bacteria have more complex cell walls compared to Gram-positive bacteria. Gram negative bacteria are composed of a thin cell wall composed of peptidoglycan, which is then surrounded by an extra layer of cells called the lipopolysaccharide outer membrane (LPS) (Figure 4) (Silhavy et al., 2010). As opposed to Gram-positive bacteria, Gram-negative bacteria do not take up and retain the color of crystal violet dye used in a staining method, which was designed by bacteriologist J.M.C. Gram (1853-1938).

Most commonly found in the gastrointestinal tract of animals, Gram-negative bacteria can be responsible for disease, and are more resistant to antibodies than Gram-positive bacteria

because of their structurally different and impenetrable cell walls (Silhavy et al., 2010). More specifically, the cell wall of Gram-positive bacteria is easily destroyed by lysosomes, which leads to the death of those bacteria, whereas Gam-negative bacteria are protected by the LPS (Silhavy et al., 2010). Over 90% of Gram-negative bacteria are pathogenic, whereas Gram-positive are predominantly non-pathogenic. In general, the presence of Gram-negative bacteria in dairy products indicates poor hygiene. Examples of Gram-negative bacteria include *E. coli*, *Salmonella*, and other *Enterobacteriaceae* species.

Figure 4. Cell structure of Gram-negative VS Gram-positive bacteria



5.3 Gram-positive Bacteria: Lactic Acid Bacteria (LAB)

Lactic acid bacteria (LAB) represent a diverse group of Gram-positive microorganisms living within plants, meats, and dairy products (Abdel-Rahman et al., 2013). LAB produce lactic acid as an anaerobic product of glycolysis (Abdel-Rahman et al., 2013). Common genera of LAB

include Lactobacilli, which are used for the production of yogurt and cheese. The optimal growth conditions for LAB vary depending on the producers, but it can grow in temperature ranges of 5-45°C and pH ranges of 3.5-10.0 (Abdel-Rahman et al., 2013).

LAB produce numerous compounds such as organic acids, hydrogen peroxide, and bacteriocin or bactericidal proteins during lactic fermentations (Talarico & Dobrogosz, 1989; Lindgren & Dobrogosz, 1990; Piard & Desmazeaud, 1991; Anderssen et al., 1998; Sholeva et al., 1998; Ouwehand, 1998; Zhennai, 2000; Oyetayo et al., 2003; Savadogo et al., 2004). Bacteriocins and bactericidal proteins are produced by bacteria to inhibit the growth of closely related bacterial strains. Many bacteriocins that are produced from LAB have received great attention recently as a novel tool for controlling pathogens in food (Savadogo et al., 2004). In addition to this, the antimicrobial effects of LAB have been used as alternatives to antibiotics in treating gastrointestinal diseases (Savadogo et al., 2004; Saez-Lara et al., 2015).

Because milk is concentrated with nutrients, it is able to support diverse, complex microbial communities (Quigley et al., 2013). The array of life that can be sustained within milk includes microorganisms that facilitate fermentation processes, cause spoilage, and protect or harm human guts (Quigley et al., 2013). Fermentation is the chemical breakdown of a substance by bacteria, yeasts, or other microorganisms.

Milk is known as a natural habitat for LAB, and milk fermentation is reliant on LAB activity (Olsen, 1990; Urbach, 1995; Maragkoudakis et al., 2006). LAB presence in milk fermentation can be either spontaneous (natural) or as inoculated culture starters (Widyastuti et al., 2014). LAB alters the texture of milk by transforming it from a fluid to a soft and more coagulated material, which allows for easier digestibility (Widyastuti et al., 2014). In addition, certain strains of LAB have been shown to have beneficial effects on gut function, and have

demonstrated the ability to improve gastrointestinal health and relieve symptoms in individuals with gastrointestinal disorders such as irritable bowel syndrome (Eales et al., 2017).

5.5 Implications: Diarrheal Diseases & Consumer Health

Foodborne diseases can be caused by microbiological, chemical, or physical contaminants (Havelaar et al., 2015). Known more commonly as 'food poisoning,' foodborne illnesses/diseases can include symptoms such as vomiting, diarrhea, fever, nausea, and abdominal pain (Langer et al., 2012). Diarrheal diseases are a major concern for the wellbeing of young children. In fact, diarrheal diseases are the second most common illnesses in children after respiratory infections, and have the greatest negative impact on infant and child growth (Lee & Middleton, 2003; Tetteh et al., 2004). Additionally, there are over 200 diseases that result from consumption of contaminated food or water, and these diseases account for nearly 2 million deaths per year (Shrivastava et al., 2015). Of those 2 million deaths per year, 1.9 million are children (WHO, 2009; Roesel & Grace, 2015).

Diarrheal diseases often coexist with nutrient deficiencies, which can lead to cycles of malnutrition and infections (Bhutta & Salem, 2012). In infants, diarrhea and improper complementary weaning foods are major contributors to undernutrition (Barrell & Rowland, 1979; Bhutta & Salem, 2012). Improper complementary feeding and recurrent infections increase the risk for malnutrition (Bhutta & Salem, 2012). Malnutrition is tightly linked to diarrhea, and acute diarrhea accounts for 35% of total diarrheal-related deaths (WHO, 2009).

The most vulnerable period for these conditions are in the first two years of life, and a large proportion of deaths from malnutrition are associated with increased susceptibility to illness (You et al., 2012). In children in rural areas of The Gambia, where weaning foods are introduced ~3 months of age, there is a significant negative relationship between diarrheal

disease and both height and weight gain, and diarrhea is a leading cause of infant morbidity and mortality (Barrell & Rowland, 1979; WHO, 2015). Growth stunting in Gambian infants parallels the appearance of diarrheal disease, starting between 3-6 months of age (Rowland et al., 1978). In addition, diarrhea in weaning infants as a result of gastrointestinal infection was found to be four times more frequent than in exclusively breastfed infants in The Gambia (Sillah et al., 2013). In addition, diarrhea did not have a significant effect on growth in exclusively breast-fed infants in this Gambian population, which suggests that breastfeeding ameliorates weight loss or compromised growth caused by diarrhea in infants (Sillah et al., 2013). It is estimated that 54% of all deaths of children under 5 years of age in developing countries are linked with malnutrition resulting from poor feeding practices in the first year of life (Sagoe-Moses & Ketsela, 2005).

Introduction to MA Thesis Research

The following pages will discuss in detail a two-part assessment of dairy consumption and handling practices throughout the milk production chain in The Gambia. First, I will describe my preliminary research study identifying milk consumption patterns and practices in rural Gambia. Next, I will discuss my main research study examining milk consumption and handling practices by Gambian herdsmen and vendors, along with an assessment of local cow's milk hygiene.

PRELIMINARY STUDY

RESEARCH AIMS & HYPOTHESES

The literature reviewed above indicates that consumption of raw milk or milk products purchased from unregulated, informal market settings could lead to morbidities in consumers. Infants consuming raw nonhuman milk and milk products in early life may have increased susceptibility to gastrointestinal issues such as digestive dysfunctions, nutrient malabsorption, and intestinal disease. Because of this, I designed a study to assess the current patterns of nonhuman milk consumption by mothers and infants living in rural Gambia. The last study to quantify consumption of these foods was published 25 years ago, and in order to determine if consumption of milk remains a potentially significant public health issue in this region, we must obtain up-to-date detailed information on how milk is being purchased, stored, and consumed today. The aims and hypotheses of my preliminary study are as follows:

Aim 1: The first aim of my preliminary study is to detail modern milk consumption practices of mothers and children in rural Gambia through a representative survey. More specifically, I am interested in investigating how milk use (consumption frequency and milk

form) by mothers and children has changed in rural Gambia (Keneba) since its last examination by Erinoso et al. (1992). I hypothesize that:

A_1H_1: The frequency of milk (in both fresh and sour form) consumption by mothers and children in rural Gambia (Keneba) has not changed since 1992 when these practices were examined by Erinoso and colleagues.

Aim 2: The second aim of my preliminary study is to describe common use and timing of introduction of non-breast milk liquids and foods to infant diet in rural Gambia. I hypothesize that:

A_2H_1: Non-breast milk liquids and other foods are regularly introduced earlier than WHO recommendations (6 months of age), which could have implications for infant health.

MATERIALS & METHODS

Survey & Opportunistic Data

In order to determine maternal and infant milk consumption patterns, a structured questionnaire (Appendix 1.1) was administered in 2015 to mothers (n=194) at the MRC in Keneba, The Gambia. Keneba is a remote village located in the Lower River Region/West Kiang region of The Gambia near the river Gambia (Figure 6). It is also the site of one of the Medical Research Council Gambia Unit's field stations. This survey was embedded in a larger on-going project in Keneba called Hormonal and Epigenetic Regulators of Growth (HERO-G, designed to identify the hormonal and epigenetic drivers of growth stunting in Gambian infants, Robin Bernstein PI), and administered by trained field workers assigned to the project. The survey focused on both mother and child milk consumption, and asked questions relating to 1) forms of milk intake – including sour versus fresh, 2) species of animal producing milk consumed, 3) frequency of milk intake, 4) milk storage conditions, 5) and sources of milk (e.g., herdsman, vendor, store).

Statistical Analyses

Coded responses to the questionnaire were analyzed using JMP Pro Statistical Software, Version 13 (SAS Institute Inc., Cary, NC, 2016). All statistical analyses discussed throughout the remainder of this thesis were conducted using JMP Pro, Version 13 unless indicated otherwise. Because the last examination of this information was 25 years ago, I tested my hypothesis of seeing no changes in milk consumption in a rural Gambian population by comparing my results to that of Erinoso et al. (1992).

RESULTS

Frequency of Milk Consumption

Results from the preliminary study survey show that 28.9% of mothers give their children cow's milk once per week, 28.4% 2-4 times per week, 2.6% once per day, and 1.03% more than once per day (Table 5). Comparatively, the survey conducted by Erinoso et al. (1992) showed that 41% of mothers give their children cow's milk once per week, 32% 2-4 times per week, 19% once per day, and 8% more than once per day (Table 7). In my survey, 11.9% of mothers report never consuming cow's milk, 39.2% once per week, 32.5% 2-4 times per week, 4.6% once per day, and 3.1% more than once per day (Table 5). A total of 30.4% of mothers never give their children cow's milk, whereas Erinoso et al. (1992) reported that 26.9% of mothers never gave their children milk. Table 7 summarizes Erinoso et al.'s (1992) findings compared to those collected in my survey. Percentage differences between the two surveys are also included in Table 7, which demonstrates the clear changes in milk consumption between the last 25 years, thereby rejecting $\mathbf{A_1H_1}$**.**

The frequency of cow's milk consumption by the mother is very weakly positively correlated with frequency of cow's milk consumption by the infant (R^2=0.371) (Table 5). Cow's milk is more frequently consumed by both mothers and children compared to goat's milk. Over 28% of children and 32.5% of mothers are reported to consume cow's milk 2-4 times per week, whereas 0.5% of children and 0.0% of mothers consume goat's milk at the same frequency. In addition, 88.1% of children and 90.7% of mothers never drink goat's milk. These results are summarized in Table 5 and Table 6.

Livestock Ownership & Milk Sources

Many survey respondents (41.8%) do not own any milk-producing animals, but of those who did, goats were the most commonly owned (26.3%), followed by cows (19.6%), and sheep

(3.1%). However, only 9.8% of respondents report using owned animals for the source of the milk they consume. Instead, 51.03% received milk from herdsmen, 23.71% from vendors, and 0.5% purchased milk from grocery stores with refrigeration.

Milk Preferences & Milk Storage

Sour milk was the most common form of milk given to children (49.0%), followed by fresh milk (25.3%), mixed into gruel or porridge (22.2%), prepackaged (0.5%), and various combinations of the aforementioned categories (4.6%). Milk is most commonly stored in containers made of plastic (62.9%) and least commonly stored in glass vessels (3.1%) (Table 4). Out of the 194 respondents, 28 report that milk is stored in "other" types of vessels. Those respondents specified that the "other" types of vessels included bowls (85.7%) and iron vessels (14.2%). Glass vessels were the least commonly used, with only 3.1% of respondents using this type of container (Table 3).

Milk is most often stored in hot spaces in The Gambia, which was defined as being in the open ambient temperatures, with 92.3% of respondents storing milk under ambient conditions. Alternatively, only 7.7% of respondents store milk in spaces cooler than ambient temperature.

Table 3. Material of Milk Storage Vessel

Milk Storage Material	% of study subjects (n=194)
Plastic	62.9%
Glass	3.1%
Other	13.3% • Bowls (85.7%) • Iron Vessels (14.2%)

Table 4. Form of milk given to children

Form of Milk Given to Children	% of study subjects (n=194)
Fresh Milk	25.3%
Sour Milk	49.0%
Mixed into Gruel or Porridge	22.2%
Prepackaged	0.5%
Various Combinations (of the above)	4.6%

According to analyses of opportunistically collected data, the earliest age at which infants were given non-breast milk foods was 4 weeks, and these foods included prepared food (i.e., gruel), tea, tinned milk, and other liquids (i.e., water). At 6 months of age, there is evidence of increased feeding of all non-breast milk liquids included in the survey (prepared food, powdered milk, cow's milk, semi-solids, tinned milk, tea, solids, water, glucose water) (Figure 6, Table 8). At 52 weeks, 23.84% of infants were receiving cow's milk.

Figure 5. Frequency of Cow's Milk Consumption – Child & Mother

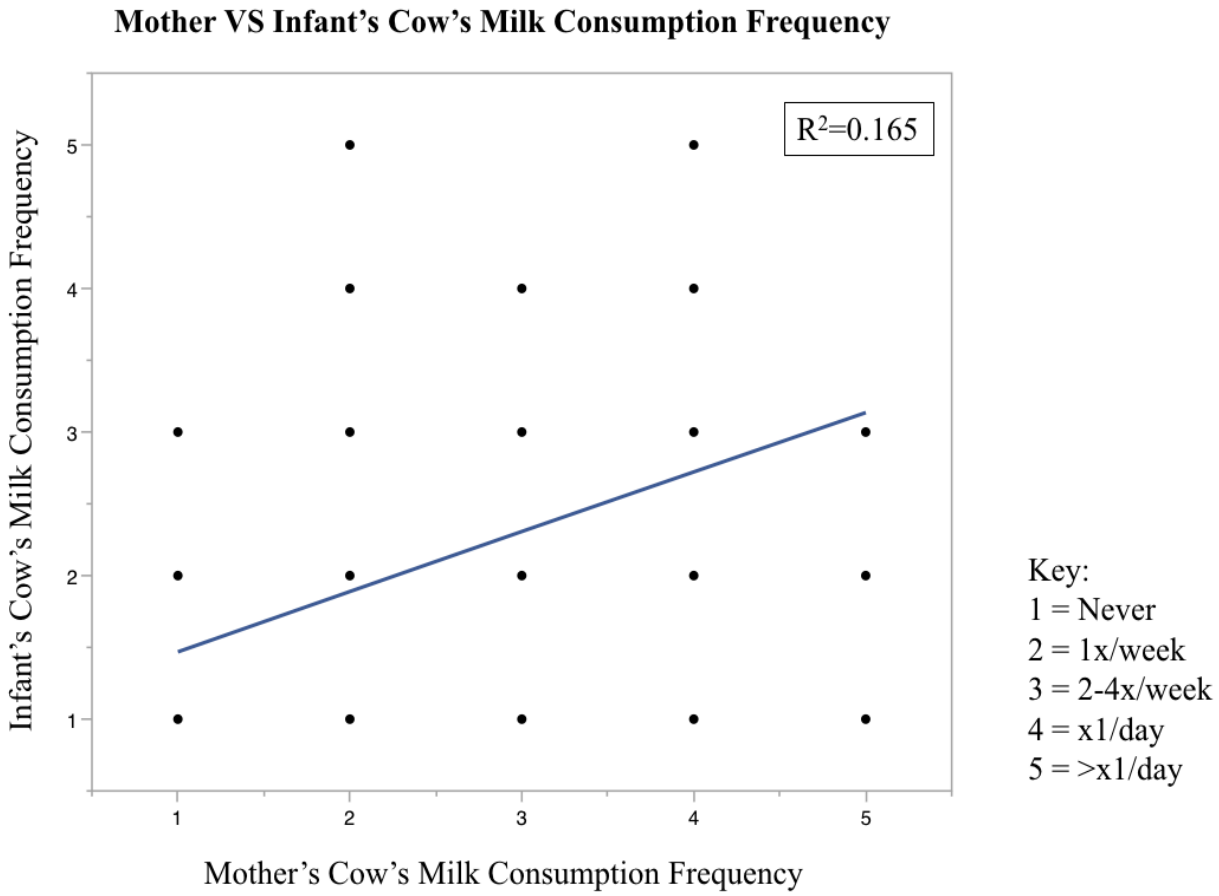


Table 5. Frequency of Cow's Milk Consumption - Child & Mother

	Never	**1x/wk**	**2-4x/wk**	**1x/day**	**>1x/day**
Child	30.4%	28.9%	28.4%	2.6%	1.03%
Mother	11.9%	39.2%	32.5%	4.6%	3.1%

