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‘Heat and Healthy Milk:’

Determining the optimal pasteurization method of Goat and Blackbelly Sheep Milk for labelling recommendations at the Greenland Livestock Research Institute, Barbados

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1. Abstract

Despite the overall popularity of pasteurized milk, many people, especially in rural areas, drink unpasteurized milk. This is the case for many people in Barbados who drink the unpasteurized milk sold at the Greenland Livestock Research Institute. The consumption of unsafe unpasteurized milk can be a severe health hazard to many people - especially sensitive populations.

The purpose of this experiment was to determine the optimal conditions for the at-home heat treatment of goat and sheep milk (filtered and unfiltered) in order to create label recommendations for the milk sold at Greenland. We hypothesized that the milk boiled at our higher temperature would yield a lower coliform count and that the milk that is unfiltered will have a greater microbial load. The results indicated that salmonella was absent in all milk samples. There was, however, high coliform counts in the filtered goat's milk and none in the unfiltered milk. This suggests that there is an issue with the filtration system and cleaning protocols. The coliforms were eradicated at the higher levels of pasteurization (73.8 degrees celsius for 10s).

In order to safeguard the safety of their customers, Greenland needs to print labels on their milk bottles indicating proper home pasteurization protocols (i.e that yielded by this experiment) and implement new cleaning strategies to reduce the bacterial load.

2. Objectives

The primary purpose of this experiment is to determine the optimal temperature and time for pasteurising goat and sheep milk (i.e. the least labour intensive method for consumers to achieve a safe coliform count according to EU Guidelines for safe milk) and to create label recommendations for Greenland's unpasteurized goat milk and potential sale of blackbelly sheep milk.

This experiment also observes the filtered and unfiltered milking processes at Greenland, placing emphasis on their sanitation measures, sterilisation of implements, storage of equipment

and milk, and the source of raw inputs such as water and cleaning chemicals. This information will form the basis of recommendations made to Greenland to reduce the microbial load present in their raw milk.

3. Hypothesis

Since high heat is able to destroy certain strains of bacteria that are detrimental to human health such as Salmonella, Staphylococcus aureus, and other coliforms, heating the milk for 10s as opposed to 15s will bring the bacteria in the milk to a safe consumption level while reducing the likelihood of the milk's macronutrients (i.e. sugars, fats and proteins) and heat labile micronutrients such as Cobalamin (Vitamin B12) and Thiamin (Vitamin B1) undergoing structural change and damage.

Moreover, the milk will be heated at two different temperatures: 45°C and 73.8°C. Though heating the milk for a longer time at a higher temperature may lead to a lower total coliform count, the macronutrients and heat labile micronutrients will be more likely to be damaged, reducing the milk's nutrient density. Therefore, it is hypothesized that boiling the milk for a shorter period of time at the higher temperature will allow the macronutrients to stay intact while effectively killing off the bacteria. Additionally, this experiment doesn't predict there to be any differences in ideal pasteurisation times and temperatures for goat and sheep milk.

Lastly, it is hypothesised that the microbial load present in the raw goat and sheep milk will exceed that suggested by EU's standards for safe milk (see Appendix) as safe drinking milk. Since this experimental design looks at three primary groups: i. Goat filtered, ii. Goat unfiltered and iii. Sheep unfiltered, it is noteworthy to take into account how the milking systems (i.e. via filter or via hand) may affect the microbial load present in the milk samples. It is hypothesized that the non-mechanical system of milking via hand will present a greater opportunity for entry of external sources of contamination in the milk due to human proximity causing unfiltered goat and sheep milk to have higher total coliform counts than filtered goat milk.

4. Literature Review

4.1 Current Health Trends and the Milk Industry in Barbados

a. Goat and Sheep Milk in developing countries

According to the FAO, cow, buffalo, and goat's milks are the three most commonly produced types of milk, with cow's milk being the most produced, studied, and consumed worldwide (Bonfim da Silva, 2019). The majority of the world's population lives in developing countries, particularly in Asia. Population growth and economic growth and development are the main drivers of increased demand for dairy products. Milk consumption has risen by 10 to 20 million tons per yer - or 1.2-1.3% or 80 million more people are drinking milk on a daily basis (FAO, 2008). As a result, dairy milk products production is increasing exponentially worldwide - rising by 13% from 2002-2007. Worldwide, goat and sheep milk consumption is at 28 million tons/ year with worldwide population of goats at 1.05 billion in 2017 and sheep is at 1.2 billion in 2017 (Cannas, 2019).

The rise in popularity of goat and sheep's milk is due to the many economic, social, and nutritional benefits that they provide to people's subsistence and livelihoods strategies in developing countries (Guo, 2003). Though milk safety is a global concern, it is a bigger concern in developing countries as the lack of adequate technologies and proper infrastructure can lead to spoilage losses and decreased yields (Yamazi, 2013). Therefore, acquiring knowledge on proper animal production, hygiene, and technological aspects of the manufacturing process can aid livelihood conservation and prevent milk spoilage (Bonfim da Silva, 2019).

Moreover, the production of milk from goats and sheep can also meet the different nutritional demands of different population groups who are more sensitive to cow's milk, such as the elderly, infants, and those suffering from gastro-intestinal issues or allergies (Verruck, 2018). The production of non-cow milks enables the opening of new markets such as specialty and value added goods like cheese, fermented milk, yogurt and soaps (Verruck, 2018). Goat and sheep milk are thus able to penetrate three different markets: home consumption, speciality or niche interests, and medical needs (Park, 2010).

b. Current health trends in Barbados

Barbados is categorised as a small island developing state, and possess similar trends to the developed world when it comes to health. According to the Global Burden of Diseases, Injuries, and Risk Factors study, mortality rates are dominated by noncommunicable diseases more so than infectious ones (Evaluation, 2010). Despite Barbados' high prevalence of obesity, overall mortality rates have been declining. The leading causes of mortality are: ischemic heart disease, diabetes, and cerebrovascular diseases (all of which can be linked to obesity issues). For these diseases, the highest risk factors are a high body-mass index, dietary risks, and high blood pressure (Evaluation, 2010). In efforts to reduce these risk factors has led to people searching for healthy alternatives to many foods, including cow's milk. Approximately 10% and 20% of the population over 20 years of age suffers from diabetes and hypertension, respectively, with prevalences more than doubling at older ages (S. Sharma, X Cao, 2009). The most striking epidemic among the Caribbean population is the high prevalence of overweight [body mass index (BMI) >25 kg m−2] and obesity (BMI >30 kg m−2). Approximately half of the adult Caribbean population is overweight, and 25% of adult Caribbean women are obese (S. Sharma, X Cao, 2009).

c. Milk industry in Barbados

The milk industry in Barbados is well developed in comparison to other developing nations, and it thus has the potential to allow the island to be self sufficient in its milk production (Baynes, et al. 1999). In 1997, fresh milk production in Barbados was approximately 6.3 million liters; however, this was not enough to satisfy local demand, and powdered milk had to be imported then, as it does now, to meet the demand (Baynes, et al. 1999). A 1999 study collected milk samples from 25 dairy farms in Barbados to test the presence of antibacterial residues in Barbadian milk since antibiotics are used commonly in Barbados to treat mastitis in animals (Baynes, et al. 1999). Along with the presence of antibiotic residues, the occurrence of Staphylococcus aureus and Streptococcus agalactiae and the pH of the milk were tested. The study found S. aureus in 44 percent of the milk, S. agalactiae in 16 percent of the milk, and that the pH of milk in Barbados ranged from 6.5 to 6.8, which is within the range of acceptable pH

values (Baynes, et al. 1999). Additionally, no microbes or residues were detected in milk samples that had undergone ultrahigh-temperature pasteurization (Baynes, et al. 1999). This data shows how milk serves as an environment in which bacteria can grow (and does grow in Barbados, as well as other places), and it also suggests the efficacy of pasteurization in ensuring the safety of milk consumption.

d. The demand for milk worldwide

The world's population is increasing. With this, the consumption, production, and demand for milk - which is cheap, energy dense, nutrient dense, and accessible to rural and urban facets of life - is on the rise. With this, is a rise in livestock production (Speedy, 2003). This increase in livestock production is particularly acute in developing countries where the production of poultry, pigs, eggs, and milk sees the largest growth (Speedy, 2003). Barbados, too, is seeing an increase in its production and consumption of animal products, including milk (Speedy, 2003). The increase in livestock production, including the production of milk, in Barbados and developing nations generally is beneficial as it improves health conditions by providing key proteins and micronutrients to the diet and makes productive use of available farm resources that might otherwise not be used (Speedy, 2003). This increase in milk production is not only beneficial for the state of food security of Barbados but also helps reduce the country's reliance on imported milk powder to meet the dairy demand (Speedy, 2003).

4.2 State of food safety in Barbados

a. Microbes in milk

The importance of food for human health is widely recognised. Eating optimal quantities of nutritious and safe food is a precondition for a healthy life. The safety of food such as milk that is from animal origin is important as a large majority of foodborne illnesses are derived from poultry. Farm animals thus play a central factor in human health (Buncic, 2006).

Various microbial groups can contaminate milk, which can impact the milk's quality, safety, and even beneficial features (Nero, A. 2019). While the emphasis of milk safety is on the

elimination of pathogenic coliforms, it is interesting to note that milk contains certain microbes that may be beneficial to health. For example, some lactic acid bacteria (LAB) are able to produce bacteriocins, which are antimicrobial peptides synthesized in the ribosomes (Cotter et al., 2005, Molloy et al., 2011). They can produce a wide variety of bacteriocins which each differ in amino acid composition, biosynthesis, transport, and mode of action and provide probiotic benefits such as enhanced gut microbiota and in turn enhanced digestion and nutrient absorption (Cotter et al., 2005, Molloy et al., 2011).

While the beneficial microbes are naturally occurring in milk, most pathogenic microorganisms may enter milk in two different ways: either from diseases that affect the dairy herd, such as the etiologic agents excreted by milk (Mycobacterium bovis and Brucella abortus) or through unhygienic milking and handling practices. Pathogens such as Staphylococcus aureus, Campylobacter spp., Salmonella, Listeria monocytogenes, Shiga toxin-producing E. coli may be introduced into the equipment, floor, soil, water, and feces and contaminate milk (M. Perin, G. Pereira, 2017) .

b. Pasteurisation techniques in Barbados

The dairy sector was one of the first food sectors to introduce processing steps as well as chemical and microbial criteria along the processing chain in order to monitor and safeguard the quality of the processed dairy products. The elimination of pathogens through pasteurisation has been the main focus of the aforementioned safety operations in the dairy industry. Commercial milk processing plants such as Pinehill Dairy in Barbados execute Ultra High Temperature (UHT) pasteurisation i.e. cooling through the entire milk chain to prevent outgrowth of spoilage bacteria, ultra high temperature treatment (UHT) and industrial sterilisation to produce commercial sterility. Recently, the most commonly used heat treatments are high temperature/short time (HTST) pasteurisation, extended shelf-life (ESL) pasteurisation and commercial sterility (Gonzales-Barron, Gonçalves-Tenório, 2017). By definition, milk that is pasteurized has received a heat treatment sufficient to minimise health hazards arising from pathogenic microorganisms associated with raw milk as well as adverse chemical, physical or organoleptic changes (Gonzales-Barron, Gonçalves-Tenório, 2017).

