**THESIS**

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# Food safety risks of raw and unpasteurised milk

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## 1. List of abbreviations

TB – Tuberculosis cause by *Mycobacterium spp.*

SCC – Somatic Cell Count; amount of leukocytes present in the milk in response to pathogens

HTST – High Temperature Short Time

UHT – Ultra High Temperature

SPC – Standard Plate Count

NTM – Non Tuberculous Mycobacteria

PCR – Polymerase Chain Reaction

PRA – Restriction Pattern analysis

EHEC – Enterohemorrhagic *E. coli*

VTEC – Verotoxin producing *E. coli*

BHI – Brain heart infusion

MLVA – Multiple locus variable number tandem repeat Assay

CC – Coliform Count

## 2. Introduction

Milk consumption has been part of human nutrition from the very beginnings of human civilization, as part of the change from a hunter-gatherer system of human society to a predominantly agriculture based system, this was due to the fact that humans eventually settled into communities instead of nomadic tribes and needed a stable source of food, such as animal by products. Before Pasteurisation and different heat treatments of milk came about it was consumed fresh, at the time the benefits and risks of consuming such milk was not known and thus many problems arose. Today with the help of science, with the discovery of pasteurisation and with better recording of data we can come to certain conclusions on the risks presented in unpasteurised milk and the products from such.

Although today with pasteurisation the amount of foodborne pathogens have been reduced, their presence are still significant in everyday life, especially on a farm environment as farmers and milk handlers still consume raw milk.

Today, due to the demand for longer shelf life, wider distribution to the public and the increase in trade throughout the world, heat treatment is necessary, which begs the question: Is the consumption of unpasteurised milk still practiced? Despite there being limited evidence to whether raw milk has enhanced nutrient properties and taste, people still consume in spite of the evidence to claim that it is a high risk of infection (Oliver et al., 2009).

History of pasteurisation and heat treatment

In 1864 Louis Pasteur discovered that heating milk to just below the boiling point reduced the number of pathogens. Eventually this developed into several different methods of treating milk with the aim of destroying harmful pathogens and removing any spoilage agents, for example HTST treated requires a temperature of 72$^{o}$C for 15 seconds as a continuous process, or 63$^{o}$C for 30 minutes, known as batch pasteurisation (Jordan and Hunt, 2012). UHT milk on the other hand is heated to 135$^{o}$C for 15 minutes in order to deactivate any bacterial spores

## 3. Literature review

Myths surrounding raw milk benefits

Regulation (EC) no. 853/2004 states that "milk produced by the secretion of the mammary gland of farmed animals that has not been heated to more than 40°C or has undergone any treatment of equivalent effect" as the definition of raw milk (Castro et al., 2017). According to scientific research provided on the Food and Drug Administration of the USA, these are the facts that come to light: Raw milk was said to have been able to cure asthma and allergies, however when experimented on animal models (Poulsen et al., 1987) and human clinical experiments (Host and Samuelsson, 1988) it was shown that this was not the case, and raw milk does not in fact stimulate any sort of anaphylactic action.

Raw milk has no scientific proof on curing osteoporosis, as there have been studies conducted on the calcium content of both raw and pasteurised milk, but no difference existed (Rolls and Porter, 1973)

Raw milk has been said to contain probiotic bacteria, however these must be non-pathogenic and must be of human origin (Teitel and Walker, 2000), and there are no such bacteria in milk as milk is normally sterile when secreted in the mammary gland. There have been reports of bifidobacter present in the milk, and this is a bacterium that is present in the gut of humans and animals (Arunchalam, 1999), but this would indicate fecal contamination rather than probiotic effects (Bereens et al., 2000).

Milk is quite active with proteases such as plasmin and somatic cell proteases (Kelly and McSweeney, 2003). However, increased amounts of these enzymes are usually correlated with mastitis (Verdi et al., 1987). Exogenous bacterial proteases could also be present in the milk. However, there is no proven physiological role to these enzymes and are normally digested in the stomach due to the acid content.

Bovine milk contains LPL, and a few other lipases from organisms and somatic cells (which are significant in mastitis) from unhygienic environments (Weihrauch, 1988). Again, these enzymes have no proven beneficial effects and therefore no nutritional value.

The amount of protein available in raw milk does not differ to pasteurised milk (Andersson and Oste, 1995), as well as digestibility of protein (Carbonaro et al., 1996).

Milk fat content also does not appear to change under pasteurisation (Rolls and Porter, 1973).