Table 6. Frequency of Goat's Milk Consumption - Child & Mother

	Never	**1x/wk**	**2-4x/wk**	**1x/day**	**>1x/day**
Child	88.1%	1.5%	0.5%	0.5%	0.5%
Mother	90.7%	0.0%	0.0%	0.5%	0.0%

Table 7. Comparison of Erinoso et al. (1992) and 2015 Preliminary Study Results

Variable	**% of subjects**		
	Erinoso et al. (1992)	**Washabaugh (2015)**	**% difference (+ or -)**
Sample Size	349 mothers	194 mothers	-
Children that consumed cow's milk 1x/week	41%	28.9%	-12.1%
Children that consumed cow's milk 2-4x/week	32%	28.4%	-3.6%
Children that consumed cow's milk 1x/day	19%	2.6%	-16.4%
Children that consumed cow's milk 1x+/day	8%	1.0%	-7.0%
Mothers that purchased milk from herdsmen	79%	52.1%	-26.9%
Mothers that obtained milk from owned cows	20%	9.8%	-10.2%
Children that consumed milk in fresh form	13%	25.3%	+12.3%
Children that consumed milk in sour form	5%	49.0%	+44.0%
Children that received pasteurized milk	2%	0.5%	-1.5%

Figure 6. Timing of Introduction of Non-Breast Milk Liquids – Opportunistic Data (n=218)

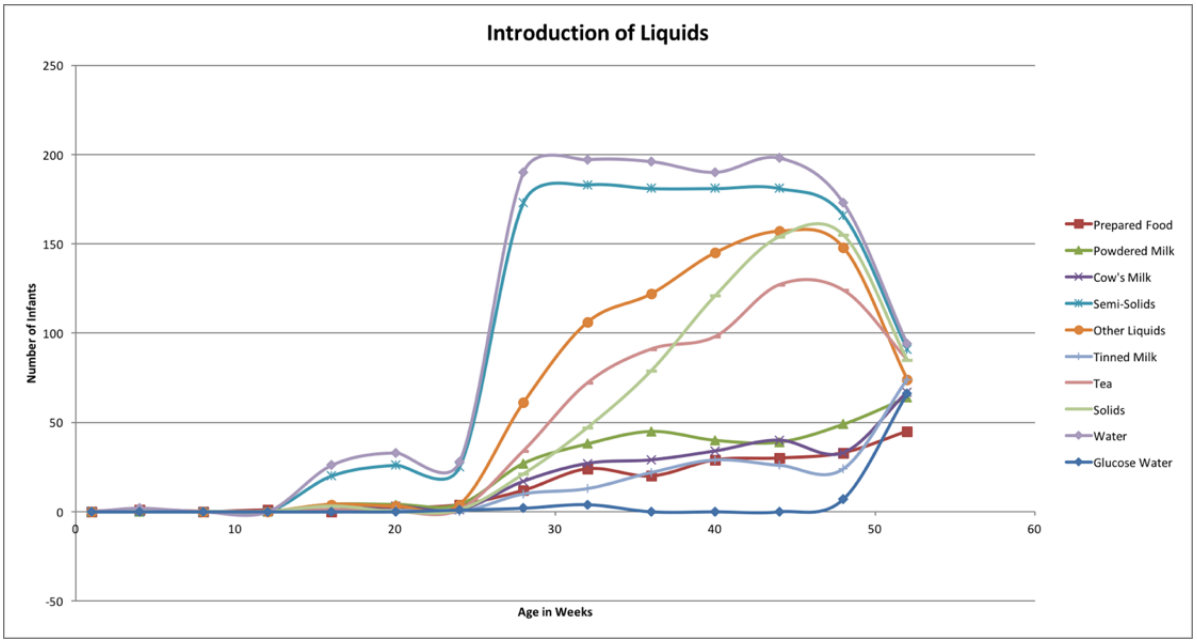


Table 8. Number of infants consuming non-breast milk liquids/foods per week – Opportunistic Data (n=218)

Wk	Powder Milk	Cow Milk	Semi-Solids	Other	Water	Tinned Milk	Tea	Solids	Prepared Food	Glucose Water
1	0	0	0	0	0	0	0	0	0	0
4	0	0	0	1	2	1	1	0	1	0
8	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	1	0
16	4	1	20	4	26	0	1	3	0	0
20	4	3	26	3	33	1	0	0	2	0
24	4	1	25	4	28	0	1	1	4	1
28	27	17	173	61	190	10	34	21	12	2
32	38	27	183	106	197	13	72	47	24	4
36	45	29	181	122	196	22	91	79	20	0
40	40	34	181	145	190	29	98	121	29	0
44	39	40	181	157	198	26	127	154	30	0
48	49	33	166	148	173	24	124	155	33	7
52	64	67	91	74	94	74	85	85	45	66

DISCUSSION

I hypothesized that the frequency of milk consumption had not changed in rural Gambian mothers and children since the last survey was published on the topic 25 years ago (Erinoso et al., 1992). In order to assess the current milk consumption practices in rural Gambia, I administered surveys to 194 mothers in Keneba in 2015. Overall, my results fail to support my hypothesis and indicate that instead, the general frequency of milk consumption has declined over time. However, an important finding from this survey is that the reported feeding of sour milk increased nearly 10-fold since 1992, which may be related to multiple biocultural factors, and moreover, may have large implications for health.

Sour milk is a food that is prepared using traditional fermentation practices, which are driven by LAB and ambient temperature. Milk fermentation alters the taste, texture, and color, and fermented milk is traditionally regarded as more palatable in many African countries where natural souring is used to prepare milk (Chelule et al., 2010). While the increase in sour milk consumption could be attributed to food preference, it may also be related to byproducts of fermentation. More specifically, fermentation softens food texture and alters its composition in such a way that minimal energy is required to cook and/or preserve the product. Chelule et al. (2010) explain that using less fuel for cooking and eliminating the need for preservation is highly advantageous in low-income countries such as The Gambia, and especially in more rural areas such as Keneba, where resources for cooking and preservation are not widely available; fermentation increases the shelf life of food.

Overall, my results suggest that milk consumption frequency and preference have changed in Keneba since 1992. The data collection for this thesis took place at the beginning of the wet season, which, as noted in Chapter 1.1, is when most livestock give birth as there is ample food and water (Jeannin et al., 1988; Jaitner et al., 2003). It is possible then that season of

survey collection could influence responses if different types of milk are available for purchase in Keneba more frequently in one form or another depending on seasonality.

In addition, the results show that fresh milk consumption by children has increased since 1992, which may be related to a potential increase in the number of cattle located in the Lower River Region. In 1978 in the LRR there were 24,444 head of cattle, and in 2014 there were 45,993 head of cattle (Trail & Gregory, 1981; Secka, 2016). According to a study conducted by Somda et al. (2005) in The Gambia, the number of local cows positively increases the market surplus of milk. More specifically, an increase in one cow increases the marketable milk surplus by 0.03 liters per household per day (Somda et al., 2005). While this number is quite low, Somda and colleagues (2005) suggests that an increase in the total milk production in a household will reduce the marginal utility of milk consumption, thereby increasing the marketable surplus. Irrespective of its small increase in milk surplus yields, the increase in number of cattle in the LRR is a relevant variable that does hold a quantifiable positive impact on milk consumption and sale; the more milk that is available, the more milk that people can consume.

Finally, some non-breast milk liquids and foods were introduced into infant diets before 6 months of age, which is the WHO-recommended length of exclusive breast feeding, providing partial support for A_2H_1. Specifically, semi-solid foods, such as gruels and cereals, tea, solids, and water were introduced by 16 weeks of age for some individuals. This information might indicate a need to improve infant feeding education, as early introduction of foods and liquids may be harmful to infant health.

Based on the results from this preliminary research, I chose to focus my field- and lab-based thesis research on milk consumption and hygiene practices in The Gambia, with a concentration on individuals playing key roles as potential contributors to milk hygiene or

contamination in the production chain – namely, herdsmen and vendors selling milk at informal markets. It is important to note that, because of logistical constraints, this portion of my MA research focused on these dynamics in an urban context, unlike the rural setting of my preliminary study. I anticipate bridging these gaps in my PhD research.

MAIN STUDY

RESEARCH AIMS & HYPOTHESES

Between 2002-2004, Hempen et al. (2004) assessed cow's milk hygiene in several countries, including The Gambia. Despite finding significant evidence of unhygienic milk in the country, no bacterial standards have been described or implemented in The Gambia.

Aim 1: The first aim of my main research project is to detail milk consumption and milk hygiene practices of herdsmen and vendors who sell milk at informal markets in The Gambia, including Bakau, Brikama, Latri Kunda, Serrekunda and other markets.

Aim 2: The second aim for the main study is to determine (a) which forms of milk are the least hygienic – as defined by milk temperature, presence of visible impurities, and concentrations of *Enterobacteriaceae* (EB) – and (b) the stage in the milk production chain (herdsmen versus vendor) that has higher concentrations of EB. I predict that:

A_2H_1: (a) Fresh milk is the least hygienic form of milk compared to powdered or sour milk because it has not undergone fermentation, a process that increases acidity (reduced pH), which can lower concentrations of pathogenic bacteria. Additionally, (b) milk collected from herdsmen and fresh milk purchased from vendors will have higher concentrations of EB compared to that of sour milk.

Aim 3: The final aim of the main study is to measure how bacterial concentrations in milk change after 24 hours in ambient temperature. This is an important step in determining how consumer practices can affect the bacterial content of milk purchased for consumption, since consumers of milk purchased at market in Gambia seldom own refrigeration systems and instead store milk in the open. I predict that:

A_3H_1: Freshly purchased milk will have greater concentrations of EB than milk sitting at ambient temperature for 24 hours, due to its higher pH value and water content, which facilitates bacterial growth.

MATERIALS & METHODS

Interviews & Laboratory Analyses

Interviews

The GBA is located within the Western Coastal Division of the country and contains the most populated cities in the country, including the country's capital, Banjul (Figure 1). In-person mixed interviews (combinations of prepared and spontaneous questions) were conducted with herdsmen (n = 12) and market vendors (n = 31) over 18 years of age in informal markets, including the Serekunda, Bakau, Latri Kunda, and Brikama markets. In addition to markets, three interviews were conducted in non-market settings, such as in vendor homes or on non-market properties (e.g., a closet attached to a building). The aim of the interviews was to gather general descriptive information about variation in herd size, experience of herdsmen/vendors, and practices of herdsmen/vendors that might influence herd health/bacterial contamination of milk. Herdsmen interviewees included only males, and vendor interviewees included 3 males and 28 females. Prepared interview questions for herdsmen and vendors can be found in Appendix 1.2a and 1.2b respectively. I conducted each interview with assistance from a translator from ITC who, where appropriate, presented the interview questions in the Fulani, Woloff, or Mandinka language. There was no attempt made to specify the ethnic group with which the interviewees identified. The interviews took place over 15-30 minute periods per participant. Interviews were voluntary, and subjects were not required to answer every question.

Interviewees were identified through opportunistic recruitment, in which unscheduled visits were made to local markets, and interviews were solicited while walking stall to stall. Pre-scheduled meetings with herdsmen involved recruitment through ITC's network with local persons involved in cattle performance practices such as herdsmen or farmers. Pre-screening was not conducted. Responses were documented (with coded answer options) by hand and then transferred to an Excel spreadsheet. The application to conduct this research was reviewed by the

University of Colorado Boulder IRB (Protocol #: 16-0463) and was granted exempt status (Category 2).

Laboratory Analyses

A total of 53 unpasteurized cow's milk samples (24 fresh milk, 10 powdered milk, and 19 sour milk) were purchased in August 2016 (wet season) from herdsmen and vendors interviewed (see Table 3). In some cases, where multiple vendors were working in a stall, I interviewed two or three vendors for every milk sample I collected. Most samples were collected in volumes around 500mL (a common volume sold in "pre-packaged" milks sold at informal markets). Milk samples were collected from Bakau (n=8), Brikama (n=5), Latri Kunda (n=5), Serekunda (n=6), and herdsmen from those same cities (n=21). Of the 21 milk samples collected from herdsmen, 8 came from Bakau, 3 from Brikama, 3 from Latri Kunda, and 7 from Serekunda. In addition, a fifth purchasing location, categorized as "Other" (n=8) is used to represent milk purchases that took place in vendor homes or on non-market properties.

Table 9. Milk Sources & Sample Sizes

	Number of milk samples purchased in market (purchased from herdsmen)				
Milk Type	**Bakau**	**Brikama**	**Latri Kunda**	**Serekunda**	**Other**
Fresh	0 (8)	2 (3)	0 (3)	1 (7)	0
Sour	4	3	3	5	4
Powdered	4	0	2	0	4
Total # Samples	16	8	8	13	8

Milk pH & Temperature

Upon collection, milk pH and temperature were recorded, and visible impurities such as dirt, mold, or insects, were documented. Milk pH levels were measured using universal indicator pH paper (Oxoid Ltd. 2015), and measurements were repeated in duplicate for each sample. Temperature (°C) was recorded using a portable probe thermometer (Traceable K., Fisher Scientific, MA, USA). The thermometer probe was thoroughly cleaned with sterile alcohol prep pads between each measurement. Temperature measurements were taken in the original containers and at the center of the milk sample. There was no attempt to determine when the milk samples sold to vendors were originally collected or purchased from herdsmen. The milk samples were transported back to the laboratory in their original containers in a portable insulated cooler. Travel time from the markets/herdsmen to the laboratory ranged from 15-minutes to 2-hours (Appendix 1.3).

Upon arrival to the lab, milk pH and temperature were recorded again using the same methods as above. Depending on milk sample volume, 10-15mL of milk was transferred from original containers into 15mL centrifuge tubes. These subsamples were homogenized for 30-seconds. Each of the aforementioned steps were completed for both t=0 and t=24 samples. T=0 represents samples at time point zero, or immediately upon collection. T=24 represents the same samples as in t=0, but specifically refers to their condition after 24-hours in ambient temperature.

Samples were left outside of the laboratory in ambient temperatures for 24 hours (during both night and day).