In Barbados, Pinehill Dairy's ultra pasteurised milk dominates the dairy market for milk consumers, yet the consumption of unpasteurised milk is significant. According to Greenland Livestock and Research Institute, the highest percentage of milk consumers of raw goat's milk from Greenland are the elderly (60 years and above). Moreover, the Institute surveyed goat milk consumers regarding how they treat raw milk prior to drinking; if anything, it was found that i. most consumers do not carry coolers to prevent microbial spoilage while transporting the milk to their homes, ii. most elderly people who buy the milk are unaware of the health hazards associated with consumption of raw milk and iii. a large proportion of the middle-aged and young milk consumers report that they don't pasteurise the milk at all prior to consumption as they believe that the nutritional status of the milk will dwindle with heat treatment. The lack of awareness around the health risks associated with raw milk may lead to the ingestion of pathogenic coliforms such as E.coli, Salmonella, Staphylococcus aureus etc.

In order to safeguard against possible illness associated with raw milk consumption at home, milk must undergo a form of heat treatment. It is especially important to pasteurise milk for people susceptible to foodborne illnesses such as pregnant women, young children, the elderly, those inflicted with HIV/AIDS and other immune system diseases (Gonzales-Barron, Gonçalves-Tenório, 2017). Yet, as Greenland reported, the largest proportion of raw goat milk consumers are the elderly. This experiment follows Oregon State University Extension Service's Home Pasteurisation of Raw Milk Guideline which suggests using the double boiler method to heat milk for the best quality pasteurisation i.e. one that will ensure the lowest final coliform count. The use of indirect heat also ensures least damage to heat labile nutrients such as Vitamin B12 and milk proteins like α-lactalbumin and β-lactoglobulin (Gonzales-Barron, Gonçalves-Tenório, 2017).

b. Health implications of contaminated milk on susceptible groups

Mesophilic organisms thrive in temperatures between 25°C to 40°C (L.A, Nero, 2015). These are also optimal conditions for spoilage and pathogenic bacteria. Mesophilic bacteria commonly associated with raw milk are Micrococcus, Staphylococcus, Enterococcus,

Escherichia, Serratia, Acinetobacter, Flavobacterium, Pseudomonas etc. among others (L.A, Nero, 2015). It is easier for these pathogens to thrive in the warm conditions of the tropics. Moreover, multiple recent epidemiological studies show that raw milk can easily be contaminated by pathogens, that may have negative short term and long term implications on health (P. Oliver et al., 2009). Yet, the number of people consuming raw milk is on the rise (P. Oliver et al., 2009).

E. coli 0157 is a common pathogen linked to health hazards. It is the leading cause of diarrhea in children from lesser developed countries (Estrada-Garcia, Hodges, 2013). It may cause toxico-infections, producing enteric and/or systemic illnesses. Furthermore, it can have severe consequences such as hemorrhagic colitis, hemolytic uraemic syndrome (HUS) and thrombotic thrombocytopenic purpura (Estrada-Garcia, Hodges, 2013). The gastrointestinal tract of farm animals, especially cattle is a carrier of the shiga toxin producing E.coli (L.A, Nero, 2015). It is estimated that up to 10% of patients with Shiga toxin producing E.coli (STEC) infection may develop HUS, with a case-fatality rate ranging from 3 to 5% (L.A, Nero, 2015). Overall, HUS is the most common cause of acute renal failure in young children. It can cause neurological complications (such as seizure, stroke and coma) in 25% of Hemolytic uremic syndrome (HUS) patients and chronic renal sequelae, usually mild, in around 50% of survivors (WHO, 2018).

Moreover, drinking raw goat milk and cheese have been associated with salmonellosis outbreaks in humans. In a large outbreak in France in 1993, consumption of cheese from unpasteurized goat milk led to a Salmonella Paratyphi B outbreak; the bacteria was isolated from milk at the processing plant on 2 of 5 occasions and was found in the milk from only 1 out of 40 farms that supplied the plant (De Buyser et al., 2001). Salmonella typhi, as tested in this experiment, is passed to humans mainly by ingestion of contaminated food, mainly beef, pork, poultry, dairy products,eggs, and fresh produce (Hald, 2013). Salmonella penetrates the intestinal epithelium, possibly proliferating in macrophages, pass into lymph nodes, liver or spleen and which causes septicaemia. Furthermore, it is one of the leading causes of gastroenteritis and

bacteremia worldwide. Symptoms include fever, a prolonged period of fatigue, and the development of gastrointestinal illness with acute diarrhea as the main symptom (Hald, 2013). Long-term irritable bowel syndrome (IBS) is an outcome of salmonellosis, which is responsible for considerable morbidity and health care costs (Hald, 2013). It also causes long term reactive arthritis which compromises one's quality of life due to the limitation of one's joints. The elderly are more susceptible to the negative consequences of the aforementioned illnesses caused by Salmonella. In the case of Barbados, which has a large elderly population, raw milk contaminated with Salmonella poses a major threat.

4.3 Nutrient composition of Goat's Milk

Fig 1.1: Table depicting macronutrient composition, energy density and calcium content in 1 cup (242mL) serving of cow, goat and sheep milk:

Milk Type	Energy (kcal/234mL)	Sugars (g/234mL)	Protein (g/234mL)	Fat (g/234mL)	Calcium (mg/234mL)
Cow	149	12	8	7.9 (4.9 of which saturated)	290 (29% DV)
Goat	168	11	9	10 (7 of which saturated)	327 (33% DV)
Sheep	265	13.1	14.7	17.2 (11.3 of which saturated)	473 (47% DV)

While goat milk and cow milk are similar in nature and structure, they possess some slight differences which can impact the final products of the different types of milk as well as their digestibility and nutritional value (amino acid composition, milk proteins' secondary structure, and other chemical properties of the milk) (Vanderpas, 2003). Goat milk is more digestible, alkaline, and has a greater buffer capacity than cow's milk which makes it a more nutritious dietary staple - especially for younger or older populations (Vanderpas, 2003). Goat's milk consumption has been proven to reduce the risk of cardiovascular disease, inhibit cancer formation, and improve immune function (Verruck, 2018). Additionally, goat's milk contains "bioactive peptides, conjugated linoleic acids and oligosaccharides – which aids in the

processing of the milk" (Verruck, 2018). These beneficial properties present in goat's milk are especially helpful to those suffering from milk allergies, gastro-intestinal problems, infants, and the elderly (Vanderpas, 2003). The presence of these health benefits can aid in the development and maintenance of metabolic, immunological, and physiological processes in humans (Verruck, 2018) .

a. Lipids in goat milk

The lipids in goat's milk are primarily composed of triglycerides (98% of total lipids) with a small amount of phospholipids (1%) and fatty acids (1%) (Verruck, 2018). The lipids are relatively small but numerous fat globules that do not aggregate naturally. The fatty acid composition of goat's milk is composed of short chains – which aids in digestibility (Guo, 2003). These short chains are formed through the polymerization of acetate produced by rumen bacteria (Verruck, 2018). The fatty acids are composed of conjugated linoleic acid – which is an essential fatty acid that is linked to a decreased risk of cardiovascular disease and increased brain function (Verruck, 2018) .

b. Proteins in goat milk

Proteins and amino acids in goat's milk are composed of caseins and whey protein (Guo, 2003). Casein proteins are smaller and more abundant in goat's milk as compared to cow's which again aids in digestibility (Guo, 2003). The protein fragments or amino acids in goat's milk also bind better and exert certain bioactive properties that aid in conserving human health – this is not observed in cow's milk (Verruck, 2018). Moreover, goat's milk contains higher amounts of 14 of the 18 essential amino acids. Of the remaining four, tryptophan, aspartic acid, glutamic acid and glycine, only tryptophan is an essential amino acid (Verruck, 2018) .

c. Allergenic capacity

Bovine milk allergies occur due to poor digestibility of the bovine proteins (Verruck, 2018). Thus, goat milk's high degree of digestibility decreases its allergenic capacity. Additionally, immunoglobulin G1 and histamine levels are lower when goat's milk is given to

milk-sensitized mice (Verruck, 2018). Moreover, alpha-s1-casein protein, the major protein and allergen in cow's milk, is absent in goat's milk (Verruck, 2018).

d. Sugars in goat milk

lactose is less prevalent in goat's milk than in cow's - 9 grams per cup compared to 12 grams in cow's milk (Guo, 2003). Additionally, goat's milk seems to be formed by several polypeptides that contain several nutritional benefits, such as promoting the gut microflora (Verruck, 2018). The better digestibility and health benefits of the goat's oligosaccharides are most likely due to the fact that they are biologically similar to those found in breast milk (Verruck, 2018) which makes them more identifiable to infants and the human body is better able to use and make sense of these polypeptides. Moreover, recent studies indicate that oligosaccharides in caprine milk ranged from 250-300mg/mL which is 4-5 times higher than that in bovine milk(W. Park, F. W. Haenlein, 2013). . Milk oligosaccharides are especially beneficial for infants as they are an easy to digest source of fiber and have antimicrobial properties. Additionally, sialic acid found in oligosaccharides have shown improvements in development of infants brains (W. Park, F. W. Haenlein, 2013).

Goat's milk, like cow's milk, is also considered to be a functional food (a food that provides additional health benefits beyond its nutritional value) since goat's milk can be fermented into cheese and yogurt, two foods that are rich in bioavailable probiotics to humans(Verruck, 2018). Probiotics aid in restoring the balance between the good and bad bacteria present in the intestinal flora, maintaining a healthy gut microflora (Verruck, 2018).