Traditional Spread Plate Methods (MacConkey Agar)

I plated 0.1mL of each dilution using traditional spread plate methods using sterile, disposable plastic spreaders on MacConkey agar plates (prepared according to manufacturer instructions, ThermoFisdher Oxoid Ltd. 2013) and incubated at 37°C for 24 hours. After 24 hours, Total Viable Cell (TVC) counts were recorded. Isolation and subculturing procedures were followed on 66.4% of samples using MacConkey agar plates and sterile disposable inoculation loops to identify specific bacterial genera. Those with identifiable bacterial genera were recorded. Plates with overgrowth were documented as Too Many to Count (TMTC). No attempt was made to differentiate specific strains of isolated genera.

Hygiena EnSURE MicroSnap EB

The Hygiena EnSURE MicroSnap *Enterobacteriaceae* is a semi-quantitative swab system that tests for presence of *Enterobacteriaceae* bacteria (EB). More specifically, this device detects *E. coli, Klebsiella, Citrobacter, Enterobacter, Serratia, Shigella, Salmonella*, and *Yersinia* (all are Gram-Negative). This device uses a luminometer to detect any light that is generated when enzymes that are characteristic of EB bacteria react with the kit's substrates. This system was designed to screen for microbial contamination of raw materials, environmental surface and equipment, with results produced in 6-8 hours after sample collection. Because of anticipated high levels of contamination based on previous analysis (see Hempen et al., 2004), the milk samples in this study were diluted 1:1000 with sterile Maximum Recovery Diluent (MRD), which was prepared according to the manufacturer's directions (ThermoFisher, Oxoid

Ltd. 2013). The remainder of the test followed the Hygiena EnSURE MicroSnap *Enterobacteriaceae* protocol for the Enrichment Device (MS1-EB) and Detection Device (MS2-EB). Briefly, 1mL of the liquid sample is put into MS1-EB tube, which contains enrichment broth – a liquid that contains nutrients and is used to culture bacteria. The enrichment broth is mixed with the liquid sample inside the MS1-EB test tube and is then left to incubate for 6 hours. After this, the incubated liquid mixture is transferred to the MS2-EB tube where it is combined with fluid containing bioluminogenic reagents. After mixing, the tube is inserted – with the liquid still inside – into the the luminometer instrument, and RLU values appear after 15 seconds. More detailed information regarding this protocol can be found in Appendix 1.4.

The bioluminometer system produces measurements in relative light units (RLUs), which are correlated to CFUs produced from traditional plating methods. The CFU to RLU conversion chart can be found in Appendix 1.5. Duplicates of each sample were run for both t=0 and t=24, and all duplicates were positively correlated (R^2=0.95).

Bioluminometer results were classified as satisfactory and unsatisfactory according to standards set in 2016 by the Food Safety Authority of the United Kingdom for unprocessed whole milk (a ready-to-eat food) for human consumption (Y et al., 2011). As aforementioned, *Enterobacteriaeae* are used in the United Kingdom as indicator organisms to determine food hygiene. For ready-to-eat foods, which are defined as foods that are ordinarily consumed in the same state as that in which it is sold or distributed and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer, the bacterial standards as written for 2016 are as follows: Satisfactory = $<10^2$ and Unsatisfactory = $>10^4$ (Food Safety Authority, 2016).

Statistical Analyses

One-way ANOVA tests were used to determine whether fresh, sour, or powdered milk had the highest levels of EB. Two-tailed T-tests were used to identify (a) the segment of the production chain at which the milk is most bacterially contaminated (upon production versus in the market for consumer purchase), (b) the time point at which milk is most contaminated following collection (t=0 compared to t=24), and (c) the purity level at which milk has the highest EB concentration (presence versus absence of visible impurities [VIs]). The significance threshold was set at = 0.05 for all statistical tests. ANOVA test was conducted with post-hoc Student's T test to determine if milk EB concentration differed significantly between location/source (markets or herdsmen).

RESULTS

Cultural Investigations: Herdsmen

Over 58% of herds consisted of 51-65 head of cattle. Half of the herdsmen have been in practice for 32 years or more, and the shortest duration of experience as a herdsman was 7 years. 41.6% of herds are owned by multiple individuals, ranging from 2-9 owners for each herd. 50% of herds were kept on property that was not owned by any of the herdsmen's family members, and 50% of herds were kept on property owned by the herdsmen's fathers. All 12 herdsmen interviewees reported that more milk is produced during the rainy season, leading to a greater surplus of milk at this time of year.

Three-quarters of the herdsmen reported that at least one of their cattle had been afflicted by either infection, sickness, birthing complications, or disease. No attempt was made to specify the particular diseases, although interviews with a veterinarian revealed that the most common diseases in the local cattle populations include trypanosomiasis and brucellosis, along with mastitis, which is an infection of the udder. If cows were experiencing sickness, 91.7% of herdsmen did not milk those individuals until their health was restored.

Cows are milked in both the morning and the evening by 75% of herdsmen, while 25% only milked in the morning. All 12 of herdsmen interviewees said that they milked their cattle every day and that the majority of the milk goes to feeding their family members. No herdsmen discard milk if it is not consumed the day it is produced/milked, and 66.7% of herdsmen kept milk for 2-6 days if not consumed. The surplus milk yields from the morning collection are bought by vendors who come directly to the farms for the purchase. The milk is taken from the herdsmen in the same containers into which the milk was collected, but the buckets are lidded before leaving the farm. The milk is taken directly to the markets. All of the herdsmen interviewed for this study were male; 41.7% of herdsmen reported knowing of women who milk

cattle, although many noted that this was a more common practice in rural areas of The Gambia (such as in Keneba).

Milk was stored in plastic containers by 66.7% of herdsmen, whereas 33.3% store milk in metal containers. Milk stored in metal containers was transferred to plastic containers before sale. No herdsmen store milk in glass containers. All herdsmen store milk at ambient temperatures. Only one herdsman washed milking buckets before milking, by swirling water inside of the buckets. Soap was not used by any herdsmen, and water used for washing by the single herdsmen was at ambient temperature and from unknown sources.

Two herdsmen poured milk from one bucket to another through cheese cloth to strain out dirt, hair, insects, and other debris before selling it to vendors, however, they both reported reusing the same cheesecloth daily until milk could no longer be strained through the material and the hardened milk residue and other debris inhibited permeability.

Cultural Investigations: Vendors

Over 67% of vendors reported washing the 500mL plastic containers before filling them with milk for consumers, although I only observed two vendors doing this. Of those who reported washing the containers, 32.3% reported washing with only water, and 38.7% used both soap and water. None of the vendors were seen washing their hands or the cups/spoons during observations. During the interviews, some vendors demonstrated their preference for one form of milk or the other by dipping their hands and/or the measuring cups into the milk and then putting their hands and/or the measuring instruments into their mouths (after which the hands and/or measuring instruments were not washed).

Vendors remain at the markets for an average of 9.29 hours per day, but some work days lasted longer. For example, one vendor reported working for 20 hours a day (median: 9 hours,

range: 7-20 hours [±2.49 hours]). The number of years in practice ranged from 5-70 years (mean: 20.65 years [±15.32 years], median: 20 years), and most began working as vendors alongside family members. Over half of vendors (51.6%) reported having family members involved in cattle-related practices.

If vendors do not sell all of the surplus milk the day that it was purchased from the herdsmen, they keep the milk for at least one day (35.5%), but most keep the milk for 2-6 days (64.5%) after purchase. Three vendors (9.7%) kept the milk refrigerated overnight, but the remaining vendors left the milk in ambient temperatures. All of the 31 vendor interviewees stored milk in plastic containers.

Vendors report that fresh milk ferments within 24 hours in ambient temperature. At the markets, the milk remains in ambient temperatures in the original collection buckets, or in larger, colorful plastic containers that sometimes have a board or sheet placed over top to protect the product from flies. The vendors use plastic cups or large spoons to measure and distribute milk to customers either into containers the customers bring themselves or into small plastic bags or plastic containers (~500mL capacities). Only 12.9% of vendors know if the cows that the milk came from were washed (udders) or if any sanitary practices were used, and only 19.3% of the sample know if the cattle were vaccinated.

Vendor & Herdsmen Consumption Patterns

Timing of Introduction of Unpasteurized Cow's Milk (UCM)

Herdsmen and vendors report introducing UCM to infants before 1 year of age (Table 10). Between birth and 3 months of age, 25% of herdsmen and 41.9% of vendors had introduced UCM to their infants. Between 3-6 months of age, 58.3% of herdsmen and 29.0% of vendors had

introduced UCM to their infants. Between 6 months-1 year of age, 16.7% of herdsmen and 25.8% of vendors introduced UCM to their infants.

Consumer Preferences

Preference for form of milk ranges between herdsmen, vendors, and their children is shown in Table 11 and Table 12. Fresh milk is preferred by 41.6% of herdsmen and 32.3% of vendors, and sour milk is preferred by 33.3% and 28.7% respectively. 25.0% of herdsmen and 29.0% of vendors report no difference in preference between the two forms. Herdsmen's children show preference for fresh (50%) over sour (6.0%), whereas vendor children prefer sour milk (35.5%) over fresh milk (29.0%). All vendors reported having consumed goat's milk, but all preferred cow's milk over goat's milk. These results are summarized in (Table 11).

Family Member Consumption

All of the interviewees (including both herdsmen and vendors) and their children consume milk, except for one vendor who did not have any children. Vendor interviewees report that family members who consume the greatest quantity of milk are as follows: elderly (grandparent or older) (38.7%), self (25.6%), female child (16.1%), male child (16.1%), mother (3.2%), and father (0.0%) (Table 13).

Table 10. Age at introduction of cow's milk for herdsmen's and vendor's children

	Herdsmen's Children	**Vendor's Children**
Birth-3mo	25.0%	41.9%
3mo-6mo	58.3%	29.0%
6mo-1 year	16.7%	25.8%

Table 11. Herdsmen & Vendor Children Milk Preference

	Herdsmen Children	**Vendor Children**
Fresh	50.0%	29.0%
Sour	6.0%	35.5%
No Difference	33.3%	32.3%

Table 12. Herdsmen & Vendor Milk Preference

	Herdsmen	**Vendor**
Fresh	41.6%	32.3%
Sour	33.3%	38.7%
No Difference	25%	29.0%

Table 13. Vendor Family Milk Consumption Patterns

Family Member #1 Milk Consumer	**Percentage of Interviewees**
Self	25.6%
Father	0.0%
Mother	3.2%
Child (female)	16.1%
Child (male)	16.1%
Elderly	38.7%

Laboratory Analyses

EB concentration in fresh, powdered, & sour milk

On average, EB concentrations were higher in fresh milk samples compared to powdered or sour milk. Average RLU values and CFU/mL equivalent values for fresh, powdered, and sour milk samples at t=0 and t=24 are summarized in (**Error! Reference source not found.**). There was no statistically significant difference in EB concentration between the three forms of milk at time of collection. After 24 hours in ambient temperature, fresh milk had significantly higher EB concentration compared to powdered milk [$F(2, 50) = 3.44$, $p = 0.0398$].

EB differences between milk from herdsmen and vendors

Milk collected directly from herdsmen had the greatest concentration of EB compared to any other source of fresh milk at t=0 ($t(38.85)=-2.03$, $p=0.0494$) and t=24 ($t(40.15)=-2.86$, $p=0.0067$). Appendix 1.6-1.8 summarizes the differences between milk contamination from herdsmen and vendors.

EB differences between t=0 and t=24 samples

Upon collection, 2 fresh, 1 powdered, and 1 sour milk sample were considered "satisfactory" for human consumption, meaning the samples contained less than 10^2 CFU/mL of EB, according to the 2016 UK standards of *Enterobacteriaceae* content in ready-to-eat foods. A total of 22 fresh, 9 powdered, and 18 sour milk samples were "unsatisfactory," meaning they contained EB concentrations greater than 10^4 CFU/mL (Table 14, Table 15).

After 24 hours in ambient temperature, 0 fresh, 0 powdered, and 2 of the sour milk samples were "satisfactory," and 24, 10, and 17 were considered "unsatisfactory," respectively (). A total of 83.3% of fresh milk, 70.0% of powdered milk, and 57.9% of sour milk sample EB concentrations decreased across the 24-hour incubation (Table 14, Table 15).

Table 14. Average RLU values, ranges, and the RLU:CFU equivalent values for fresh, powdered, and sour milk samples at time point 0, or upon collection (t=0) and after 24 hours at ambient temperature (t=24).

	t=0			**t=24**		
	Mean RLU	**Range**	**RLU:CFU/mL Equivalent**	**Mean RLU**	**Range**	**RLU:CFU/mL Equivalent**
Fresh (n=24)	2040.29	0-6364	5×10^6 CFU/mL – TMTC	2671.5	18-8753	5×10^6 CFU/mL – TMTC
Powdered (n=10)	731.7	1-4126	$1 \times 10^6 – 5 \times 10^6$ CFU/mL	234.6	14-936	$1 \times 10^6 – 5 \times 10^6$ CFU/mL
Sour (n=19)	999.26	0-6545	$1 \times 10^6 – 5 \times 10^6$ CFU/mL	1152.05	7-6743	$>1 \times 10^7$ CFU/mL

Table 15. Hygiene status of Gambian UCM compared to UK standards (Enterobacteriaceae)

	t=0		**t=24**	
	Satisfactory $<10^2$ CFU/mL	**Unsatisfactory $>10^4$ CFU/mL**	**Satisfactory $<10^2$ CFU/mL**	**Unsatisfactory $>10^4$ CFU/mL**
Fresh	2	22	0	24
Powdered	1	9	0	10
Sour	1	18	2	17

Milk pH and Temperature

Upon collection, the mean pH values were 6.88 (6-7, SD= ±0.24), 4.25 (2.5-5.5, SD= ±0.54), and 4.39 (3.5-6; SD=±0.70) for fresh, powdered, and sour milk samples respectively (Table 16). On site pH was not significantly different between powdered and sour milk samples, but both were significantly lower than the pH of fresh milk [$F(2, 50) = 164.88$, $p<.0001$].

The mean milk temperature taken on site was 28.48°C (29.2-34.8°C, SD= ±1.37°C) for fresh milk, 25.2°C (13.2-29.9°C, SD= ±6.82°C) for powdered milk, and 30.86°C (14-33°C; SD= ±5.51°C) for sour milk (Table 16). To create the powdered milk product, the powder is mixed with both water and sour milk. It was not possible to identify the water sources used by vendors for the samples purchased for this study.

After a 24 hour incubation in ambient temperatures, the pH of all samples decreased (Figure 7). The mean pH for t=24 fresh milk samples was 5.04 (4.0-6.0), powdered 3.85 (3.5-4.0), and sour 3.86 (3.5-5.5). Fresh milk samples had the largest reduction in pH level after the 24-hour period, dropping from 6.88 to 5.04. Milk pH at t=24 is significantly lower compared to pH at t=0 ($F(1, 51) = 171.81$, $p<.0001$).

Average temperature of t=24 milk was 27.68°C for fresh samples, 27.4°C powdered, and 27.2°C sour (Table 16). Broken down by form of milk, the mean temperature was 27.8°C for fresh milk (26.3-28.8°C, SD= ±0.63), 27.4°C (26.3-28.4°C, SD= ±0.74) for powdered milk, and 27.1°C (26.1-28.5°C, SD= ±0.81) for sour milk. Average temperature decreased for each form of milk except for powdered milk, which increased by over 2 degrees.

Average temperature (°C) and pH value of fresh, sour, and powdered milk samples are summarized in Table 14. Results from Hempen et al. (2004) are in the far-right column for comparison. Hempen et al. (2004) did not collect powdered milk samples nor did they assess milk samples after 24 hours in ambient temperature.

Table 16. Average temperature (°C) and pH of fresh, sour, and powdered milk samples compared to same results from Hempen et al. (2004).

	Average Temperature		**Average pH**		**Results from Hempen et al. (2004)**	
	t=0	**t=24**	**t=0**	**t=24**	**Temperature (t=0)**	**pH (t=0)**
Fresh	28.48	27.68	6.88	5.04	29.7	6.1
Sour	30.86	27.2	4.39	3.86	28.6	4.2
Powdered	25.20	27.4	4.25	3.85	-	-

*Figure 7. Milk pH change over 24 hours in ambient temperature (***P < 0.001)*

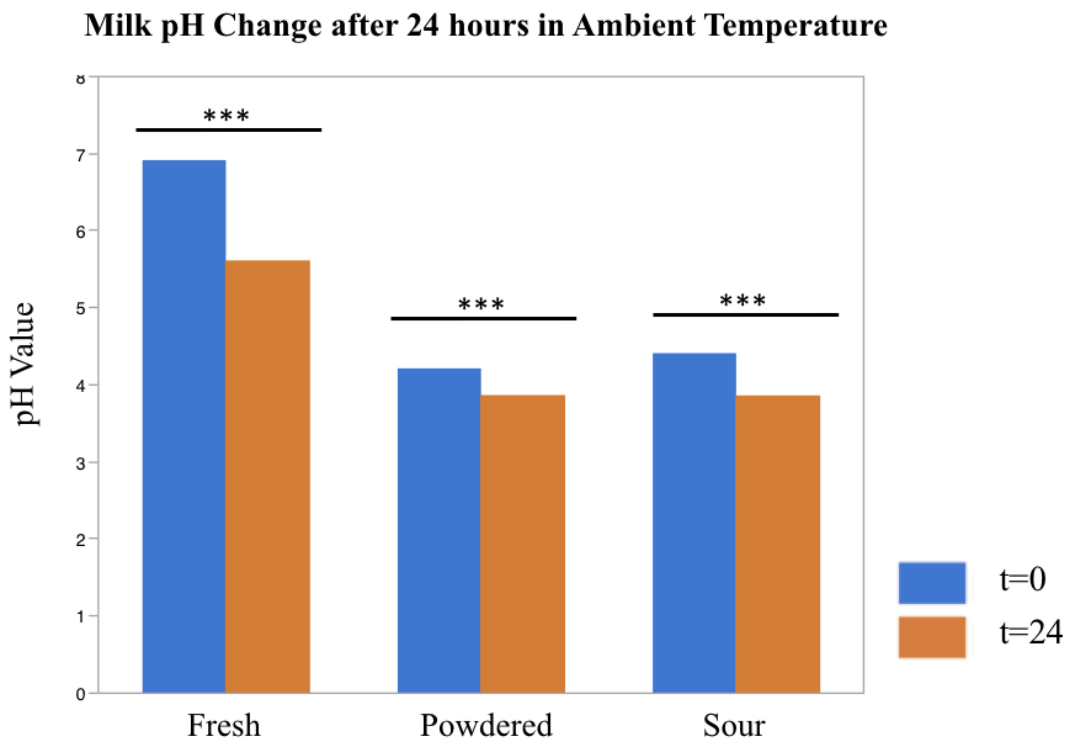


Figure 8. Milk Temperature at t=0 and t=24

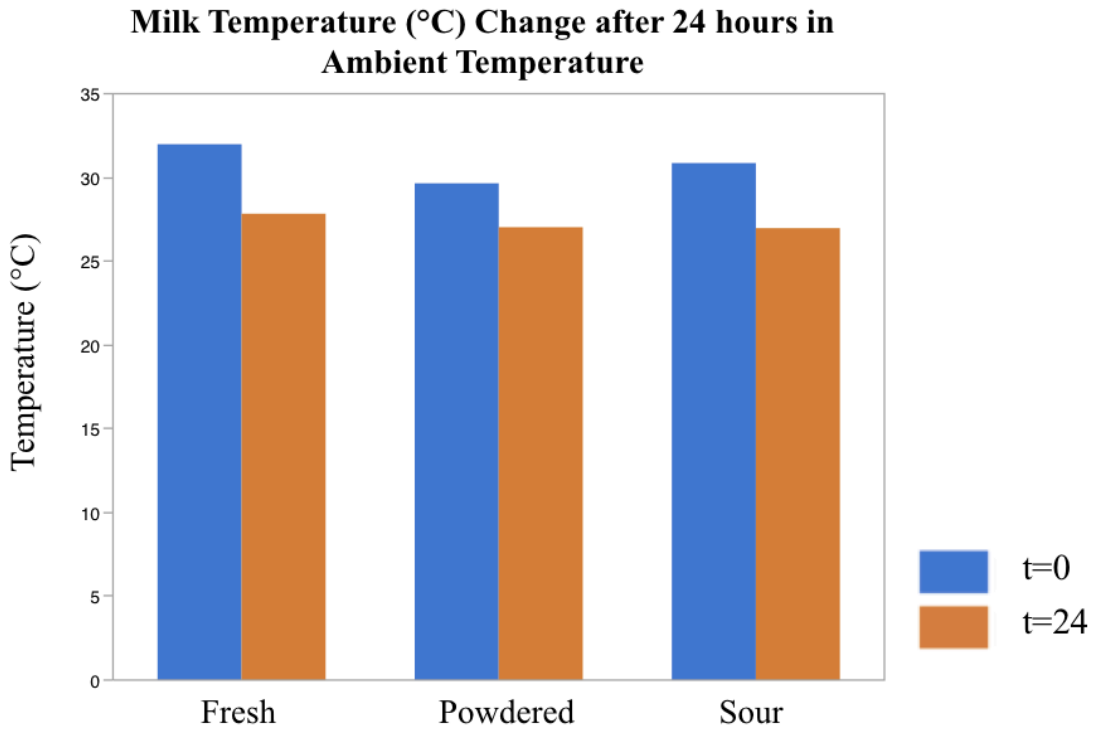
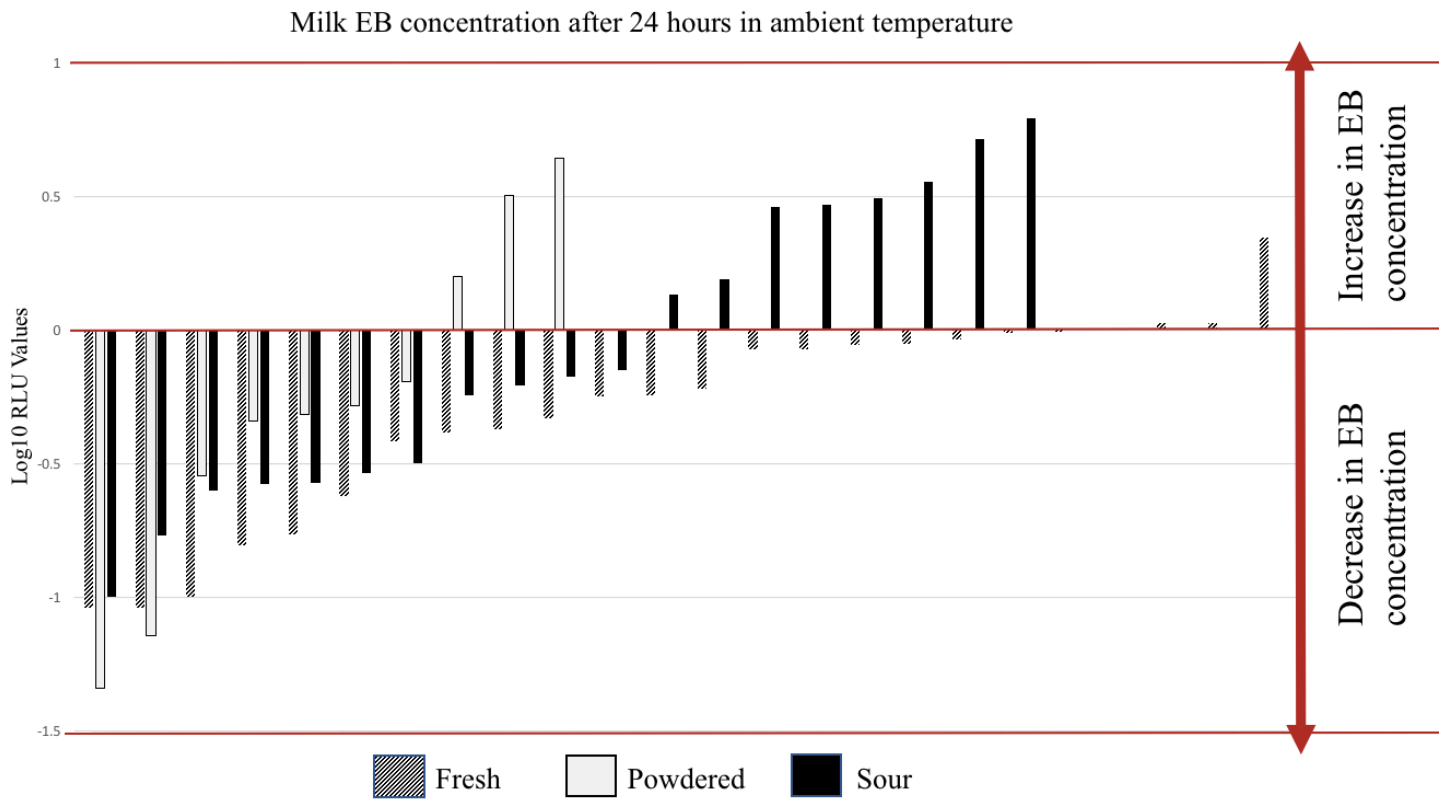


Figure 9. Milk contamination after 24 hours in ambient temperature for fresh, sour, and powdered milks. Log_{10} RLU value of 0.00 indicates no change in EB concentration from t=0, >0.00 indicates increase in EB concentration, <0.00 indicates decrease in EB content.



Visible Impurities (VIs) in Milk

A total of 31 samples contained visible impurities (58.5% of total): 11 fresh (45.0% of all fresh samples), 7 powdered (70.0% of all powdered samples), 13 sour (68.4% of all sour samples). Visible impurities were present in 60.4% of milk samples from markets, whereas 39.6% of samples collected directly from herdsmen contained visible impurities. Samples without VIs had higher RLU values than those with VIs at t=0 (t(49)=-2.19, p=0.033) and t=24 (t(43.4)=-2.37, p=0.022) (Figure 10).

Results by Location/Source

An one way analysis of variance showed that the effect of location/source (markets or herdsmen) on EB concentration in milk at t=0 was significant (F(5, 45) = 3.86, p=0.0054). Post hoc analyses using Students T test indicated significant differences between locations/sources of milk and the resultant EB concentrations at t=0 (t(-2.03)=38.85, p=0.0494]. Latri Kunda market milk samples contained significantly lower EB concentrations (M=0.58, SD=±0.44) compared to Bakau (M=2.68, SD=±1.12); t(-3.53)=45, p=0.001, Brikama (M=1.80, SD=±0.44); t(-1.90)=45, p=0.318, Herdsmen (M=2.67, SD=±1.16); t(-4.11)=45, p=0.0002), Other (M=2.35, SD=±1.14); t(3.05)=45, p=0.0038), and Serekunda (M=2.15, SD=±0.74); t(2.55)=45, p=0.0144).

Source of milk (specific market or herdsmen) was also significant in determining EB concentration of t=24 milk samples (F(5, 47) = 3.90. p=0.0049). After 24 hours at ambient temperature, Latri Kunda market milk samples contained significantly lower EB concentrations (M=1.28, SD=±0.34) compared to Bakau (M=2.23, SD=±0.86); t(-1.88)=47, p=0.0036), Herdsmen (M=2.87, SD=±0.99); t(-3.58)=47, (p=0.0008), Other (M=2.62, SD=±1.16); t(2.65)=47; (p=0.0110), and Serekunda (M=2.39, SD=±0.68); t(2.05)=47; (p=0.0457). Brikama market milk samples contained significantly lower EB concentrations (M=1.51, SD=±0.25)

compared to Bakau (t((p=0.0286), Herdsmen (t(1.72)=47; p=0.0036), and Other (t(2.19)=47, p=0.0335).

Appendix 1.6-1.8 includes a table comparing general information about milk samples broken down by location/source for reference.

*Figure 10. Milk EB Concentration in Presence and Absence of VIs (*P < 0.05)*

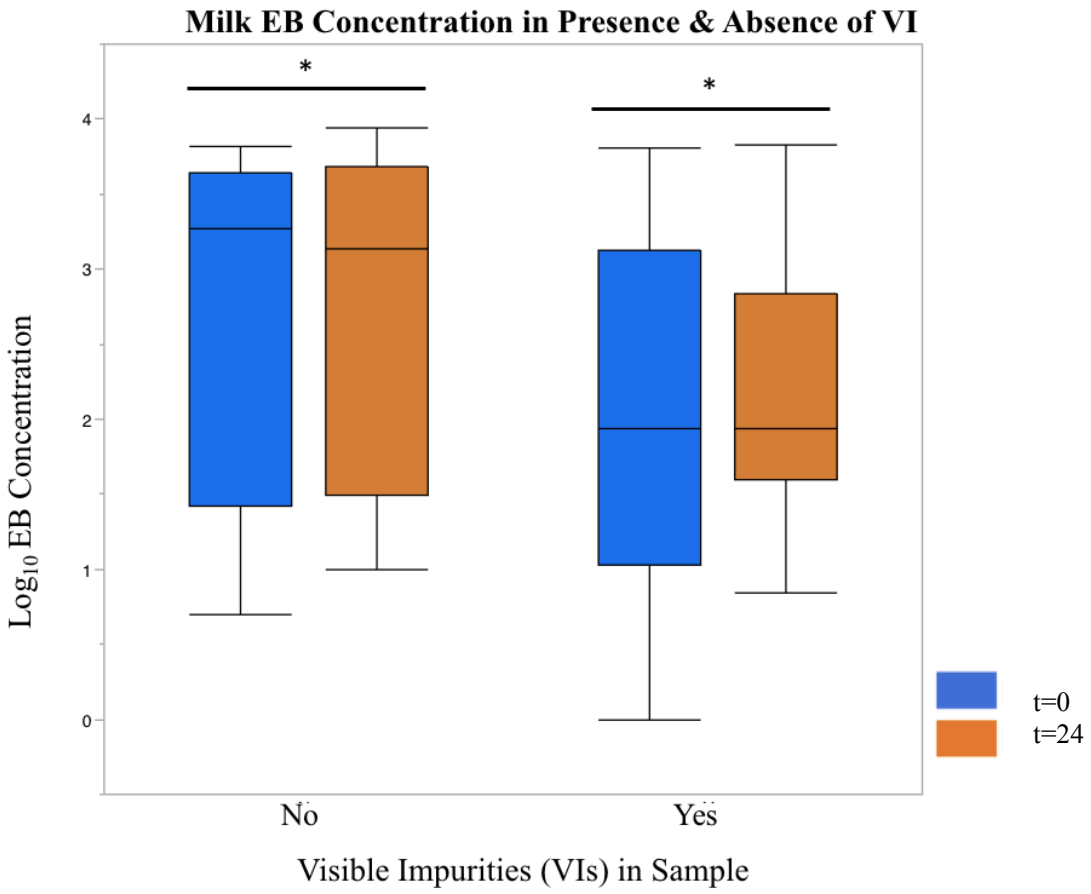


*Figure 11. Milk EB Concentration by location/source of milk (markets or herdsmen) t=0 (*P < 0.05, **P < 0.01, and ***P < 0.001)*