4.4 Agricultural importance and significance of Blackbelly Sheep in Barbados

a. Usage of Sheep Milk

Raising dairy sheep requires a high degree of management skill. It involves two production systems – one for the sheep and one for the milk (Hale, 2006). Marketing sheep's milk also possess unique challenges, ones which may be amplified in Barbados. In the United States, sheep milk cheese was not commonly sold until 20 years ago, and it is still largely considered a niche market (Hale, 2006). However, the market is evolving and now sheep's milk

is being transformed into other products such as yogurt, ice cream, soap and cheeses such as Manchego that consumers have come to love (Hale, 2006). In Barbados, however, there are cultural and knowledge barriers to the drinking of sheep milk. According to both the employees at Greenland and the employees at the Ministry of Agriculture's Veterinary Services Lab, the consumption of sheep's milk is not something one would see as a desirable option. This lack of cultural acceptance can similarly be seen in North American countries, despite the changing landscape there.

b. Barbados Blackbelly Sheep

The Blackbelly sheep proves to be a highly prolific and important breed for the Barbadian landscape. The Blackbelly sheep has traditionally been used as a source of money and meat for peasant farmers in Barbados, and the breed continues to be important thanks to its special characteristics (Rastogi, R. K., et al.). For one, it is specifically adapted to the production systems in the tropics. Tropical conditions are characterized by a long dry season that can be prone to droughts and poor pasture quality and therefore seasonal weight loss (Almeida, 2017). Additionally, pastures are composed essentially of fast-growing grasses, high in fibre and low on protein, and in the case of the Caribbean, the best soils were traditionally used for sugarcane plantations and animal production was done essentially on roadsides and poorer terrains (Almeida, 2017). Finally, animals are subjected to different tropical diseases and parasitosis that hinder animal production in the region (Almeida, 2017). The Barbados Blackbelly sheep deals well with these conditions as they are highly tolerant to parasitic infestation, have good mothering instincts, and have "low fat on the longissimus dorsi (rib-eye) muscle" (McClean, et al. 2011). These traits are important as they are "characteristics of high prolificacy" (McClean, et al. 2011). The Barbados Blackbelly sheep is an extremely adaptable animal that is able to thrive in the tropical and difficult conditions of Barbados (Almeida, 2017).

In a 2014 study, the preferences of 78 farmers in Macedonia and Northern Greece were tested to determine what the goals of sheep breeding programs are. The goals of breeding programs are important as they give farmers the characteric sheep that will maximize their

profits and their utility (Ragkos and Abas, 2014). The results showed that farmers have a high variability in what traits they are looking for in their sheep depending on the farmers' needs and the characteristics of the farm such as acreage and herd size (Ragkos and Abas 2014). For example, longer lactation periods and high fertility rates are desired by farmers with large herds as opposed to those with small herds (Ragkos and Abas 2014). Extensive farmers, who use animals with short lactation periods as they use too much land to work year round efficiently, want sheep with higher milk production so that they can get as much milk out of their limited season as possible (Ragkos and Abas, 2014). Sheep with low fat deposits are preferred by the majority of farmers - though not all since desire is variable which is exemplified by the fact that highland sheep farmers actually prefer sheep with fat coverage (Ragkos and Abas, 2014). Higher milk production rates, however, are typically seen as a positive feature (Ragkos and Abas, 2014). By the nature of the variability of farmers' preferences, disease resistance and low fat on the muscle are not universally desired, but in the tropical conditions of Barbados, these are seen as positive features as disease incidence is much higher in tropical climates (Ragkos and Abas, 2014). The Barbados Blackbelly Sheep is important in Barbadian agriculture as the traits that allow them to be productive in the Barbadian environment are sought after and carefully preserved by Barbadian subsistence farmers who need productive sheep (McClean, et al. 2011). The Barbados Blackbelly sheep is primarily raised for meat, but these sheep are prolific producers of milk and could be a potential new source of milk for the island once more is known about sheep's milk and it is more commonly consumed.

The Barbados Blackbelly sheep is important to the agricultural landscape of Barbados as it is well suited to the environment and can thus be raised well and utilized widely in Barbados, but the sheep bread is also gaining popularity among other nations because of its positive characteristics. The Blackbelly sheep's resistance to major diseases and internal parasites, coupled with the fact that the breed has a high prolificacy rate and can birth continuously throughout the year without seasonal restriction, make the sheep a popular breed to import (Rastogi, R. K., et al.). The Barbados Blackbelly sheep has been exported to many Caribbean islands such as St. Lucia, Antigua, Trinidad and Tobata, and Guyana as well as countries further

away from Barbados such as Venezuela, Mexico, Panama, Taiwan, Canada, and the United States (Rastogi, R. K., et al.). For example, there is now a sizable population of sheep in Texas that are crosses of the Barbados Blackbelly and the Rambouillet sheep (Rastogi, R. K., et al.). Exports of the Blackbelly sheep were stopped from 1974 to 1976 by the Barbadian government in an effort to build up national numbers, but export of the animals picked up after the ban when their export value was seen and the Barbados Sheep Farmers Association was formed in 1975 (Williams and Youssef). The export of the Blackbelly sheep in turn increases its importance to Barbados as it also serves as a source of additional revenue.

4.5 Nutrient composition of Blackbelly Sheep Milk

Sheep milk has a higher specific gravity, viscosity, and refractive index than cow's milk since it has a greater total solids content in the milk, and this can greatly influence the taste and texture of final products - such as the firmness of a yogurt (Bonfim da Silva, 2019). The macronutrient profile of sheep's milk is similar to that of goat's milk. However, it does contain higher levels of lactose and lower levels of fat and protein (Bonfim da Silva, 2019) . Similarly to goat's milk, the lipids are present in the form of small and numerous globules. Their small size can aid in digestibility and efficacy of lipid metabolism when consumed (Bonfim da Silva, 2019). Additionally, the fatty acid profile is composed of shorter chains which are also easier to digest (Bonfim da Silva, 2019). Nutritionally speaking, sheep's milk also has high levels of Vitamins A and B but low levels of Vitamin E and folic acids (Bonfim da Silva, 2019).

a. Lipids in Sheep Milk

The average fat globules in sheep milk are the smallest amongst small ruminants, making it more efficient in terms of digestion and lipid metabolism (W. Park, F. W. Haenlein, 2013). Moreover, sheep's milk has a higher proportion of short chain triglycerides (SCTG) and a lower proportion of long chain triglycerides (LCTG) as opposed to cow's milk. This has implications on the physicochemical properties of sheep milk - it is less energy dense, has a lower melting and boiling point than cow's milk. The medicinal and nutritional values of these fatty acids are under

research, thus far having demonstrated benefits in different diseases (W. Park, F. W. Haenlein, 2013).

Additionally, oleic acid, the second most predominant fatty acid in sheep's milk, is an anti atherogenicagent and decreases blood LDL cholesterol level which is linked with a higher incidence of cardiovascular disease (CVD) (W. Park, F. W. Haenlein, 2013). Since the leading cause of mortality in Barbados is ischemic heart disease (Evaluation, 2010), sheep's milk consumption may be a beneficial dietary addition in order to improve blood lipid profile.

Another interesting consideration regarding lipid composition in sheep milk is it's conjugated linoleic acid (CLA) content, which is higher than that in goat and cow's milk (W. Park, F. W. Haenlein, 2013). CLA competes with arachidonic acid in a biochemical pathway and thus reduces the concentration of pro-inflammatory agents like thromboxane and prostaglandins. Besides being anti-inflammatory and thus reducing the risk of CVD, CLA is associated with lean body mass enhancing properties which is of importance in reducing the risk of obesity (W. Park, F. W. Haenlein, 2013); noteworthy in Barbados.

b. Proteins in sheep milk

Enzymatic hydrolysis of milk proteins can release bioactive peptides which a able to exert specific biological activities, such as antihypertensive, antimicrobial, opioid, antioxidant, immunomodulatory, or mineral binding (Mine, Shahidi, 2005). Sheep derived bioactive peptides are known to provide a nonimmune disease defense and control of microbial infections. Angiotensin - Converting Enzyme (ACE) peptides are a significant class of sheep milk proteins. They are well documented for potential beneficial effects in the treatment of hypertension, which is one of the leading causes of mortality in Barbados (refer to section 2.1). Recent studies found that ovine casein fractions and their hydrolysates are able to exert antioxidant activity (W. Park, 2009). Antioxidants fight free radicals generated in the body and therefore reduce the risk of RNA, DNA or protein damage and faulty metabolism and thereby reduce the risk of various cancers.

Additionally, the total protein content in sheep milk - 14.7g/cup is significantly higher than that found in cow and goat milk - 8g/cup and 9g/cup respectively (refer to Fig 1.1). A child is almost two times more likely to meet their protein nutritional needs (RDA) by drinking one glass of sheep's milk as opposed to goat or cow milk.

c. Micronutrients in Sheep Milk

Fig 1.2: depicting mean and median daily energy intake of Barbadian children (Gaskin et al., 2019).

Table 2 Comparison of the mean and median daily energy and nutrient intakes of children aged 9–10 years (*n* 362), Barbados Children's Health and Nutrition Study (BCHNS), September 2010–April 2011

	Boys				Girls			
	RDA	Mean	SE	Median	RDA	Mean	SE	Median
Energy (kJ)	7531	8644	174·5	8502	6694–7531	8912	169·9	8690
Energy (kcal)	1800	2066	41·7	2032	1600–1800	2130	40·6	2077
Carbohydrates (g)	130	286·7	6·0	279·2	130	298·9	5·9	297·3
Protein (g)	34	71·2	1·7	68·2	34	73·0	1·7	72·6
Fat (g)	25–35	72·7	1·8	68·3	25–35	73·7	1·8	71·9
Saturated fat (g)	–	24·3	0·7	23·8	< 10	24·6	0·7	23·4
Trans fat (g)	–	0·6	0·1	0·3	–	0·7	0·1	0·4
Cholesterol (mg)	<300	248·6	10·0	230·4	<300	249·4	10·6	206·1
Sugar (g)	–	127·7*	3·6	123·5	–	139·1	3·7	135·1
Fibre (g)	25	14·0	0·4	12·6	22	14·6	0·4	13·5
Vitamin A (RE)	600	490·2	35·8	331·6	600	565·4	46·8	371·8
Vitamin C (mg)	45	96·3	5·5	87·4	45	108·0	16·2	81·5
Vitamin D (μg)	15	1·3	0·1	0·9	15	1·5	0·1	1·2
Na (mg)	<2200	2723·0	67·5	2604·4	<2200	2688·5	63·5	2662·4
Ca (mg)	<1300	581·2	18·4	544·9	1300	610·5	31·3	569·0
Fe (mg)	8	12·2	0·3	11·5	8	12·2	0·3	11·4
% Energy from carbohydrate	45–65	55·7	0·5	56·1	45–65	56·3	0·5	56·4
% Energy from protein	10–30	13·8	0·2	13·6	10–30	13·7	0·2	13·5
% Energy from fat	25–35	31·4	0·3	31·3	25–35	30·9	0·4	30·7
% Energy from saturated fat	<10	10·5	0·2	10·4	<10	10·2	0·2	10·1
% Energy from *trans* fat	–	0·3	0·0	0·1	–	0·3	0·0	0·2
% Energy from sugar	–	24·6	0·5	24·1	–	26·1	0·5	26·0
% Energy from added sugar	–	18·1	0·5	18·0	–	19·7	0·5	19·7
% Energy from empty-calorie foods	–	11·3	0·5	10·9	–	11·7	0·5	11·2
% Energy from empty-calorie sugar-added beverages	–	8·8	0·5	8·1	–	8·9	0·5	7·9

Mean intake of boys significantly different from that of girls: *$P < 0·05$.

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The levels of calcium, Phosphorus, Magnesium, Zinc, Iron and Copper are higher in sheep than in cow milk (W. Park, 2009). A 1 cup serving of sheep milk contains 47% of the Calcium recommended daily allowance (RDA) which goat and cow milk provide 33% and 27% of the Calcium RDA respectively. This is of special importance in Barbados as most of the

population falls below the RDA for Calcium (Fig 1.2), which may pose negative health implications such as a higher risk of osteoporosis or hip fractures due to inadequate bone mineralisation.

4.6 Optimal feeds for small ruminants

a. Optimal feeds for small ruminants

Small ruminants such as sheep and goats present unique feeding challenges due to their wide capacity to adapt to a variety of nutritional and environmental contexts (Cannas, 2019) – these ruminants (sheep and goats) are raised through a variety of "farming and feeding systems, diverse ecological backgrounds, breeds, populations and crosses" (Cannas, 2019) due to their large capacity for adaptation. However due to this large capacity for adaptation, the production efficacy of the sheep and goat can be difficult to predict in the long run (Cannas, 2019). Feed recommendations are usually based around cattle, and although all three ruminants (cows, goats and sheep) share many similarities, they are also very different in terms of feeding strategies and the physiological functions that they provide (milk, meat or wool) (Cannas, 2019). The most important difference between them is body size and weight which are proportionally related to the energy requirements that they would need. Since goats and sheep are smaller than cows, they have "higher maintenance requirements and a lower ratio between reticulorumen volume and energy requirements then cattle" (Cannas, 2019). This is because they have less rumen available and so they need to eat more to satisfy maintenance requirements. Additionally, goats also have more selective feeding requirements which will cause the lower rumen to fill (Cannas, 2019). Sheep and goat also need to spend more time eating and chewing their food – in order for the food to be ground down to the proper particle size that can be passed through the rumen (Cannas, 2019). Production efficiency refers to the maximization of obtained product or outputs (such as milk) related to inputs (such as dry matter intake, nutrients and energy). Inputs are lost when there are inefficiencies in the system – such as surplus of feed or extensive environmental impacts (Cannas, 2019). When residual feed intake is reduced, there is less spending on feeding costs and the environmental impact of the animals is also reduced (in the form of emissions reductions) (Cannas, 2019). Farm and animal conditions can affect residual feed intake (breed,

age, body composition, digestion, etc) (Cannas, 2019). Feed conversions and optimizing the amount of feed used for adequate conversion to milk is important in reducing methane emissions (Cannas, 2019). For lambs this can be achieved by feeding a pasture composed of rye-grass with increasing levels of dry matter intake (Cannas, 2019). This would increase the energy that is accessible through grain and lead to a reduction in emissions. Additionally, accounting for the amount of emissions each animal produces can serve as a proxy for the animals' efficiency – less gas indicates greater metabolic efficiency (Cannas, 2019).

b. Goat feeding system

In developing countries – the goat feeding systems are overall characterized by low input of poor quality feeds and pastures and lead to overall low productivity (Nampanzira, 2015). This is especially true in countries that have a very rainy and dry season which can affect the availability and quality of feed (Nampanzira, 2015). Therefore, it is important to improve the feeding and nutrition of the animals in order to enhance goat productivity. Since land can be scarce – this can limit the amount of quality pastures available for the goats and this will call for improving feed technologies (Nampanzira, 2015). One example of this is adding leguminous trees. This can improve daily weight gain and the nutrition values of the pasture, and ultimately increase the productivity of the animals (Nampanzira, 2015). Feed availability is also seasonally dependant. In the wet season – goats are able to have an abundance of feed (Nampanzira, 2015). However, in the dry season – their feed needs to be supplemented since most forage has dried out and is lacking in crude protein (Nampanzira, 2015).

c. Sheep feeding system

In one study done on hairsheep – grasses and forages were supplemented to assess whether or not this would impact the productivity of the animals (Macías-Cruz, 2009). This was done due to the seasonal and annual variations in forage quality and amounts. A forage supplements was fresh orange pulp derived from a local juice factory (Macías-Cruz, 2009). This was an ideal alternative because it was low-cost and could replace the traditional grain and forage in the lambs' diet. The fresh orange pulp is similar to grains and is easily digested by the

hairsheep (Macías-Cruz, 2009). Additionally fresh juice pulp releases pectin which is a carb that can be fermented in the rumen and release energy for rapid microbial growth (Macías-Cruz, 2009). It is also a soluble fiber – which means that less lactate is produced, and this leads to a healthier rumen. Unfortunately, citrus pulp is highly perishable and requires heavy monitoring over time (Macías-Cruz, 2009). The citrus pulp was found to be readily fermentable and a better source of fiber then the dry forages because citrus pulp had a higher pectin to lignin content. The FOP (fresh orange pulp) also lead to a more fattening diet which would aid in weight gain of the animals (Macías-Cruz, 2009). Additionally, the FOP also stimulated chewing – which aids in rumination and the energy density of the diet overall decreases as forage levels increase (Macías-Cruz, 2009). The downside to using citrus was that it would need to be coupled with an other forage source to activate the positive effects on digestion (Macías-Cruz, 2009). Citrus also contains phenols and tannins which can reduce palatability- reinforcing the fact that the FOP needs to be used in conjunction with another forage source (Macías-Cruz, 2009).

d. Feeding systems at Greenland Livestock and Research Institute

At Greenland both the sheep and goats are fed the same imported feed which is locally mixed. The feed is also enriched with soya and corn in order to increase the protein and quality of the feeds. Additionally, the goats are let out to pasture to graze the local grass on a daily basis and are able to roam free after milking for a few hours a day as they have extensive pasture land to graze on. Greenland is also located in the northeast part of the island which is not as badly affected by drought conditions as some other parts of the island. This preferable location allows for grazing to be done during most parts of the year. However, the pasture grasses are insufficient as feed is imported to provide additional nutritional support to the animals. The sheep on the other hand are exclusively fed the imported mixed feed and only go out to pasture for breeding. This may be because the sheep are primarily raised for meat consumption and therefore may need additional caloric intake and a more fattening feed. The feed for both the animals is at times enriched with local supplements such as orange juice pulp in order to increase the quantity of outputs from the animals.

4.7 Milk safety and pasteurisation techniques in Barbados

a. Milk safety standards

The microbial status of raw milk determines its quality and chemical composition (Robinson, 2008). Counts and types of microorganisms influence the keeping quality of raw milk and the efficacy of heat treatments. Milk delivered from the farm to processing plants contains microbes in varying amounts determined by the health of cow or non bovine cattle, their environment, feed, milking equipment, water supply, labour, cooling and transport conditions (Haenlein, 2013). The microbial status of raw milk intended for human consumption is determined by law (Eurpean Union, 2006). Barbados uses the European Union safety standards for determining acceptable milk for consumption (Appendix 1). This experiment too uses milk safety standards established by the EU in order to assess milk safety of raw and pasteurised Goat and Blackbelly Sheep milk at the Greenland Livestock and Research Centre. Moreover, amongst the aforementioned causes of milk contamination in bovine and non bovine ruminants, a malistic udder and milking equipment are the most serious, since they can increase the bacterial count of raw milk by several cfu per milliliter (Haenlein, 2013).

b. Milk study of pathogens

Raw milk is a rich medium for bacterial and pathogenic growth that can lead to several foodborne diseases. Pathogenic development on cheese and milk can be hazardous to the health of consumers, especially to children or the elderly population (Foscino, 2001).

Milk contamination can occur through many pathways For one, it can pass through the blood due to an infection in the udder or during and after the milking process due to poor handling (Gonzales-Barron, 2017). The main pathogenic bacteria found in cow, sheep, and goat's milk are Bacillus cereus, Campylobacter, Staphylococcus aureus, E. coli, Salmonella, etc. Although many sheep and goat's milk undergoes further processing to be turned into cheese and yoghurt, the heat stable pathogens can withstand these processes (Gonzales-Barron, 2017).

In a study done by Gonzales-Baron, the incidence of L. monocytogenes, Salmonella, and S. aureus in goat and sheep cheeses was assessed. Results indicated that Salmonella had the

lowest rate of contamination in both sheep and goat milk, while Listeria had a higher level of contamination in both raw milks. It was analysed that the routes of contamination for both bacteria were through poor quality feeds, cross contamination of feed and manure, poor quality housing, contaminated milking equipment and mastitis (Gonzales-Barron, 2017). Additionally, E. coli was the second most frequent contaminant with a high prevalence in raw sheep and goat milk.

The equipment used in developing countries is sometimes more rudimentary and less sophisticated; there is more hand milking, longer milking times, and poor milking facilities (Gonzales-Barron, 2017). Additionally, cheese manufactured on the same farm where the milk is produced can be expected to have a higher incidence of pathogenic contamination and therefore would require stricter preventative and sterilization practices (Gonzales-Barron, 2017).

In a study done in Italy, the microbial content of raw goat milk intended for cheese making was assessed (Foscino, 2001). The main microbial groups (Salmonella, Listeria, and E. Coli) were analyzed. Foscino did not detect either Salmonella or Listeria bud did detect one incidence of E. Coli. The most prevalent contaminant detected was S. aureus. Since heat treatment can be difficult to carry out (due to production capacities and economies of scale), improving the hygienic conditions of the animals, milking stages, and cheese making process is essential to controlling contamination and hazardous products (Foscino, 2001). The main microorganisms that were regularly found were yeast and coliforms (Foscino, 2001) The origin of the farm was a big factor in determining the microbial composition of goat milk (this is due to differences in feeding, hygienic conditions, practices on farm and location of the farm). The amount of coliforms was proportionally related to the hygienic practices done during the production and cooling of the milk (those with little to no refrigeration had a higher coliform count) (Foscino, 2001).

c. water as a possible source of contamination:

Access to clean and safe drinking water is a major health concern for many developing regions in the world. Poor water quality can cause many gastro-intestinal and infectious diseases when consumed (Amenu, 2016). Exposure to these pathogens can result through two channels - either direct consumption of the water or indirect consumption of water when it is used in the cleaning, processing and handling of food products- this is especially crucial or dangerous when non-potable water is used in the production of raw milk which is an excellent media for bacterial proliferation (Amenu, 2016).

Hygienic practices are of utmost importance when it comes to minimizing microbial contamination (Amenu, 2016) and ensuring the quality of the milk. However, when a contaminated water source is used these hygienic practices can be nulled out if the water that is used to clean the equipment and the animals is not sanitary and/ or not regularly checked as a source of pathogens. As emphasized by Kebede Amenu's study: "the water used during handling and processing of milk products can be a potential source of microbial contamination with possible negative consequences to food safety". This indicates that the quality of the water is just as important as the other raw inputs - such as the milk in production.