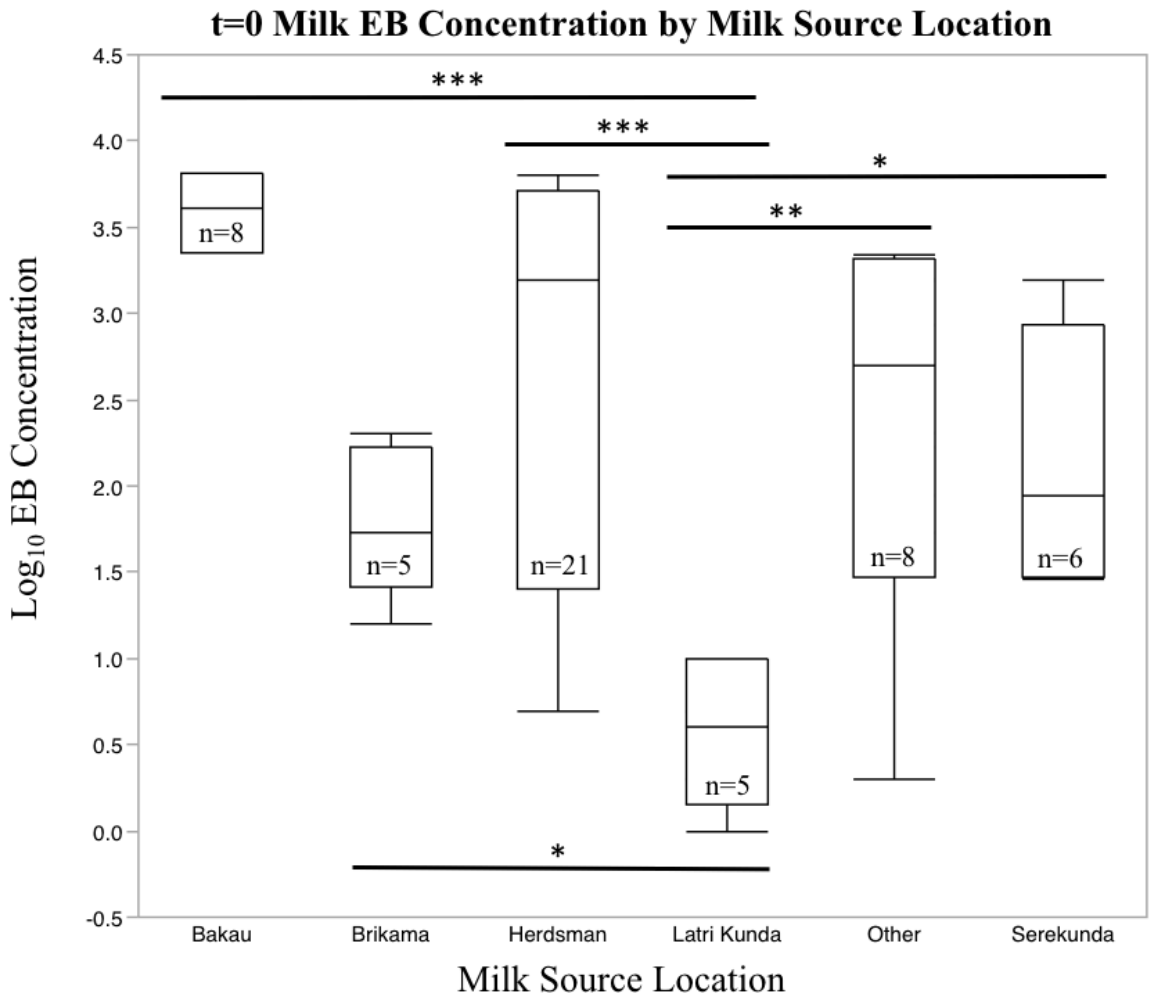
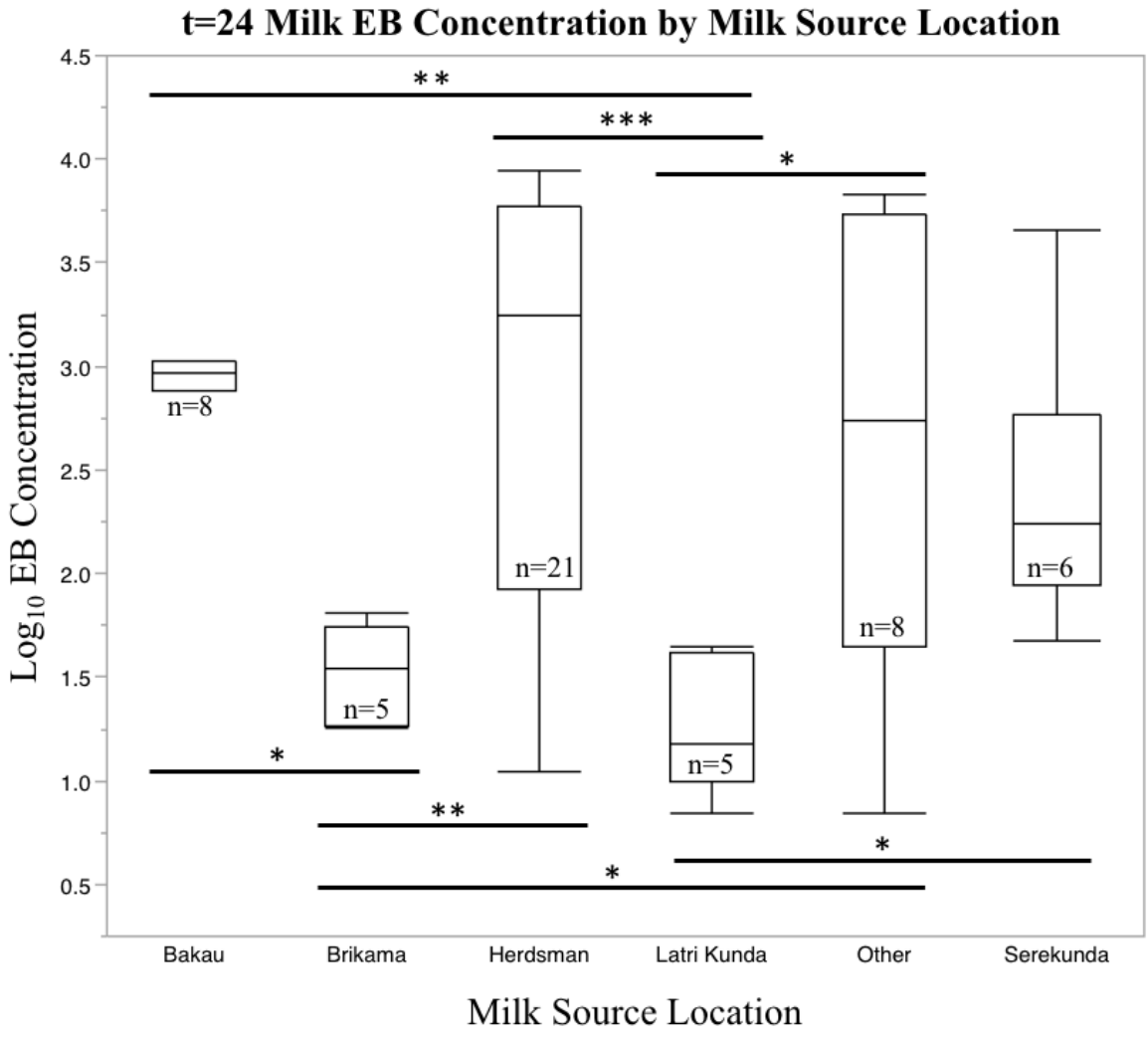


*Figure 12. Milk EB Concentration by location/source of milk (markets or herdsmen) t=24 (*P < 0.05, **P < 0.01, and ***P < 0.001)*



Results of Luminometer VS Traditional Plating Techniques

Although I completed standard plating procedures for each milk sample to examine bacterial growth, the great variation in bacterial concentrations produced cultures with overgrowth. Each sample was serially diluted as follows: neat; 1:10; 1:100; 1:1000; 1:10000; 1:100000 in attempt to find the best dilution to determine TVC. *E. coli* growth was present in 69.8% of samples; however, due to the high concentration of total bacteria, the *E. coli* were occasionally outcompeted by other bacterial species. For plates with successful *E. coli* growth, the total viable cell counts ranged from 1.7×10^5 - 3.6×10^8. Images of bacterial growth on MacConkey agar plates can be found in Appendix 1.9.

DISCUSSION

Interview & Observation Findings

My first aim for the main study of this thesis was to detail milk consumption and hygiene practices of herdsmen and vendors who sell milk at informal markets in The Gambia, which has not been documented in detail previously. My results show that all herdsmen and vendor families (of those interviewed) are frequent milk consumers, with elderly family members most frequently reported as the greatest milk consumers. This may reflect a generational eating behavior or consumer preference that may be fading over time, or may be related to nutritional needs; either way, this particular finding has not been noted in the literature to my knowledge and requires additional investigation.

In addition, male and female children were equally voted by interviewees as the second greatest milk consumers in the family. When asked which family members consumed the most milk, one interviewee laughed and said, "My children! They run here [to the market stall] every day after school demanding milk. Sometimes they even come [to the stall] during lunch." When I asked another interviewee if her children liked milk, she laughed and said, "They like it too much!"

More than 70% of all interviewees introduced UCM to infants before 6 months of age. The WHO recommends that cow's milk not be introduced until after 1 year of age, so it is possible that the early introduction of this non-breast milk food may have implications for infant gut and immune system development in these cases. Non-human milk consumption before one year of age can lead to occult intestinal blood loss, which may be related to the lack of immune tolerance to the foreign milk proteins (Sullivan, 1993, Ziegler, 2007). If this bleeding occurs, there is an increased risk for iron deficiency in the infant, which can result in long-term health consequences such as impaired immunocompetence and brain development (Wegmüller et al.,

2016). In one study, anemia was found in around 79% of preschool aged children in The Gambia, and other studies report that anemia affects more than 50% of young children in the country (WHO, 2009; Wegmüller et al., 2016). Thus, it will be important for future research to gain a better understanding of the health impacts of early introduction of cow's milk – especially raw milk – on Gambian infant health and development.

In terms of hygiene practices, most interviewees do not boil milk before they sell it or before they consume it. This is, in part, attributed to taste preference, but it is also related to cultural traditions. According to Roesel and Grace (2015), research in West Africa has documented that cattle owners believe that if milk is heated or boiled it is 'bad' and has no nutritional value. During my interviews with herdsmen, three individuals told me that there was a traditional belief that if they boiled their milk, then their cattle would become sick or would stop producing milk. Additionally, one study in West Africa found that the Fulani believed that milk was in its nature pure and could not be a source of disease (Roesel & Grace, 2015).

Personal observation and interviews showed that personnel involved in dairying practices in The Gambia do not regularly implement hygienic practices, and absence of handwashing, vessel cleaning, and storage in hot conditions likely contributes to product contamination. As an example, fecal matter is present on the hind legs and tails of the cattle as they are individually tethered and not able to move about freely. While being milked, the cows introduce fecal matter directly into the milk buckets and to the herdsman's hands via their hindlimbs and tail. Fecal-oral pathways, including ingestion of feces-contaminated food or water, or direct contact with an infected person, cause most diarrheal diseases (Curtis et al., 2001; Thapar & Sanderson, 2004; Black et al., 1981). According to Guzewich and Ross (1999), 89% of foodborne illness outbreaks caused by food contaminated by food workers could be traced back to pathogens transferred to

food by the workers' hands. Handwashing with soap is considered the most effective method for reducing the occurrence of diarrhea (Fewtrell et al., 2005). In fact, general handwashing can reduce diarrhea risk by 47% (Curtis & Cairncross, 2003).

However, Aihara et al. (2014) report that handwashing with poor quality water without soap – what would likely be available to most herdsmen in the fields - does not have effect on removal of bacteria from hands. In fact, the number of viable bacteria and coliforms on palms increased when study participants washed hands with only water of poor quality. Because clean water is not widely available in The Gambia, handwashing recommendations may not be the most effective intervention strategy for this particular hygienic concern. Pinfold & Horan (1996) and Curtis et al. (2001) suggest that hygiene promotion programs are more likely to be effective if they are based off of locally conducted research and use locally appropriate channels of communication. Thus, attempts to improve hygienic practices throughout the milk production chain in The Gambia should consider local traditions, available resources, and sustainability of the interventions when developing intervention approaches.

In addition, contamination can easily be introduced from impurities on teat skin or the hindlimbs of cattle as they are being milked. For example, calves are allowed to suckle their mother in order to stimulate milk production. After a short duration of suckling, the herdsmen remove the calf from the mother and promptly begin milking into their collection vessels. Due to this stimulation technique and because there is no attempt to clean the udders before milking, the bacteria from inside and around the calves' mouths and snouts are able to easily mix with the milk. Additionally, the containers used for milk collection and storage are not washed immediately before use, and also contain dirt and debris, which could introduce additional

bacteria to the milk. Milk collection is done outdoors, which exposes milk to various environmental sources of contamination, including insects, dirt, hair, and other debris.

Once the milk collection process is complete, the buckets of milk are sold to vendors (generally women) who obtain the milk directly from the herdsmen, and then transport the milk to different informal markets and other selling locations. These markets are outdoors, but some stalls have roofs or at least partial coverage from direct sunlight. At selling locations, some buckets are left open, but generally only if customers are consistently stopping at the stall. If no customers are at the stalls, the buckets are generally kept closed. While open, the milk is exposed to environmental contamination such as dirt, insects, and other debris. Fresh and sour milk is transferred from buckets using plastic cups into plastic bags or into repurposed plastic containers (e.g., empty mayonnaise containers). In some cases, the milk may be kept in a dark space (such as a closet or shed), and only rarely is refrigeration available to or used by vendors. Additionally, there is an absence of enforcement of food safety regulations and sanitary measures, as demonstrated by the lack of requirements for permits to sell milk or any hygienic testing of milk and milk products before sale (Touray, 2016).

Finally, it is important to note the apparent dearth of communication between herdsmen and vendors regarding cattle health, and milk hygienic status. After asking a vendor about her knowledge regarding this information, the translator said, "she does not know. But these vendors, they have no way of knowing such things." This communication gap creates a barrier for information to travel from the source of milk to the final consumer, and this lack of awareness could come with health consequences. One interviewee reported that the reason that most people do not boil milk before consuming is because, "…they just don't know," and three others reported that the government does not mandate it in The Gambia, whereas it is a law in

Senegal, so it is not yet necessary to boil it. This suggests a need to enhance food safety and risk communication between producers and consumers, and an overall need to design and strengthen food safety regulations at the level of the government.

Similarly, in one interview, a herdsman said that he had received hygiene training from ITC more than a decade ago on how to properly wash milking buckets using hot water and soap, and to wash hands and udders before and after milking. However, he did not implement any of those hygienic practices. I asked if there had been any follow-up interviews or training sessions, to which he replied, "No. That is not how research goes here. People come, they try to make their changes, and then they just leave and don't come back." He suggested that the best way to alter poor hygiene practices is to live with the people directly involved in the dairying practices and work with them until the practices become second nature.

Milk Bacterial Contamination Findings

As I hypothesized for Aim 2a for my main study, fresh milk samples did in fact contain higher concentrations of EB compared to sour and powdered milk samples**.** After 24 hours in ambient temperature, the EB concentrations of milk samples reduced in more than 50% of samples for each form of milk. Additionally, the pH levels in all milk samples decreased after the 24 hour incubation, facilitating fermentation and thereby reducing EB concentrations within the product. Similarly, milk collected directly from herdsmen has greater EB concentrations than milk collected from vendors, supporting the hypothesis for **Aim 2b** for my main study regarding the segment of the production chain at which milk is least hygienic, and milk at t=0 has greater concentrations of EB compared to t=24 samples, which supports the hypothesis for **Aim 3** regarding the effect of ambient temperature on EB growth. All of these findings can be explained using the same reasoning: fresh milk contains a higher pH-value and has a greater liquid content,

which is more conducive to Gram-negative bacterial growth than that of sour milk (and the powdered milk samples are made up of sour milk, milk powder, and water). In this way, the processes of fermentation appear to reduce the contamination of EB.