It is recommended that the water that is used in cleaning the equipment and machines and in the processing of the final outputs (the milk) should be held to a "drinkable" standard - therefore safe for human consumption even when it is just used for cleaning purposes (Amenu, 2016). This can pose a challenge to many smallholder farmers who are unable to verify or obtain safe and drinkable water therefore increasing the pathways to contamination of their products and decreasing the food safety of their final products (Amenu, 2016).

d. Quality assurance of Raw milk:

Good quality Grade A milk needs to be able to fulfill certain requirements put in place by the European Union's IDF (international dairy regulations) guidelines in order to maintain the quality of the milk and to insure that it is safe for sale and consumption (Park, 2010). These requirements are that the milk is safe to drink; has good flavor; is free of spoilage bacteria and somatic body cells; and its composition is regulated (Park, 2010). There are certain methods put in place such as pasteurization - a process that is able to remove pathogens - to ensure the safety

of the milk (Park, 2010). However, this can be a costly and time consuming practice for most small farms to adopt. Therefore, since the quality of milk is highly impacted by many factors that exist on the farm – these factors (feeding, handling and milking practices for example) need to be safeguarded, regulated and controlled for biosafety due to their ability to affect the quality and perishability of milk down the line (Park, 2010).

Moreover, good quality milk needs to be free of pathogens or microorganisms which can damage the milk – this includes foreign substances such as antibiotics and pesticide residues (Park, 2010). The main parameters which are routinely monitored and regulated are nutritious constituents in milk; somatic cell count and bacterial count (which is linked to the hygiene levels of the facility); pesticide residue content; flavor, taste and appearance (Park, 2010).

When a farm is able to properly care for its animals and implement good management practices such as promoting healthy lactation and sanitary milking procedures and equipment, having quality feed for the animals can heavily influence the taste and quality of the product (Park, 2010). Good quality milk begins with a good balanced diet, good cleaning of the living and milking quarters of the animals, and proper ventilation before and during milking (Park, 2010). Workers should also practice proper cleaning practices of the milking equipment and routinely check for the cleanliness and functionality of the milking equipment (Park, 2010).

Moreover, the fundamental differences in milk secretion systems between dairy species influence the pathogenic susceptibility of the milk they produce. Goats secrete milk through the apocrine process while cows and sheep secrete milk through the merocrine process. This contributes to goat's milk having a higher somatic cell count compared to cow's milk. Yet, the somatic cell count regulations for milk quality standards are enforced for both species. Though Goat milk has a higher somatic cell count than cow's milk due to the apocrine system milk production, this isn't an indicator for leukocyte count (indicator of infection) (Park, 2010). Due to this problem, goat producers have actively pursued the problem of SCC legal thresholds.

Based on this, this experiment assumes a higher coliform count in raw milk for goat's milk, as opposed to sheep milk.

d. Quality assurance at Greenland:

Greenland tries to implement these principles and prevent disease in their milk by making sure their animals are in good health and by continuously cleaning everything involved in the milking process. The goats' udders and feet are cleaned with iodine before milking, and the part of the milking machine that mimics a hand automatically cleans itself after every use. An alkaline acid is pumped through the filtration system everyday to remove any fat buildup that may have occurred during milking. Every two weeks, the machine is given a more thorough cleaning and is taken apart so that individual contact surfaces can be cleaned with chlorine and hydrochloric acid. Greenland's current cleaning practices were prescribed to them by an outside company they consulted. The machine came with a cleaning liquid, but the employees at Greenland did not find that it worked as it did not successfully remove fat solids or calcium buildup from the piping. The company they brought in gave them new procedures to follow, and those procedures are their current protocol.

4.8 Treatment of milk

a. Cold storage influences on microbial composition of raw goat milk:

Standards and specifications for proper management of milk are crucial in avoiding negative effects on the milk's quality (Yamazi, 2013). To preserve the quality of the milk, it should be cooled in a refrigerator after milking until consumed. Despite the slowed growth of microorganisms that comes with cooling, it is still easy for psychotropics to create spoilage enzymes which may directly impact the quality of the milk and its products (Yamazi, 2013). The enzymes produced damage milk quality by interfering with coagulation of milk during cheese production. This can be avoided when care of animals and proper milking practices are safeguarded (Yamazi, 2013).

b. Heat treatment of milk:

Heating milk promotes aggregation and decreases acidification which allows for the correct associations of proteins in fermented milk to appear (Bonfim da Silva, 2019). Milk heated to 65°C and then rapidly cooled (which is the standard protocol for at home boiling pasteurization) causes a temporary decrease and then an increase in milk viscosity. This renders the proteins of the milk insoluble (Bonfim da Silva, 2019) . However, these changes to the texture profile occur on a micro level and are only detectable in milk that is exposed to high heat or superheated, such as condensed milk (Bonfim da Silva, 2019). There is no noticeable change of texture when sheep milk is heated (to temperatures of 90-95° C for 5-10 minutes) since it has a high total solids content (Bonfim da Silva, 2019).

Pasteurization is a common practice for killing harmful and pathogenic organisms in raw milk. Pasteurization can improve shelf life as well as transportation ease and time (Bonfim da Silva, 2019). Controlling for cross contamination before, during, and after pasteurization is important in ensuring the food safety of the milk (Bonfim da Silva, 2019). However, goat milk is sensitive when it comes to heat treatment, more specifically to UHT (ultra-high temperature) methods of pasteurization (Bonfim da Silva, 2019). Different forms of pasteurization have been recommended for dairy farmers since UHT can destabilize and change the taste profile of the milk (Bonfim da Silva, 2019).

The importance of the use of heat to treat food has been known for a long time - almost all of human history, but the process of milk pasteurization has only relatively recently been refined and popularized (Steele, 2000). Heat was used in the preservation of vinegar in 1782 by Scheele, a Swedish scientist, but it wasn't until 1824 that Professor Willam Dewees of the University of Pennsylvania recommended feeding babies with milk that had been heated and cooled (Steele, 2000). However, the pasteurization process largely in place now is largely the result of the work of French scientist Louis Pasteur (Steele, 2000). His technique of parboiling, or under boiling, was pioneered during his efforts to solve the problem of wine spoilage (Steele, 2000). This parboiling technique involves heating the liquid to a level sufficient enough to kill the spoilage organisms while retaining the quality of the liquid. Pasteur perfected this method

with wine in 1864, and he later applied it to beer (Steele, 2000). There is no evidence of Pasteur having used this method on milk, but his work set the basis for what milk pasteurization would become (Steele, 2000). In the late 19th century, Nathan Straus adopted the issue of milk sterilization as a philanthropic mission as he was discouraged by the number of infant deaths he saw resulting from the consumption of raw milk. Straus worked tirelessly to popularize the process of milk pasteurization both throughout the United States and in Europe (Steele, 2000).

There are a few different methods of pasteurization. Flash pasteurization involves heating milk to temperatures below its boiling point, usually between 70 and 80 degrees celsius, for just a second before cooling it back down (Steele, 2000). This form of pasteurization was one of the earliest forms of commercial pasteurization, and vat or holder pasteurization, which involves holding milk at a slightly lower temperature (60 degrees celsius) for a longer period of time, followed as the more popular and effective method of commercial pasteurization (Steele, 2000). A method of pasteurization that can be done on the household level is cooking or boiling: a method which people have turned to for a long time (Steele, 2000). In this experiment, emphasis has been made on home pasteurising i.e. stove top heating of milk through a double boiling method where the milk is indirectly heated through heat conduction from boiling water (Steele, 2000).

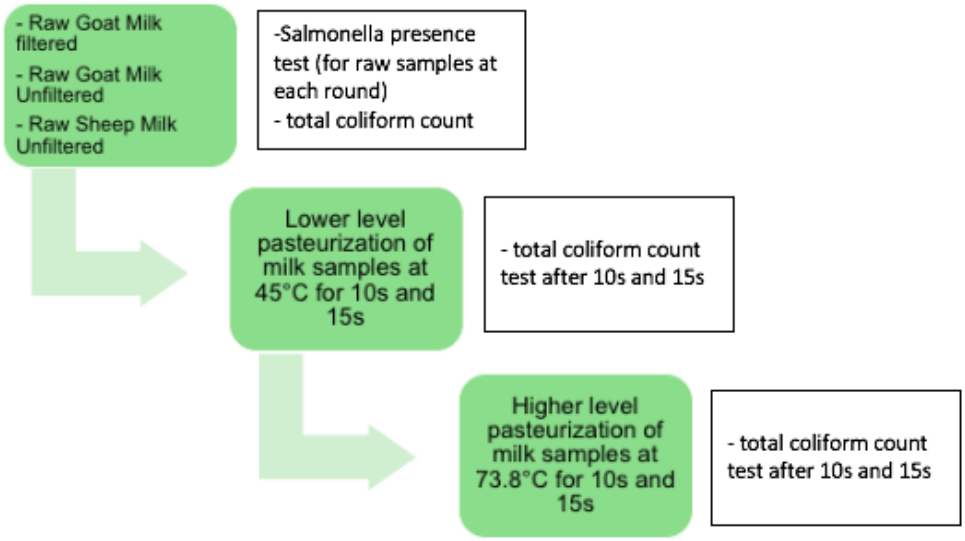
Currently, none of the milk that is produced at Greenland is pasteurized. This means that many people who buy from Greenland consume raw milk or home boiled milk. This consumption of raw milk can lead to disease, and there are risks to drinking boiled milk if the boiling is not done properly. Pinehill Dairy, owned by international beverage-giant ABInbev, uses ultra-high pasteurization on all of the milk that passes through its facility. Pinehill receives milk from a variety of local farmers, tests the milk to make sure it is up to quality (not watered down and contains the correct amount of cream), and pasteurizes it before making it into one of their signature products. Pinehill supplies a large amount of pasteurized milk to the island, but the presence of unpasteurized milk in Barbados is not negligible as Greenland and other small farms sell their products without pasteurization.

5. Materials and Methodology

In this Experiment, three types of milk were tested: i. black belly sheep unfiltered, ii. goat unfiltered and iii. goat filtered. As there is no equipment to milk black belly sheep at Greenland currently, this experiment lacks a sheep filtered treatment group. Milk from two to three sheep was pooled together for sampling. Goat Milk was derived from marked goats belonging to Seeaanen, Togenburge and Alpine breeds. The milks were sampled on three different days over a period of three weeks.

In the first round, raw goat and sheep milk was sampled for total coliforms and Salmonella spp. During the second round, a lower level pasteurisation was conducted at 45 °C for 10s and 15s in addition to another round of raw milk testing. During round three, a higher level pasteurisation was executed at 73.8 °C for 10s and 15s in addition to a third and final round of raw milk testing. A negative and positive control for total coliforms and Salmonella spp. Was executed on each day of testing.

Fig A: A visual representation of the experimental protocol followed during three rounds of testing milk samples. Raw, lower level pasteurised and higher level pasteurised goat filtered, goat unfiltered and sheep unfiltered milk were tested for total coliform counts. Raw goat filtered, goat unfiltered and sheep unfiltered milk samples were tested for the presence of Salmonella at each round.



<u>Total coliform counts:</u>

5.1 Preparing Violet Red Bile Agar (VRBA)

Reagents and Apparatus:

Yeast extract - 3.0 g, Peptone or gelysate - 7.0 g, NaCl - 5.0 g, Bile salts or bile salts No. 3 - 1.5 g, Lactose - 10.0 g, Neutral red - 0.03 g, Crystal violet - 0.002 g, Agar - 15.0 g, Distilled water - 1.0 liter

Method of preparation:

Suspend ingredients in distilled water and let stand for a few mins. Mix thoroughly and adjust to pH 7.4 ± 0.2. Heat with agitation and boil for 2 min. Do not sterilize. Before use, cool to 45°C and use as a plating medium. After solidification, add a cover layer above the agar of approximately 3.0 to 4.0 ml to prevent surface growth and spreading of colonies.

5.2 Solid Medium method: coliforms

Prepare violet red bile agar (VRBA) according 1.1. Cool to 48°C before use. Prepare, homogenize, and decimally dilute samples. Place 1 mL of the milk sample into peptone broth using a finnpipette. Secure the jar shut and swirl 30 times. Label this as the 10^{-1} dilution. Place 1mL from this jar into another peptone broth jar. Close it, swirl and label as 10^{-2} Carry this out until 10^{-5} for raw milk samples and till 10^{-3} for heated milk samples. Then, Label petri dishes with the milk sample and heating time. Transfer two 1 mL aliquots of each dilution to two petri dishes to create two duplicates for each sample. After this, pour 10 mL VRBA tempered to 48°C into plates, swirl plates to mix, and let solidify. To prevent surface growth and spreading of colonies, overlay with 5 mL VRBA, and let solidify. After solidification, invert plates and incubate 18-24 h at 32°C (2). Finally, examine plates under magnifying lens and with illumination. Count purple-red colonies that are 0.5 mm or larger in diameter and surrounded by zone of precipitated bile acids. Plates should have 25-250 colonies. Lastly, to confirm that the colonies are coliforms, pick at least 10 representative colonies and transfer each to a tube of BGLB broth. Incubate tubes at 35°C. Examine at 24h and 48 h for gas production.

Salmonella spp. Test:

5.3 Preparing media for Salmonella testing

Reagents and Apparatus

Blender and sterile blender jars, Sterile, 16 oz (500 ml) wide-mouth, screw-cap jars, sterile 500 ml Erlenmeyer flasks, sterile 250 ml beakers, sterile glass or paper funnels of appropriate size, and, optionally, containers of appropriate capacity to accommodate composited samples, Sterile, bent glass or plastic spreader rods, Balance, with weights; 2000 g capacity, sensitivity of 0.1 g, Balance, with weights; 120 g capacity, sensitivity of 5 mg, Incubator, 35 ± 2 °C, Refrigerated incubator or laboratory refrigerator, 4 ± 2°C, Water bath, 49 ± 1°C, Water bath, circulating, thermostatically-controlled, 43 ± 0.2°C, Water bath, circulating, thermostatically-controlled,42 ± 0.2°C, Sterile spoons or other appropriate instruments for transferring food samples, Sterile culture dishes, 15 × 100 mm, glass or plastic, Sterile pipettes, 1 ml, with 0.01 ml graduations; 5 and 10 ml, with 0.1 ml graduations, Inoculating needle and inoculating loop (about 3 mm id or 10 5l), nichrome, platinum-iridium, chromel wire, or sterile plastic, Sterile test or culture tubes, 16 × 150 mm and 20 × 150 mm; serological tubes, 10 × 75 mm or 13 × 100 mm, Test or culture tube racks, Vortex mixer, Sterile shears, large scissors, scalpel, and forceps, Lamp (for observing serological reactions), Fisher or Bunsen burner, pH test paper (pH range 6-8) with maximum graduations of 0.4 pH units per color change, pH meter, Plastic bags, 28 × 37 cm, sterile, with resealable tape. (Items 23-24 are needed in the analysis of frog legs and rabbit carcasses.), Plastic beakers, 4 liter, autoclavable, for holding plastic bag during shaking and incubation, Sponges, non-bactericidal (Nasco cat # B01299WA), or equivalent, Swabs, non-bactericidal, cotton-tipped.

5.4: preparing buffered peptone water (BPW)

Reagents and Apparatus:

Peptone - 10g, Sodium chloride - 5g, Disodium phosphate - 3.5g, Mono-potassium Phosphate - 1.5g, Distilled water - 1L

Method of preparation:

Autoclave at 121°C for 15 min. Final pH 7.2 ± 0.2.

5.5 Isolation of Salmonella:

Add 25mL of milk sample in 225mL buffered peptone water (as prepared in 1.4) using a 50mL pipette and pipette filler. Tighten lid and gently shake incubated sample. If the sample is slightly cloudy post incubation, this may be an indication of the presence of Salmonella spp. Regardless, the next step should be executed. Then, transfer 0.1 ml mixture to 10 ml Rappaport-Vassiliadis (RV) medium and another 1 ml mixture to 10 ml tetrathionate (TT) broth. Vortex. After vortexing, incubate RV medium 24 ± 2 h at 42 ± 0.2°C (circulating, thermostatically controlled, water bath). Incubate TT broth 24 ± 2 h at 35 ± 2.0°C. Vortex and streak 3 mm loopful (10 µl) incubated TT broth on bismuth sulfite (BS) agar, xylose lysine desoxycholate (XLD) agar, and Hektoen enteric (HE) agar. Prepare BS plates the day before streaking and store in dark at room temperature until streaked. Repeat with 3 mm loopful (10 µl) of RV medium. Use inoculating loop to streak colonies of bacteria onto each quadrant of the petri dish. Ensure sterility by flaming the inoculating loop each time. Incubate plates 24 ± 2 h at 35°C. Finally, examine plates for presence of colonies that may be Salmonella.

5.6: Typical Salmonella colony morphology

Pick 2 or more colonies of Salmonella from each selective agar plate after 24 ± 2 h incubation. Typical Salmonella colonies are as follows:

If typical colonies are present on the BS agar after 24 ± 2 h incubation, then pick 2 or more colonies. Irrespective of whether or not BS agar plates are picked at 24 ± 2 h, reincubate BS agar plates an additional 24 ± 2 h. After 48 ± 2 h incubation, pick 2 or more typical colonies, if present, from the BS agar plates, only if colonies picked from the BS agar plates incubated for 24 ± 2 h give atypical reactions in triple sugar iron agar (TSI) and lysine iron agar (LIA) that result in culture being discarded as not being Salmonella.

6. Results

Section A: Coliforms

The EU Guide's Standard for Safe Drinking milk (see Appendix) is used as a reference for determining acceptable levels of microbes in milk samples in this experiment. The values highlighted in Fig 1.1-1.3 depict valid microbial counts based on the general acceptable range of countable colonies on a standard agar plate i.e. 25-250 for most bacteria (Peeler, J. T., 2001).

Fig 1.1: depicting no. of colonies counted per agar plate (n,n) for unheated, Raw (no pasteurisation) samples of Filtered Goat, Unfiltered Goat and Unfiltered Black Belly Sheep milk samples; Round 1.

Milk Condition	**Type of Milk**	**No. of colonies at n Dilution**				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Raw, unheated	Filtered goat	**31,41**	7,3	2,0	0,0	0,0
	Unfiltered goat	0,0	4,8	1,0	0,0	0,0
	Unfiltered sheep	0,0	0,0	0,0	0,0	0,0

Fig 1.2: depicting no. of colonies counted per agar plate (n,n) for Raw unheated, lower level pasteurisation at 45°C for 10s) and lower level pasteurisation at 45°C for 15s) for filtered goat, unfiltered goat and unfiltered Black Belly Sheep milk samples; Round 2.

Milk Condition	**Type of Milk**	**No. of colonies at n Dilution**				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Raw, unheated	Filtered goat	**60,42**	0,0	0,0	0,0	0,0
	Unfiltered goat	**0,0**	4,8	1,0	0,0	0,0
	Unfiltered sheep	0,0	0,0	0,0	0,0	0,0
Lower level pasteurisation at 45°C for 10s)	Filtered goat	**61,39**	0,0	0,0	N/A	N/A
	Unfiltered goat	0,0	4,6	0,0	N/A	N/A
	Unfiltered sheep	0,0	0,0	0,0	N/A	N/A

Lower level pasteurisation at 45°C for 15s)	Filtered goat	**31,31**	0,0	0,0	N/A	N/A
	Unfiltered goat	0,0	7,1	0,0	N/A	N/A
	Unfiltered sheep	0,0	0,0	0,0	N/A	N/A

Fig 1.3: depicting no. of colonies counted per agar plate (n,n) for raw unheated, higher level pasteurisation at 73.8°C for 10s) and higher level pasteurisation at 73.8°C for 15s) for filtered goat, unfiltered goat and unfiltered Black Belly Sheep milk samples; Round 3.

Milk Condition	**Type of Milk**	**No. of colonies at n Dilution**				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Raw, unheated	Filtered goat	**114,101**	11,15	1,2	0,0	0,0
	Unfiltered goat	0,0	0,0	0,0	0,0	0,0
	Unfiltered sheep	0,0	0,0	0,0	0,0	0,0
Higher level pasteurisation at 73.8°C for 10s)	Filtered goat	0,0	0,0	0,0	N/A	N/A
	Unfiltered goat	0,0	0,0	0,0	N/A	N/A
	Unfiltered sheep	0,0	0,0	0,0	N/A	N/A
higher level pasteurisation at 73.8°C for 15s)	Filtered goat	0,0	0,0	0,0	N/A	N/A
	Unfiltered goat	0,0	0,0	0,0	N/A	N/A
	Unfiltered sheep	1,1	0,0	0,0	N/A	N/A

Fig 1.4: depicting coliform confirmation tests results for coliforms on petri dishes that showed growth during Round 2 and Round 3: Raw Filtered Goat milk on Monday and Tuesday, Filtered Goat Milk pasteurised under low heat conditions (45°C, 10s) and Filtered Goat Milk pasteurised under low heat conditions (45°C, 15s). Positive (+) is analysed qualitatively by a cloudy green appearance and the presence of gas. Negative (-) is indicated by a clear green gasless solution. No coliform confirmation test was executed for Round 1 due to aforementioned limitations.

Colony n	Coliform Confirmation Test Results			
	Filtered Goat, Raw (Round 2)	Filtered Goat, Raw (Round 3)	Filtered Goat, 45°C for 10s	Filtered goat, 45C° for 15s
1	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	+	+	+
9	+	+	-	+
10	+	+	-	-
Percentage of true coliforms (%)	100%	100%	80%	90%

Sample calculations for Enumeration of Coliforms:

1.7. Samples that tested positive (+) for 100% true coliforms as depicted in Fig 1.4 were enumerated using standard CFU count method:

CFU=(no.of colonies x dilution factor)/(volume of culture plate)

No. of colonies = average of colonies = (n colonies from sample 1 + n colonies from sample 2)/(2)

No. of colonies= (60+42)/(2) = 51

E.g. for Filtered Goat, Raw (Round 2):

CFU= $(51 \times 10^1)/1mL$

= **510 colonies/mL**

1.8 Samples that tested below 100% for true coliforms as depicted in Fig 1.4 are enumerated by alternatively i.e. including %confirmed coliforms.