These findings are supported in other regions of dairy research. For example, a study in Ethiopia showed the significant role of traditional fermentation in preventing staphylococcal poisoning (reducing by 90%) (Roesel & Grace, 2015). A low pH in fermented milk inhibits the growth of pathogenic *E. coli* (Frank & Marth, 1977; Hempen et al., 2004). In one study in Ghana, 48 hours of fermentation eliminated all microbial pathogens, however, the milk was too sour for consumers (Donkor et al., 2007; Akabandu et al., 2010). Saalfeld et al. (2016) found that fermentation of cow colostrum inhibited growth of bacteria including *B. abortus*, *E. coli*, *L. interrogans*, *M. bovis*, *S. Enteritidis*, *S. Typhimurium*, and *S. aureus*, which are all major bacteria of concern in the dairy industry.

However, certain types of bacteria are not as inhibited by fermentation as others. For example, *Salmonella spp*. growth is not as affected by low pH value of sour milk compared to other bacterial types (Roesel & Grace, 2015). Also, in Ghana, while fermentation reduced certain types of bacterial contamination, it did not reduce the *Listeri*a risk (Donkor et al., 2007; Akabandu et al., 2010). Ultimately, a high bacterial count reduces the shelf life of milk and enhances the risk of milk-borne bacterial infections if the milk is not heat treated properly.

Some research has also demonstrated that fermentation is more effective in acting as a deterrent for Gram-negative than Gram-positive bacteria (Mensah, 1997). As described in Chapter 5, the most common foodborne pathogens are Gram-negative bacterial species. Gram-negative bacteria also include the EB family, which this thesis measured using luminometer technology, and research has repeatedly demonstrated that lactic acid – central to fermentation –

is able to inhibit growth of species of the EB family (Doores, 1993). In addition, during the fermentation process, lactic acid bacteria produce protein antimicrobial agents that are able to elicit protective activity against food spoilage organisms and foodborne pathogens (Aymerich et al, 2000; Carolissen-Mackay et al., 1997). Because of this information, most researchers agree that fermented foods could be used to control diarrheal diseases in children (Guandalini, 2006; Szajewska et al., 2006).

EB concentrations in some milk samples did not decrease over time. This may be attributable to the fact that there are a range of bacterial survival capabilities that are strain dependent, and that these survival capabilities may be largely context dependent. For example, Saidi et al. (2014) found that EB species from cow's milk presented significantly distinct antimicrobial resistance profiles.

Finally, samples without VIs had higher EB concentrations compared to those with VIs, which may be related to the type of visible impurity in the milk. More specifically, if the VI included known antimicrobial substances (e.g., mold with antibiotic properties), it could contribute to the EB reduction in the milk in addition to the ongoing fermentation processes. Like LAB, certain yeasts and molds also produce antimicrobial proteins and can deter growth of certain pathogenic bacteria (Chelule et al., 2010). In addition to this, mold VIs may indicate that a sample has been fermenting longer than those without mold VIs. In this way, the milk samples containing VIs may have been undergoing fermentation longer than those without, thereby reducing EB concentrations in those samples.

PRELIMINARY & MAIN STUDY CONCLUSIONS

This thesis examined unpasteurized cow's milk consumption and hygiene practices in The Gambia. Overall, the results demonstrate that unpasteurized cow's milk is regularly consumed in The Gambia, and it contains bacterial contamination that could lead to possible public health risks. A survey completed by 194 mothers from Keneba reported that children are not drinking milk as frequently as they were 25 years ago when the last milk consumption assessment occurred. However, there has been a sharp increase in the percentage of children consuming sour milk since then, which may reflect changes in milk availability, consumer preferences, seasonality, economic-related variables, or differences in survey design between this and the prior study. In addition, opportunistic data collected from 218 mothers demonstrates that some infants are receiving non-breast milk foods such as semi-solids (gruels and cereal), tea, water, and solids by 16 weeks of age, which is two months earlier than the WHO infant feeding recommendations.

Based on in-person mixed interviews with herdsmen and vendors in 2016, children in the greater-Banjul area of the Gambia are regularly consuming milk. Non-human milks are being introduced well before the recommended age of one year, with some individuals introducing it shortly after birth. This may have negative health consequences because the infant gut has not yet been exposed to new foods or certain environmental factors. In addition, based on bacterial analyses of 53 raw milk samples (fresh, powdered, and sour milks) collected from herdsmen (n=12) and vendors (n=31), the milk available for purchase in informal markets in The Gambia have concentrations of EB that exceed hygiene standards. Contamination with potentially harmful bacterial species may put infants at even greater risk of infection or illnesses such as

diarrheal diseases, dehydration, and malnutrition due to consumption of these milks or milk products.

However, traditional fermenting practices, such as exposing food products to ambient temperature over a 24 hour period, have been shown to reduce bacterial contamination in milk products in other research and is suggested by the results of this study as well. In essence, milk bacterial concentration appears to be responsive to environmental changes in the milk, such as reduction in pH-level and liquid content. During the fermentation process, growth of certain pathogenic bacterial strains can be reduced or even inhibited, thereby preserving the quality of the milk product. In this way, it could be the case that consumption of fermented cow's milk in The Gambia is benign or even beneficial to human health. Overall, this work has identified the potential for milk contamination by pathogenic bacteria species, which could have negative effects on consumer health. Additional research is needed to better understand illnesses associated with milk consumption and pinpoint microbial contamination in milks from small-holder farmers in The Gambia.

By addressing the aims described above, this research could greatly inform our current understanding of milk consumption practices in rural Gambia and in vendor and herdsmen families. Foremost, this work will provide a proxy for milk consumption patterns, which could be helpful in determining how much of a public health concern raw milk consumption could be in this country. Similarly, this information may provide insight into populations that are most likely to be impacted by effects of raw milk consumption. In addition, by documenting herdsmen and vendor hygienic practices, results of this work could identify sources of possible contamination and design educational training programs accordingly. Alternatively, this work

may set the stage for a reevaluation of milk products as sources of food-borne illness, and it may provide insight into the roles and potential benefits of traditional food fermentation systems.

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Appendix

1.1 – 2015 Milk Consumption Survey Questions

A. In general, how often does/would your infant consume cow's milk?
    1. Never
    2. 1x/week
    3. 2-4x/week
    4. x1/day
    5. >x1/day
B. How often does the mother consume cow's milk?
    1. Never
    2. 1x/week
    3. 2-4x/week
    4. x1/day
    5. >x1/day
C. In general, how often does/would your infant consume goat's milk?
    1. Never
    2. 1x/week
    3. 2-4x/week
    4. x1/day
    5. >x1/day
D. How often does the mother consume goat's milk?
    1. Never
    2. 1x/week
    3. 2-4x/week
    4. x1/day
    5. >x1/day
E. In what form is the nonhuman milk given?
    1. Fresh
    2. Sour
    3. Pre-packaged
    4. Various combinations
    5. Mixed into porridge or gruel
F. Does the infant's family own milk-producing cows, goats, or sheep?
    1. No
    2. Yes, they own cows
    3. Yes, they own goats
    4. Yes, they own sheep
G. Where does the infant's family get the milk?
    1. Owned animals
    2. Purchased from herdsmen
    3. Purchased from store
    4. Other
        i. Freetext
H. In what type of container do you store the milk?
    1. In a glass vessel
    2. In a plastic vessel

3. If Other, specify:

I. In what conditions is the milk stored?
   1. In a cool space
   2. In a hot space (out in the open)
   3. If other, specify

1.2a – Herdsmen Interview Questions

VARIABLE	DESCRIPTION	MANDATORY	FORMAT/RANGES
SubjectID	Subject/Participant ID number	Y	HGM999X/HGI999Y
FamilyInvolvment	Is your family involved in cattle performance practices (i.e. milking, vaccinations, birthing, etc.)?	N	1=Yes 2=No
FamilySpecifics	Are you the only person who milks the cattle?	N	1=Yes 2=No
HerdSize	How many cattle are in your herd?	N	1=Less than 5 2=6-20 3=21-35 4=36-50 5=51-65 6=66-80 7=81-95 8=96+
CowOwnership	How many owners own the cattle in your herd?	N	1=1 2=2-5 3=6-9 4=10+
LandOwnership	Who owns the land where cattle graze?	N	1=No family members 2=Father 3=Mother
PastureRotation	Do you practice pasture rotation when allowing your cattle to graze?	N	1=Yes 2=No
MilkRainyVSDry	Do your cattle produce more milk during the	N	1=Rainy 2=Dry 3=No difference

	rainy or dry season?		
SeasonalPriceFluctuation	Do these prices fluctuate depending on season?	N	1=Yes 2=No 3=Other
SeasonalPriceFlucSpecify	If yes, specify	N	1=More $ in rainy 2=More $ in dry
SelfConsumption	Does your family practice self-consumption of your cow's milk?	N	1=Yes 2=No 3=Sometimes
MilkConsumptionChildren	Do your children consume cow's milk?	N	1=Yes 2=No 3=Occasionally
MilkConsumptionChildrenAge	If yes, at what age do	N	Freetext
MilkShelfLife	Do you throw away milk if it is not purchased at the market the day it is milk from the cow?	N	1=Yes 2=No 3=Occasionally
MilkShelfLifeSpecifics	If not, how long do you keep it?	N	1=1 day 2=2-6 days 3=1 week 4=1 week+ 5=Other
VaccCattle	Do you vaccinate your cattle?	N	1=Yes 2=No
VaccSpecify	If yes, which vaccines?	N	Freetext
CattleMorbidities	In the last 6mo, have any of the cattle you have owned been afflicted by any type of infection, sickness, birthing complication, or disease?	N	1=Yes 2=No
MorbSpecify	If so, do you milk them at that time still?	N	1=Yes 2=No

ConsumpYou	Do you drink milk?	N	1=Yes 2=No
ConsumpPreference	If so, which type do you prefer (F or S)?	N	1=F 2=S 3=Both
MilkStorageContainer	In what type of container do you store your milk?	N	1=Glass 2=Plastic 3=Metal 4=Other
cMilkStoredCondition	In what conditions is the milk stored?	N	1=In a cool space 2=In a hot space (out in the open) 3=Other
cMilkConditionSpecify	If Other, specify:	N	Freetext
MilkTimes	When do you milk your cattle?	N	1=Morning 2=Midday 3=Evening 4=Morning & Evening
MilkDay	Do you milk your cattle everyday?	N	1=Yes 2=No
MilkSell	Do you milk directly into the container you sell milk in?	N	1=Yes 2=No
HerdsTime	How long have you been a herdsman?	N	1=Less than 1 year 2=2-6 years 3=7-11 years 4=12-16 years 5=17-21 years 6=22-26 years 7=27-31 years 8=32+ years
Women	Do women ever milk the cattle?	N	1=Yes 2=No

1.2b – Market Vendor Interview Questions

VARIABLE	DESCRIPTION	MANDATORY	FORMAT/RANGES
SubjectID	Subject/Participant ID number	Y	HGM999X/HGI999Y
FamilyInvolvment	Is your family involved in cattle performance practices (i.e.	N	1=Yes 2=No

	milking, vaccinations, birthing, etc.)?		
MilkRainyVSDry	Do you buy more milk during the rainy or dry season?	N	1=Rainy 2=Dry 3=No difference
MilkVolumes1	In what volumes do you buy the milk from the herdsmen?	N	Freetext
MilkVolumes2	In what volumes do you sell your milk?	N	1=Cup or less 2=Cup-Liter 3=Liter+
FreshVSSour	In what form is the milk sold?	N	1=Fresh 2=Sour 3=Both
SellingFreqFreshVSSour	Which form of milk is sold more frequently (F or S)?	N	1=Fresh 2=Sour 3=No difference
SeasonalPriceFluctuation	Do these prices fluctuate depending on season?	N	1=Yes 2=No 3=Other
SeasonalPriceFlucOther	If Other, specify:	N	Freetext
SelfConsumption	Does your family practice self-consumption of your cow's milk?	N	1=Yes 2=No 3=Sometimes
FamilyConsumptionSpecifics	Who in your family consumes the most milk?	N	1=Self 2=Father 3=Mother 4=Child (Female) 5=Child (Male) 6=Elderly
MilkConsumptionChildren	Do your children consume cow's milk?	N	1=Yes 2=No 3=Occasionally
ChildComp	Do children drink more F or S?	N	1=Fresh 2=Sour 3=No difference
MilkConsumptionChildrenAge	If yes, at what age do	N	Freetext

SelfConsumption2	Do you drink cow's milk?	N	1=Yes 2=No 3=Occasionally
SelfConsumption3	If so, which form do you prefer (F or S)?	N	1=Fresh 2=Sour 3=No difference
MilkShelfLife	Do you keep or discard the milk if is not purchased at the market the day it is milk from the cow?	N	1=Keep 2=Discard 3=Combination
MilkShelfLifeSpecifics	If not, how long (in days) does it stay on the market?	N	1=1 day 2=2-6 days 3=1 week 4=1 week+ 5=Other
cMilkShelfLifeSpecify	If Other, specify:	N	Freetext
MilkStorageContainer	In what type of container do you store your milk?	N	1=Glass 2=Plastic 3=Metal 4=Other
PurchaseContainer	Where do you purchase these containers?	N	Freetext
ContainerCleaning	Do you clean these containers before filling with milk?	N	1=Yes 2=No 3=Occasionally
CleaningSpecify	If so, how do you clean the containers?	N	Freetext
cMilkStoredCondition	In what conditions is the milk stored?	N	1=In a cool space 2=In a hot space (out in the open) 3=Other
cMilkConditionSpecify	If Other, specify:	N	Freetext
WorkingHours	How many hours do you stay in the market to sell milk (per day)?	N	1=1-3 2=4-6 3=7-9 4=10-12 5=12+
SouringProcess	How do you get the milk to sour?	N	1=Leave outside 2=Tablets 3=Other

OutsideProcess	If leaving outside, how long does it take for the milk to sour?	N	1=Less than 4 hours 2=5-9 hours 3=10-14 hours 4=15-19 hours 5=20-25 hours 6=26+ hours
MilkToHome	Do you take milk home with you if it is not sold?	N	1=Yes 2=No 3=Occasionally
MilkToHomeSpecify	If Other, specify:	N	Freetext
VendorTime	How long have you been a milk vendor?	N	1=Less than 1 year 2=1-4 years 3=5-8 years 4=9-12 years 5=13-17 years 6=18-21 years 7=22+ years
Vaccination	Do you know if the cows the milk comes from are vaccinated?	N	1=Yes 2=No
HerdsmenHygiene	Do you know if the cows the milk comes from are washed or if any sanitary practices are used during milking processes?	N	1=Yes 2=No
MaleVendors	Do you know of any men that sell milk at markets?	N	1=Yes 2=No
MaleVendors	If so, how many?	N	Freetext

1.3 – Table of travel times from markets/herdsmen to the laboratory (range: 15min- 2 hours)

Location	**Travel Time (minutes)**
Bakau	15min
Brikama	60min
Herdsmen	(matched the distance to the city in which the herdsmen lived)
Latri Kunda	30min
Serekunda	120min
Other	60min

1.4 – Hygiena EnSURE MicroSnap *Enterobacteriaceae* protocol

MicroSnap *Enterobacteriaceae* (MS-EB) Standard Operating Procedure

1. Purpose

The purpose of this SOP is to describe the processes of the Hygiena MicroSnap EnSURE Enterobacteria (EB) test. MicroSnap EB is a rapid test for detection and enumeration of *Enterobacteriaceae* (EB) bacteria. The test uses a bioluminogenic reaction that generates light when EB bacteria are present. The light signal is then quantified in the EnSURE luminometer. The light output is directly proportional to the concentration of bacteria present. The luminometer is part of an ATP Hygiene/Sanitation Monitoring System intended to detect ATP found in organic matter and microorganisms. The Hygiena luminometer, in conjunction with the MicroSnap test devices, measures levels of contamination on surfaces, water and product samples. Organisms are detected in 6 – 8 hours.