CFU= (no. of colonies x dilution x % confirmed colonies)/(volume of culture plate)

E.g 1. Filtered Goat, 45C for 10s (lower heat pasteurisation):

CFU= $(50x10^{1}x0.80)/(1mL)$

= **400 colonies/mL**

Fig 1.5: depicting true CFU counts for coliform positive samples i.e. Filtered Goat, Raw (Round 1), Filtered Goat, Raw (Round 2), Filtered Goat, Raw (Round 3), Filtered Goat, 45°C for 10s - lower level pasteurisation and Filtered Goat, 45°C for 15s - lower level pasteurisation.

Milk Treatment	Colony Forming Unit (Colonies/mL)
Filtered Goat, Raw (Round 1)	360
Filtered Goat, Raw (Round 2)	510
Filtered Goat, Raw (Round 3)	1075
Filtered Goat, 45C for 10s - lower level pasteurisation	400
Filtered Goat, 45C for 15s - lower level pasteurisation	279

Section B: Salmonella spp.

According to 1.6, which describes the protocol adhered to for determining the presence of Salmonella in milk samples, It was determined that Salmonella was absent in all milk samples tested. Refer to 1c, Appendix.

7. Discussion

The results of bacterial distribution in the samples are presented in Section 1.6. All the raw filtered goat's milks had high bacterial loads which average ranged from 360-1000 c.f.u./ml after incubation, plating, and enumeration using the CFU formula to establish number of coliforms per unit milk during the three rounds of testing. The term "total coliforms" refers to a wide range of

gram-negative, rod-shaped bacteria that share similar features. This includes thermotolerant coliforms, bacteria that may be of faecal origin and some that may be isolated from environmental sources such as milking filters. As the microbial load was highest in the raw filtered goat's milk, it is assumed that environmental contaminants through the filter, implements, handling equipment or the source of the water and chemicals to clean and sterilise the filtration system respectively may have presented an entry pathway for pathogens into the milk.

Moreover, the microbial load per mL found in unheated goat's milk varied considerable during each round of testing. The coliform count in raw goat's milk at round 2 was 41.7% than it was at round 1, and the coliform count in unheated goat's milk at round 3 was 199% higher compared to the coliform count in round 2. Taken that, based on the results, it is assumed that the source of contamination at Greenland is the filter, the increasing trend of microbial load over three consecutive rounds of testing may indicate that the filter was not being cleaned adequately, leading to a build up and proliferation of microbes in the filter or bulk tank. The uncleanliness of the bulk tank and filtration system may be a result of a lack of cleaning, a lack of thoroughness of cleaning, or the difficulty of keeping things bacteria free in the hot and humid tropical environment of Barbados.

It is also noteworthy that not all counted data indicating microbial presence in milk samples is representative of true coliforms. Thus, to confirm the true number of coliforms a coliform confirmation test was executed. 10 colonies were randomly picked from coliform containing agar plates and placed into individual BGLB broth containing test tubes. If gas was produced post incubation, the colony represented a true coliform. This percentage of true coliforms in the random sample test was used to adjust the formula used to enumerate the number of coliforms per mL of milk, as executed in Fig 1.5. Thus, the data analysed in this experiment is based on true coliforms. Additionally, some milk samples, such as the raw filtered goat milk at the 10^{-2} dilution in round one (Fig 1.1), contain the presence of microbes at counts (i.e. 7,3) that fall

below the acceptable countable range of 25-250 (Peeler, J. T., 2001). The math to undo the dilution factor puts the number of coliforms around the one obtained from the 10^{-1} Dilution. However, the 10^{-1} dilution is used since only plates with counts between 25 and 300 are used.

The hypothesis of this experiment states that the higher level of pasteurisation at 73.8°C for 10s would form the ideal pasteurisation condition, ensuring that the microbial load in goat and sheep milk would be brought down to a level safe for human consumption. As depicted in Fig 1.5, there were microbes present in milk samples after it was heated at the lower level of pasteurization at 45°C at 10s and 15s with colony forming units of 400 colonies/mL and 279 colonies/mL respectively. From these results, it can be inferred that though there was a 30.25% reduction in microbial load when the heating time was increased from 10s to 15s, the total coliform count at each time was higher than that present after heating the milk at the higher temperature i.e. 73.8°C.

Moreover, while the emphasis of this experiment is bringing down microbial load in raw milk, it is based on the assumption that all raw milk contains a certain level of naturally occurring microbes due to the physiology of small ruminants such as goats and black belly sheep, the various gateways for the entry of microbes presented by the process of milking - manually or mechanically. Thus, the absence of any significant coliforms in raw unfiltered goat and black belly sheep milk in all three rounds of testing was peculiar. According to the Ministry of Agriculture Food and Veterinary Services Lab, it was established that it is rare for raw milk, especially in the tropical humid environment of Barbados, to be free of any microbes. For one, this may be due to the prolific nature of black belly sheep and goat at Greenland. At Greenland, goat and black belly sheep rearing do not involve the injection of hormones to regulate (increase the frequency of) lactating cycles as the species of small ruminants are sturdy and prolific in the Barbadian setting. Antibiotic residues in milk are caused by the direct treatment of dairy cattle with antibiotics in feed. A retrospective observational study conducted at Wisconsin dairy farms found a positive correlation between antibiotic residues and high somatic cell counts (SCC) in

milk (P.L.Ruegg, T.J.Tabone, 2000). This may form part of the explanation for raw goat and sheep unfiltered milk at Greenland being virtually free of any coliforms.

Secondly, Greenland's manual milking measures may be hygienic and pose minimal gateways of opportunity for entry of microbes. According to Greenland Research and Livestock Institute, the measures taken to maintain sterility of the manual milking system include the usage of gloves, regular changing of gloves, and the sanitising of milking containers with chemicals. Moreover, the milk is stored at refrigerator temperature of 4°C right after milking to prevent thermophilic microbes from proliferating and causing milk spoilage.

All of the Salmonella tests came back negative indicating the absence of Salmonella in Greenland's goat and sheep milk. However, in the unfiltered raw goal milk sample from July 16th, there was black growth on the petri dish that warranted further testing to determine if it was Salmonella or not. When the black growth was placed on triple sugar iron agar (TSI) and lysine iron agar (LIA) (which are both agars that allow for the growth of hydrogen sulfide producing bacteria - of which Salmonella is one), some black hydrogen sulfide was produced. The coloration of the agar after incubation, however, did not change in a way that indicated the presence of Salmonella (refer to 2a and 2b in the appendix). This leaves open the possibility that the milk contains either a hydrogen sulfide producing Cirtobacter or E. coli species. Further testing should be conducted on the milk at Greenland, and specifically on the goats numbered 9011, 00922, and 00889 from which the raw milk samples were taken.

8. Potential Sources of error

To collect the milk samples for testing, the samples had to be transported from Greenland to a Ministry of Agriculture building in Speightstown where ice was collected before being transported past Bridgetown to the Ministry of Agriculture's Veterinary Services Lab. The samples were not on ice for the ten minutes it took to get from Greenland to the Ministry of Agriculture building. While this is a short time for the samples to not have been cooled, this time

gap in cooling could be a potential source of error in that it allowed more colonies to grow than had the samples been refrigerated since immediately after milking.

We were also unable to conduct a number of samples that would have made statistical analysis possible for this experiment. We had planned on conducting three samples a week over a series of three weeks which would have given us nine total samples. However, we were only able to collect seven samples since we did not receive a sufficient amount of milk the first week we went into the lab. Additionally, we tested a lower level of pasteurization the second week that resulted in us not having repeat results from the higher level pasteurization we conducted. We would have liked to have made up for this by continuing our trials for an extended period of time, but unfortunately we did not have access to the lab after the third week. This results in our data being preliminary and in the possibility that our counts are outliers and not a normal reaction to the levels of pasteurization we conducted since we have no nothing to compare our trials to.

Another factor to consider is the potential of the milk containers at Greenland to contaminate the milk samples. The milk samples were given to us in emptied and cleaned out half gallon milk jugs. Upon arrival to the lab, the milk had to be pipetted out of the milk jugs into sterile glasses to avoid touching the rim of the milk jug and any potential contaminants there. Despite the fact that these precautions were taken, there is still the possibility that more than just the rim of the milk jugs could serve as a source of contamination and that by simply putting the milk into the jugs the milk was being contaminated. This would mean the results did not accurately reflect the level of bacteria in the milk but in the milk and the jug as well.

Another study researched the effect of boiling milk on microbial content of cow and buffalo milk. The research was carried out with a similar goal to our work – to "determine the proper boiling period which results in the required safety through a proper shelf life with minimum quality deterioration" (Metwally, 2011). The experiment looked at cow's and buffaloes' milk while ours looked at goat and sheep's milk. However, they still heated the milk at 100deg C, 80deg C and 90deg C for 30 seconds, 1 minute, 5 and 10 minutes and then cooled in

an ice bath. The results of this experiment was that there was a subsequent decrease in microbial content from the untreated milk to the sample that was heated at 80deg C, however, there were smaller improvements when the milk was heated for longer. Boiling the milk, brought the lethality level of the milk down to acceptable grade A quality milk suitable for drinking. This is similar to our findings – where the number of colonies decreased the more the milk was heated.

Additionally, the contaminated milk came from the bulk tank milk and not from the animals themselves. Research from the FAO indicates that "the milk contact surfaces of milking and cooling equipment are a main source of milk contamination and frequently the principal cause of consistently high bacterial counts. Simple, inexpensive cleaning and disinfecting routines can virtually eliminate this source of contamination" (FAO). This indicates that the surfaces that the milk comes into contact with – the bulk tank or the milking equipment is not getting cleaned as effectively as it should and the source of the problem is the equipment itself and not the animals. The FAO also indicates that the "water for Dairy use must be either an approved, piped supply or chlorinated (50 ppm) before use. In hard water areas, milking and ancillary equipment must be de-scaled periodically" (FAO). This suggests that even if the cleaning methods used at Greenland are effective, their efforts could be undone if they are using a contaminated water source. The water that is used in cleaning the milking equipment would therefore need to be held at the same water safety standards that drinkable/ potable water is held at. This is to ensure that the water that is being used to clean the filtration system and milking equipment is not leading to an increased microbial load in the final products. Therefore, if the cleaning protocols and chemicals are properly used - however, contamination persists it would be worthwhile for Greenland to check their water (as well as water storage areas) for waterborne pathogens (E. coli) as well as treating the water source (through a similar home pasteurization technique) before being used. Additionally, proper handling of the water would also need to be ensured to safeguard against increased introduction of pathogens due to unhygienic practices implemented during water collection and storage (Amenu, 2016).

9. Conclusion

This experiment taught us many things about Barbados and about designing and conducting an experiment. Firstly, we learned about the information gap between the producers and consumers- especially around pasteurizing milk. Producers know that boiling the milk is a crucial step to take before consumption - however, many consumers are unaware that this is important to do or the health risks that are associated with raw milk consumption, which is because many consumers are unaware that milk is the perfect medium for bacterial growth and proliferation. There is also a big information gap in the population. Most elderly people are more likely to drink milk raw and from the farmer then they are to buy pasteurized milk at the store. This could be due to generational and cultural differences as well as a lack of trust in the UHT pasteurization process - believing that when milk is pasteurized it is considered “dead” milk.
We also learned about the strong cultural preferences and dislike towards sheep milk, cheese and yogurt consumption. Many of the lab technicians at the Veterinary Services Lab and the workers at Greenland have indicated that sheep milk is something that either they personally would never consume nor would any one they know - especially not the elderly. However, some young people may be more open to trying it.

Conducting an experiment in Barbados has been a unique experience since this research required a lot of patience, coordination, delays and misunderstanding. Given that we did not have an established topic and had to build the experiment ourselves - we ultimately needed to navigate our way around the infrastructure of Barbados- such as contacting a government lab to be able to test samples - which did not inform us that they would be going on vacation thereby cutting down lab time for us; equipment malfunctioning at Greenland with little recommendations or help finding alternatives. Through these inconveniences we learned that conducting experiments takes time and patience to make sure everything is going smoothly.
Since Barbados is a tropical country - we learned how crucial it is to have proper preservation techniques due to hot and humid environment, and therefore it is important to be able to reduce contamination sources.

We recommend that the staff at Greenland to figure out where the source of the contamination for the milk in the bulk tank is coming from - whether it is from an animal in particular, a specific element of the equipment that is contaminated (the bulk tank, or the other milking equipment), or the staff - improperly washing their hands and not wearing gloves. Additionally, until the coliform count is reduced to an acceptable level (less than 100 CFU according to the EU's standard), routine microbial testing should be done in order to ensure the quality of the milk that they are selling.

We also recommend that Greenland print labels on their bottles indicating the ideal milk boiling time and temperature (boiling the milk in a double boiler until it reaches 78 degrees Celsius). We also recommend that these labels indicate that boiling the milk does not impact the quality of its nutrients. Additionally, the label should include the associated health risks with drinking unpasteurized milk such as (gastrointestinal and stomach problems as well as long term risks and complications which can be especially harmful to sensitive groups such as the elderly, young children and pregnant/breastfeeding women).

Another recommendations for the staff at Greenland is to investigate new cleaning protocols. The manufacturer recommended protocols were considered defective which lead to the implementation of new ones; however, these new protocols are either not followed properly or they have also become defective. Therefore, we recommend that Greenland implement and enforce an easy to follow standard operating protocol that the staff can follow on a weekly and daily basis to ensure that the machines are actually cleaned. We believe that the machines at Greenland are not properly taken care of. The lacticheck machine – which is used every three months to check the protein content of the milk - was defective when we went to sample our milk samples for total fat and protein content of the goat and sheep milk. Additionally, residue and buildup came out of the machine when attempts to clean it were made making it appear as if the milk is not regularly checked per their recommendations (every 3 months). Since this machine was not well taken care of – it is fair to wonder whether the rest of their machines are

properly cleaned on a bi-weekly basis as described. A proper cleaning schedule and oversight is a crucial step to ensuring the quality of the milk.

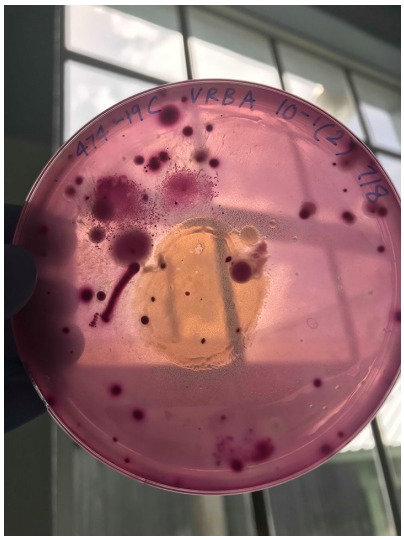
Future experiments would ideally be centered around testing the nutritive content of the milks before and after boiling - such as the fat and protein content and composition. If issues with milk quality persist, testing different cleaning products or methods that effectively reduce contamination can also be done. Additionally, the water that Geenland uses could be looked at as a possible contamination source, and so could the treatment of the animals there. Additionally, since Blackbelly sheep milk is not a culturally popular drink - it could be interesting to find a way to promote it and its health benefits amongst local populations or amongst the tourism sectors.

10. Acknowledgements

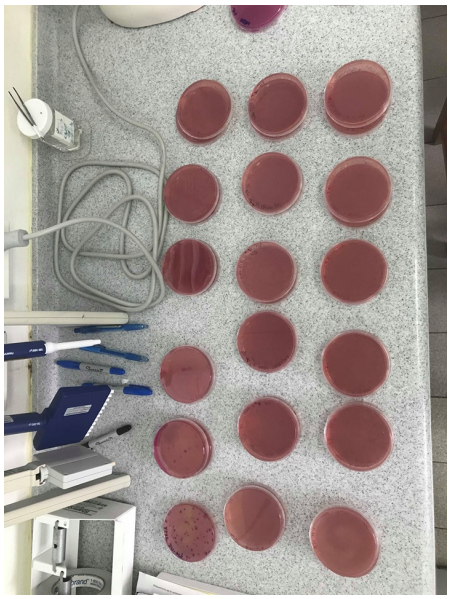
We would like to thank John Vaughn and Debra Rollins of the Greenland Livestock Research Station of the Ministry of Agriculture, Food, Fisheries and Water Resource Management for helping us conceptualize this project and for providing us with milk samples and cultural information about milk in Barbados. We would like to thank Corey Lowe, a dairy technician at Greenland, who collected all of the milk samples and walked us through the procedures Greenland uses to clean its equipment. We also extend a big thank you to David Elcock, Pamela Whitehall, Kerrilyn Pilgrim, and Maxine Morris of the Ministry of Agriculture's Veterinary Services Laboratory for allowing us to use their laboratory to conduct our coliform and salmonella testing and for giving us the knowledge of how to carry out those procedures. Thank you to David Elcock, the coordinator of the Veterinary Services Lab, for allowing us to use the facility and for coordinating the required permission for us to work there.

11. Appendix

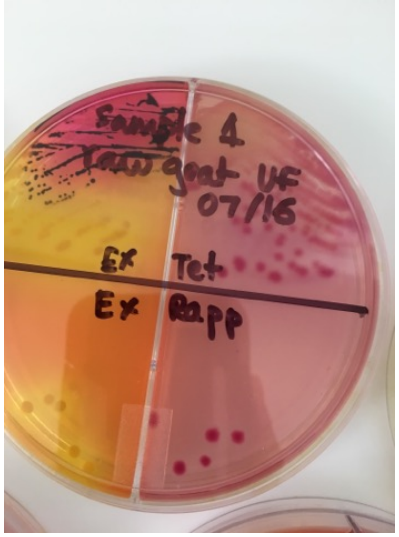
1a. VRBA enrichment media showing growth of coliform colonies in agar. Colonies are characterised by pink regular circles surrounded by a thin fuzzy pink halo.



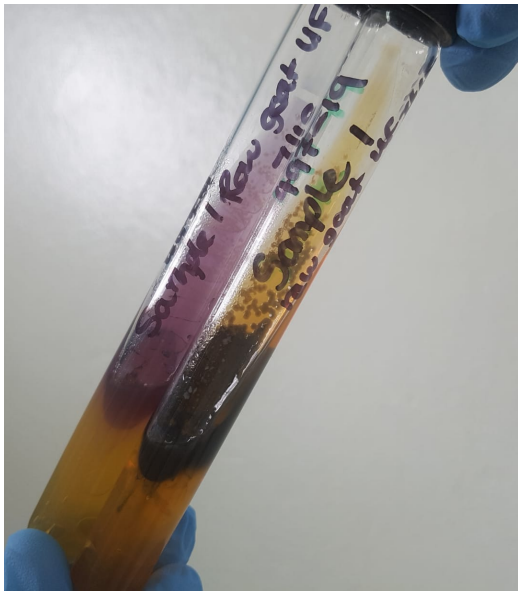
1b. Petri dishes grouped according to dilution and milk condition before counting colonies of coliforms.



1c. Petri dish with Rappaport-Vassiliadis (RV) and tetrathionate (TT) media to test for the presence of salmonella. On the top left quadrant, a black striated colony indicated that Salmonella spp. may be present in the raw goat unfiltered sample from Round 3 of testing. The Food and Vet services lab executed another test to confirm if the colonies were Salmonella. Results were negative; they may, however, indicate the presence of a Citrobacter species of an H2S producing E.coli. Additionally, coliforms grew on these plates as well.



2a. A negative salmonella reaction for the raw goat unfiltered sample taken on July16th 2019.



2b.

The positive control reaction and coloration of Salmonella - notably different from the negative reaction above in that the purple tube has no yellow butt like the tube above and in that there is pink in the second tube while there is no pink in the same tube above.

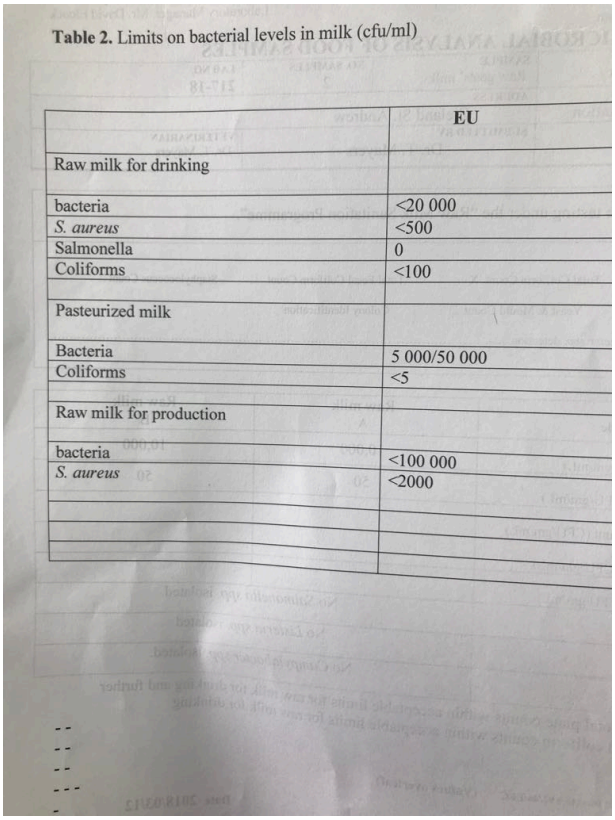


Table 2. Limits on bacterial levels in milk (cfu/ml)

	EU
Raw milk for drinking	
bacteria	<20 000
S. aureus	<500
Salmonella	0
Coliforms	<100
Pasteurized milk	
Bacteria	5 000/50 000
Coliforms	<5
Raw milk for production	
bacteria	<100 000
S. aureus	<2000

Table depicting the EU standard levels of acceptable bacterial profiles used by the Barbados Ministry of Agriculture's Veterinary Services Laboratory

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