2. Required Materials

Required Materials:

- MicroSnap EB Enrichment Device (Part # MS1-EB)
- MicroSnap EB Detection Device (Part # MS2-EB)
- EnSURE luminometer
- Incubator at 37 ± 0.5 °C (or a waterbath at 37 ± 0.5 °C)

For product samples:

- Diluents e.g.
    - Buffered Peptone Water
        - ThermoFischer Scientific Cat. No. CM0509B
    - Maximum Recovery Diluent
        - ThermoFischer Scientific Cat. No. CM0733B
    - Butterfield's Phosphate Buffer
        - ThermoFischer Scientific Cat. No. R112526
    - Other validated diluents of user's choice

3. Procedure

This is a two-part procedure. Perform all processes aseptically. Diagrams of procedure can be found on page 5 of this document. Diagrams were created by Hygiena.

Step 1: Enrichment

(1) Collect sample
    a. Samples can be:
        i. Surface - Swab a 4 x 4 inch square area, or for irregular surfaces, as much of surface as possible to collect a representative sample.
        ii. Liquid - 1mL liquid food, beverage or water samples added directly to Enrichment Device.

iii. Product – 1mL of appropriate suspension, e.g. 10% w/v (weight/volume) food homogenate added directly to Enrichment Device. Food homogenate should be prepared using standard microbiological procedures. For unknown sample contamination, dilutions below 10% should be made and tested.

(2) Place collected sample in the MicroSnap EB Enrichment Device.
(3) Re-attach swab back on to swab tube. Device should look the same as it did when first pulled from bag.
(4) Activate Enrichment Device by holding swab tube firmly and using thumb and forefinger to break snap-valve by bending bulb forward and backward.
(5) Separate bulb and swab tube about 1-2 inches from each other, relieving internal pressure, and squeeze bulb to flush all media to bottom of swab tube. Ensure most of enrichment broth is in bottom of swab tube.
(6) Re-attach swab back on to swab tube firmly to seal device.
(7) Shake tube gently to mix sample with enrichment broth.
(8) Incubate at 37 ± 0.5 °C for 6 to 8 hours.
    a. Incubation period of 6 and 7 hours reports RLU levels (see corresponding CFU on page 6 of this document)
    b. Incubation period of 8 hours reports presence/absence of EB (see corresponding CFU on page 6 of this document)
    c. Samples can be incubated in a standard dry incubator or in a waterbath. If incubating in a water bath, be sure that the water does not exceed the sample level within tube.

<u>Step 2: Detection</u>

Before beginning Step 2, turn on EnSURE luminometer. Allow the MicroSnap EB Detection Device to equilibrate to room temperature (~10 minutes). Shake test device by either tapping on palm of hand 5 times, or forcefully flicking in a downward motion once. This will bring extractant liquid to bottom of tube.

(1) Transfer enriched sample from Enrichment Device to Detection Device. Enrichment Swab can be used as a pipette for convenience.
    a. Squeeze and release Enrichment Device bulb to mix and draw sample into bulb.
    b. Remove Enrichment swab from tube.
    c. Open Detection Device by twisting and pulling to remove bulb. Set aside.
        i. Insert Enrichment swab tip into top of Detection Device tube (approximately 1 inch) and lightly squeeze Enrichment Device bulb to trickle enriched sample into tube until volume reaches fill line marked on bottom of Detection Device tube. *Avoid adding excess sample above fill line, as this can increase variation of test results*
    d. Reassemble Detection Device to original state.
    e. Activate Detection Device by holding swab tube firmly and using thumb and forefinger to break snap-valve by bending bulb forward and backward. Squeeze bulb 3 times to release all liquid to bottom of swab tube.
    f. Shake gently to mix.

g. Immediately insert whole device into luminometer; close lid and holding unit upright, press "OK" button to initiate measurement. Results will appear after 15 second count down.
h. Result will be displayed in RLU (Relative Light Units). Refer to "Definitions" below for clarification on RLU meaning.
i. Document each RLU value with its corresponding sample ID.

Disposal:

Disinfect before disposal. MicroSnap devices can be disinfected by autoclaving or by soaking in 20% bleach for 1 hour. Then, they can be placed in the trash. Alternatively, MicroSnap devices may be discarded at a biohazard waste disposal facility.

Storage & Shelf Life:

- Store at 2 – 8 °C.
- Devices have a 12 month shelf life.
- Check expiration date on label.

Safety & Precautions:

Components of MicroSnap devices do not pose any health risk when used correctly. Used devices confirming positive results may be a biohazard and should be disposed of safely in compliance with Good Laboratory Practice and Health and Safety Regulations. Do not use devices after expiration date.

1. MS-EB Detection Device is designed for a single use. Do not reuse.
2. Do not use devices after expiration date.
3. Sampling should be done aseptically to avoid cross contamination.
4. Ensure proper dilution of sample to be read within the luminometer's dynamic range.
5. Ensure proper incubation temperature and time for the test application

4. Definitions

RLU (Relative Light Unit):

The luminometer displays results in Relative Light Unit (RLU) values. The light produced from the Luciferin/Luciferase and ATP reaction in the swab is emitted in the form of photons. A photon is an elementary particle and the basic unit of light. The luminometer detects these photons and displays them directly as RLU values. The more light detected by the luminometer, the greater the RLU value. The quantitative RLU reading is then compared against user programmable thresholds to provide an overall qualitative pass ✓, caution **!**or fail ✗ result.

The luminometer detects total ATP, not just ATP from bacteria, yeast, and mold but also the ATP from anything organic in the sample. Therefore, an RLU value is not the same as a microbial colony forming unit (CFU). Since the luminometer is detecting total ATP, it is unknown whether the RLU result displayed by the luminometer is due to the detection of microbial ATP, residual ATP, or a combination of both. Therefore, a comparison cannot be drawn between RLU values from ATP and standard plate counts (SPC); or rather RLU does not equal CFU.

Table 2: Correlation between RLU and CFU at 6 hours

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1mL liquid sample	10% suspension of solid sample
<10	<50/mL	<500/g
<25	<120/mL	<1,000/g
<50	<250/mL	<2,500/g
<100	<500/mL	<5,000/g
<250	<1,200/mL	<12,000/g
<500	<2,500/mL	<25,000/g
<1,000	<5,000/mL	<50,000/g
>1,000	TNTC	TNTC

Table 3: Correlation between RLU and CFU at 7 hours

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1mL liquid sample	10% suspension of solid sample
<10	<5/mL	<50/g
<25	<12/mL	<100/g
<50	<25/mL	<250/g
<100	<50/mL	<500/g
<250	<120/mL	<1,200/g
<500	<250/mL	<2,500/g
<1,000	<500/mL	<5,000/g
>1,000	TNTC	TNTC

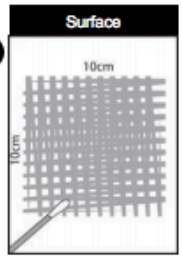
Table 4: Correlation between RLU and CFU at 8 hours

EnSURE RLU	Equivalent CFU	
	Direct sample e.g., surface swab or 1mL liquid sample	10% suspension of solid sample
<10	Absence	Absence
<25	Absence	Absence
<50	Absence	<25/g
<100	<5/mL	<50/g
<250	<12/mL	<120/g
<500	<25/mL	<250/g
<1,000	<50/mL	<500/g
>1,000	TNTC	TNTC

MicroSnap™ EB (Enterobacteriaceae)

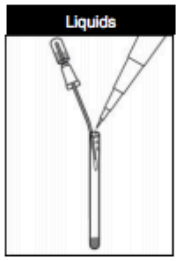
Step: 1 Enrichment of Environmental Surface Swab, Liquid and Solid Samples

1



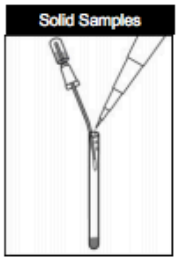
1-i. **Surface:** Swab a 10x10cm area with Enrichment Device.

OR



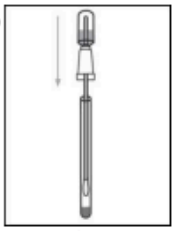
1-ii. **Liquids:** Add 1mL liquid food, beverage or water sample directly to Enrichment Device.

OR



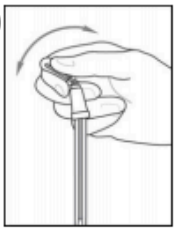
1-iii. **Solid Samples:** Add 1mL of appropriate dilution of solid samples directly to Enrichment Device.

2



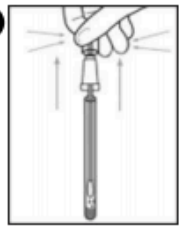
2. Re-insert Snap-Valve bulb into swab tube.

3



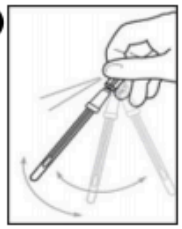
3. Activate Device. Bend bulb, breaking Snap-Valve.

4



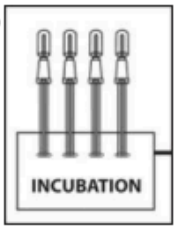
4. Lift bulb up (about 1 – 2 inches) and squeeze bulb to release liquid into bottom of tube. Replace bulb on to tube. Liquid should now be in bottom of tube.

5



5. Shake tube gently to mix sample in liquid.

6



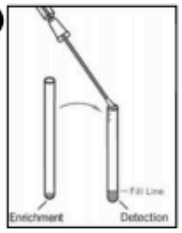
6. Incubate at 37 ± 0.5 °C for 6 - 8 hours. Proceed to Step 2.

Step: 2 Detection / Measurement

1

1. Allow Detection Device to equilibrate to room temperature. Shake to bring liquid in tube to bottom of tube.

2



2. Transfer enriched sample from Enrichment Device to Detection Device.

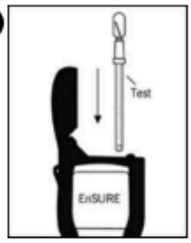
3

3. Activate Detection Device by breaking Snap-Valve. Squeeze bulb to release liquid into tube. Liquid should now be in bottom of tube.

4

4. Shake tube gently to mix sample in liquid.

5



5. Insert Detection Device into a luminometer and initiate measurement.

6

6. Record results as RLUs. Refer to Table 2 for interpretation of results.

Instructional Video:
www.youtube.com/HygienaTV

1.5 – CFU to RLU Conversion chart

Table X. CFU Equivalents to RLU Values (according to Hygiena website)

CFU/mL	***Enterobacteriaceae* RLU***
	Direct sample (e.g., surface swab or 1mL liquid sample)
<10	NA
<20	NA
<50	<10
<100	<20
<200	<40
<500	<100
<1,000	<200
<5,000	<1,000
<10,000	TNTC**

*Data reflects dynamic range after 6-hour incubation.

**TNTC = Too Numerous To Count

The CFU equivalents should be divided by .001 in order to get the actual equivalents (which will be recorded as ranges since we cannot get specific numbers) so, an RLU of 650 would have a CFU equivalent of 1,000-5,000 CFU/mL ….. divide by .001 and the true range is 1,000,000-5,000,000 CFU/mL or $1 \times 10^6 – 5 \times 10^6$

1.6 – Milk Analyses

Temperature, pH, RLU at t=0 and t=24 for each field site – these are avgs

		Bakau	Brikama	Latri Kunda	Herdsmen	Other	Serekunda
Fresh	**Temp t=0**	-	32.1	-	32.1	-	29.2
	Temp t=24	-	27.7	-	27.9	-	26.3
	pH t=0	-	6.5	-	6.98	-	6.5
	pH t=24	-	5.25	-	5.71	-	4
	RLU t=0	-	29	-	2294.95	-	715
	RLU t=24	-	18.1	-	2854	-	6622
	CFU t=0	-	$1x10^5$-$2x10^5$	-	$5x10^6$-$1x10^7$ (TMTC)	-	$1x10^6$-$5x10^6$
	CFU t=24	-	$5x10^4$-$1x10^5$	-	$5x10^6$-$1x10^7$ (TMTC)	-	$5x10^6$-$1x10^7$ (TMTC)
Sour	**Temp t=0**	22.4	32.5	30.1	-	31.87	29.5
	Temp t=24	28.3	27.7	26.9	-	26.72	26.24
	pH t=0	5	5.3	3.83	-	4.62	3.8
	pH t=24	3.87	4.8	3.67	-	3.75	3.5
	RLU t=0	2202.75	108	66	-	1604.25	543.8
	RLU t=24	356.75	27.3	14.3	-	4758.25	123.2
	CFU t=0	$5x10^6$-$1x10^7$ (TMTC)	$5x10^5$-$1x10^6$	$2x10^5$-$5x10^5$	-	$5x10^6$-$1x10^7$ (TMTC)	$1x10^6$-$5x10^6$
	CFU t=24	$1x10^6$-$5x10^6$	$1x10^5$-$2x10^5$	$5x10^4$-$1x10^5$	-	$5x10^6$-$1x10^7$ (TMTC)	$5x10^5$-$1x10^6$
Powdered	**Temp t=0**	16.3	-	29.85	-	29.55	-
	Temp t=24	28.2	-	26.7	-	26.97	-

	pH t=0	4.37	-	4	-	4.25	-
	pH t=24	3.75	-	4	-	3.87	-
	RLU t=0	1772	-	1.5	-	56.5	-
	RLU t=24	316.25	-	72	-	62.75	-
	CFU t=0	$5x10^6$-$1x10^7$ (TMTC)	-	$<5x10^4$	-	$2x10^5$-$5x10^5$	-
	CFU t=24	$1x10^6$-$5x10^6$	-	$2x10^5$-$5x10^5$	-	$2x10^5$-$5x10^5$	-

1.7
Average RLU : CFU equivalent – satisfactory or unsatisfactory EB levels compared to UK standards (t=0)

	Bakau		**Brikama**		**Latri Kunda**		**Serekunda**		**Herdsmen**		**Other**	
	S	**U**	**S**	**U**	**S**	**U**	**S**	**U**	**S**	**U**	**S**	**U**
Fresh	-	-	0	2	-	-	0	1	***2****	19	-	-
Sour	***1****	3	0	3	0	3	0	5	-	-	0	4
Powdered	0	4	-	-	0	2	-	-	-	-	***1****	3

*Because the results are in ranges, the samples that qualify as "satisfactory" are only potentially satisfactory. The RLU results indicate that these samples have $<5 \times 10^4$ … so they may not even be satisfactory! But, they also could be satisfactory.

The only t=0 samples that *possibly* met satisfactory standards ($<5x10^4$ EB)=
Sour: M025S 8/15/16 Market: Bakau
Powdered: M044P 8/17/16 Market: Other (from the nice woman?)
Fresh: M036D 8/16/16 HM TT
M037D 8/16/16 HM TT
^Joseph?

1.8
Average RLU : CFU equivalent – satisfactory or unsatisfactory EB levels compared to UK standards (t=24)

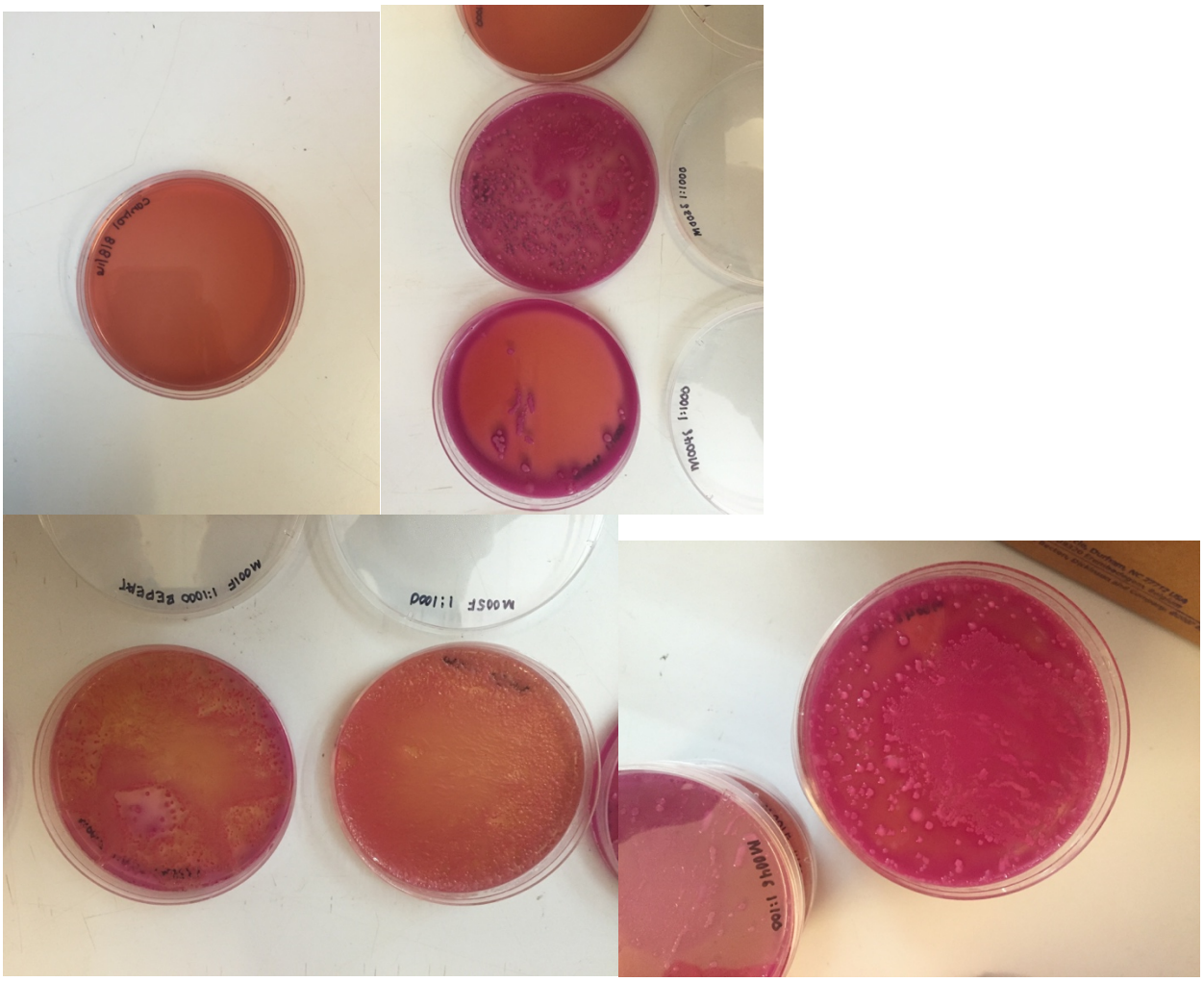
	Bakau		**Brikama**		**Latri Kunda**		**Serekunda**		**Herdsmen**		**Other**	
	S	**U**	**S**	**U**	**S**	**U**	**S**	**U**	**S**	**U**	**S**	**U**
Fresh	-	-			-	-					-	-

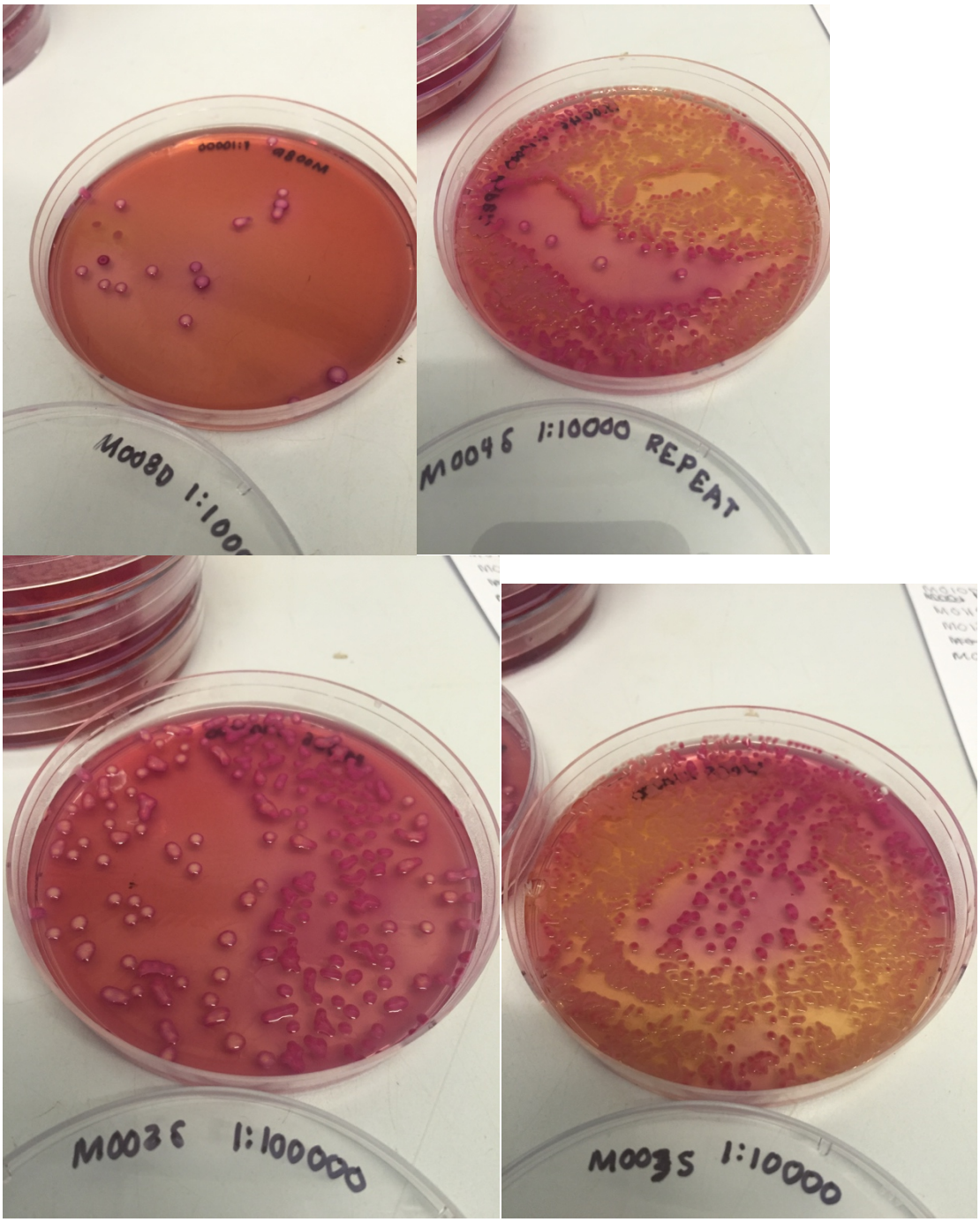
Sour					1*		1*		-	-		
Powdered			-	-			-	-	-	-		

*Because the results are in ranges, the samples that qualify as "satisfactory" are only potentially satisfactory. The RLU results indicate that these samples have <5 x 10^4 … so they may not even be satisfactory! But, they also could be satisfactory.

The only t=24 samples that *possibly* met satisfactory standards (<5x10^4 EB)=
Sour: M020S 8/12/16 Serekunda Plastic
M011S 8/10/16 Latri Kunda Plastic

1.9 MacConkey Agar Plates

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M0046 1:10000 REPEAT
M0036 1:100000
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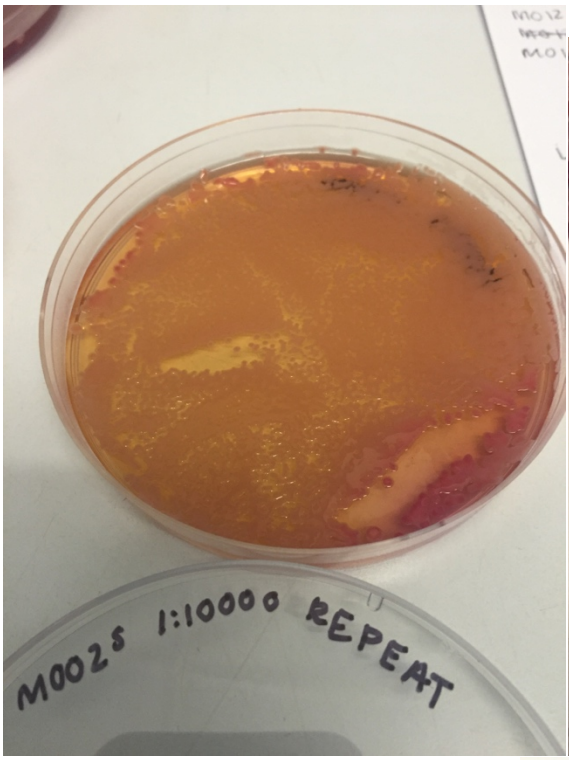
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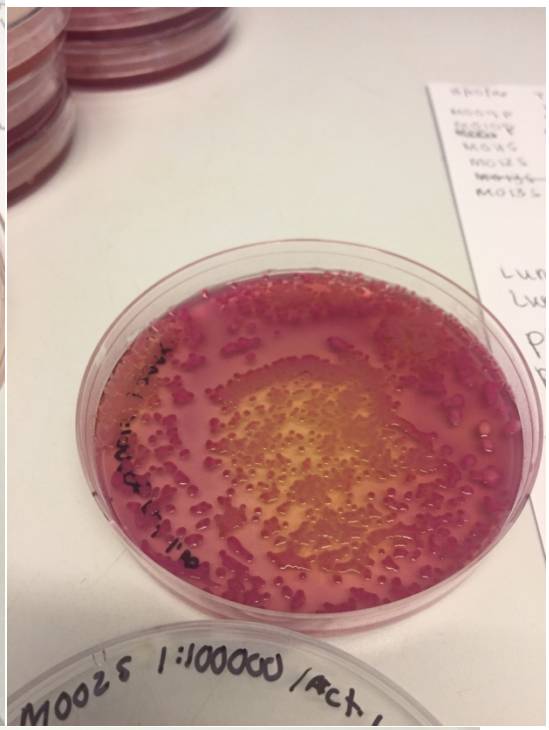
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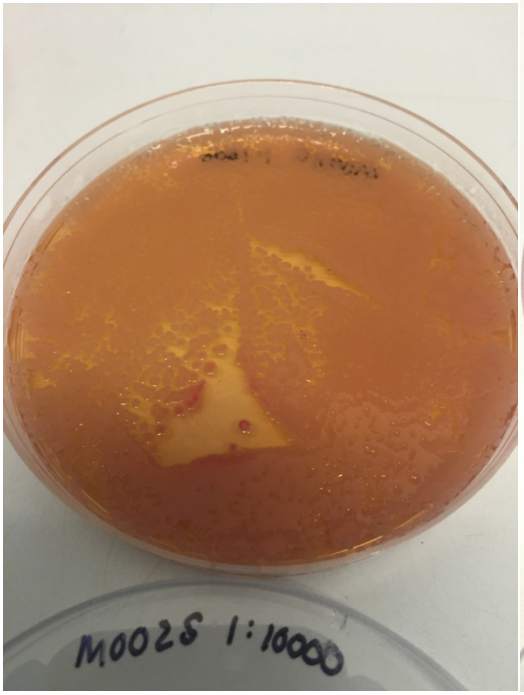
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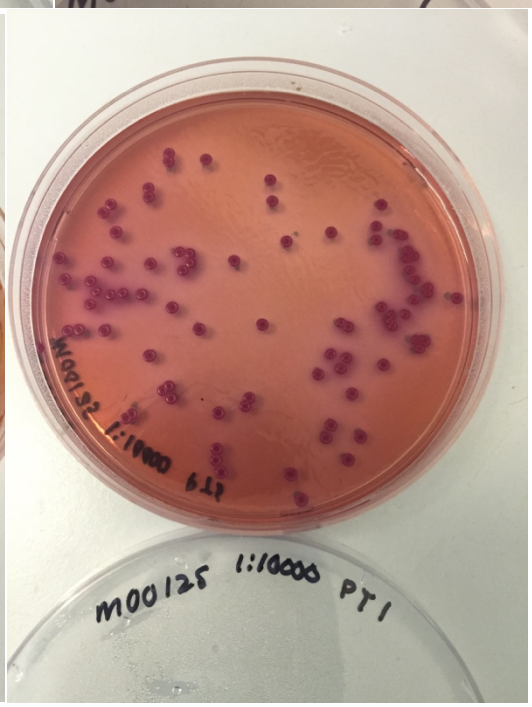
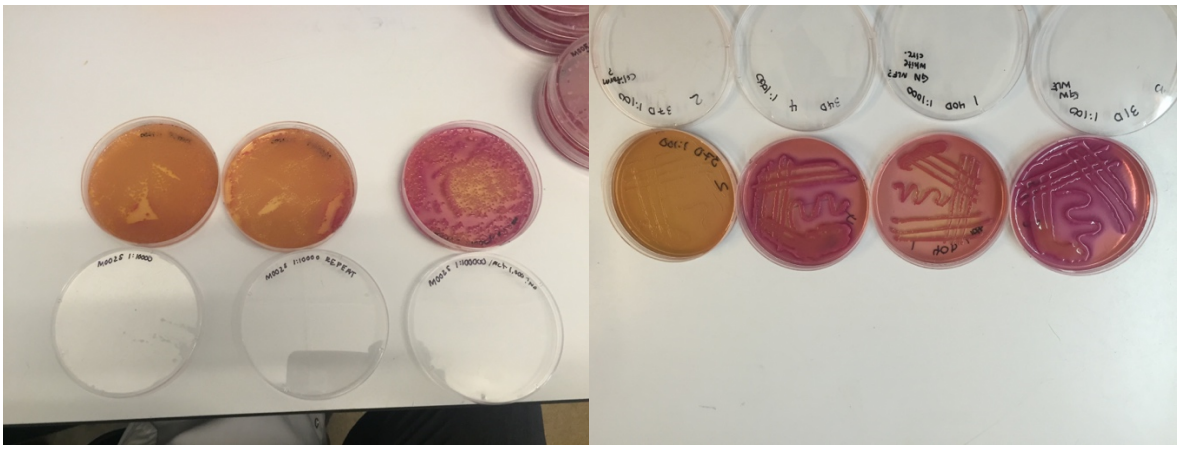

M00125 1:10000 PT1



Washabaugh (2016) results compared to Hempen et al. (2004)

	Washabaugh (2016)	Range	Hempen et al. (2004)	Range
Fresh	6.88	6-7	6.1	5-7
Sour	4.39	3.5-6	4.2	3.6-6
Powdered	4.25	3.5-5.5	-	-

	Washabaugh (2016)	Range	Hempen et al. (2004)	Range
Fresh	28.48	29.2-34.8	29.7	20-39
Sour	30.86	14.0-33.0	28.6	19-33
Powdered	25.20	13.2-29.9	-	-

	Average 0hr pH	Range	Average 24hr pH	Range
Fresh	6.88	6.0-7.0	5.04	4.5-6.5
Sour	4.39	3.5-6.0	3.86	3.5-5.5
Powdered	4.25	3.5-5.5	3.85	3.5-4.0

	Average temp (t=0)	Range	Average temp (t=24)	Range
Fresh	28.48	29.2-34.8	27.68	26.3-29.0
Sour	30.86	14.0-33.0	27.2	26.1-28.3
Powdered	25.2	13.2-29.9	27.4	26.5-28.2