Milk minerals remain stable under pasteurisation and the amount is not altered significantly at all (Rolls and Porter, 1973).

Milk contains a wide arrange of vitamins, and pasteurisation has been shown to have no particular effect or degradation in them (Bendicho et al., 2002).

Raw milk has been said to contain many natural antibiotic substances such as xanthine oxidase, lactoferrin, lactoperoxidase etc. but there is no evidence to substantiate the claim that these substances truly kill pathogens. In fact, studies show that elevation in these substances actually indicates the presence of mastitis as the cow's immune system reacts to pathogenic organisms and their metabolites (Chaneton et al., 2008; Schmitz et al., 2004; Farkye, 2003).

There is another myth that raw milk contains a higher concentration of an enzyme called nisin, which has antimicrobial properties (although is not very effective against gram negative bacteria, fungi and viruses). This is produced by an overgrowth of *Lactococcus* bacteria (Arauz et al., 2009), but has no effect when refrigerated at the temperatures used to preserve milk. Thus, if detected, means the milk was not stored properly.

Raw milk under the Hazard Analysis Critical Control Point procedures (HACCP) is not safe either, as there is no ability to pinpoint specific parts of the farm where one can determine hazards for raw milk, since it contains so many different pathogenic agents it is too unpredictable to control (Cullor, 1997; Sperber, 2005).

Major risks involved in consuming raw milk

With all that being said, the major risks present in raw milk are innumerable. In a study conducted by Zeinhom and Abdel-Latef and published in 2014, in an attempt to identify the major public health riks associated with drinking raw milk, by culturing 150 samples of farm and market based raw milk along with milk handler stool samples and hand swabs and using specific agar for culturing pathogenic strains of certain bacteria had found that the most common organism was the strain O157:H7 *Escherichia coli*, Along with members of *Salmonella*, *Yersinia* and *Aeromonas* spp (Zeinhom et al., 2014). The study had concluded that the presence of these organisms is suggestive of fecal contamination (as they are indicators of such) and that raw milk was microbially unsatisfactory to consume without further processing.

A study conducted by Blaser et al. Published in 1979, followed a family of five people, four

of which were known to have been consuming raw milk from their dairy cattle, in order to find correlation with the presence of *Campylobacter fetus ss jejuni*. Three of the family members had started showing clinical signs and were sampled serologically, to which a four times increase in antibody specific to *Campylobacter* was found (Blaser et al., 1979). The study had shown that there was a correlation between infection and consumption of raw milk, as an index patient had been used as a visitor to the farm and all other factors were ruled out.

*Mycobacterium* complex is also very present in raw milk and proves a significant threat to human health. The disease is very difficult to detect and is subject to many tests to detect non-clinical animals (Di Pinto et al., 2006)

*Coxiella burnetti* is the causative agent of Q fever and is very well-known to be associated with raw milk. The disease in acute form usually presents itself as infective endocarditis (Shah et al.,2015).

*Brucella*, one of the more well-known pathogens present in raw milk (although more frequently associated with drinking raw goat or sheep milk), causes severe systematic disease in humans and is subject to many eradication programmes all over the world (Oliver, 2009).

*Salmonella* spp. Is one of the more frequently occurring pathogens and one of the more preventable ones with pasteurisation. Several serovars are shed intermittently by carrier cows, and may cause bloody diarrhea and in severe cases systemic disease (Poppe, 2011)

Milk quality

Milk quality is normally quantified using several indicators found in the milk itself. SPC is one method used to quantify milk hygiene by measuring the amount of “aerobic bacteria present in raw milk” (Oliver et al., 2009). This technique is used as a way to check the health of the herd, udder and milk hygiene practices and as a way to check mastitis prophylactic measures. According to Oliver et al. A low SPC count is “<5000 colony forming units [CFU] of bacteria=mL” and a high count would be indicative that bacteria found their way into the milk through specific routes (Oliver et al., 2009).

Good quality milk can is usually identified using SCC as an indicator for quality. In Europe the maximum limit for good quality milk is 400,000/mL (National milk producers federation, 2007). According to Oliver et al.: “high milk SCC is associated with a higher incidence of antibiotic residues in milk, and the presence of pathogenic organisms and toxins”. SCC is a

count of inflammatory cells present in milk.

Milk quality also dictates that no pathogenic bacteria such as *Campylobacter* spp., *Escherichia coli*, *Listeria monocytogenes*, *Salmonella*, *Staphylococcus aureus*, *Mycobacterium bovis, Brucella spp., Coxiella burnetti, Yersinia enterocolitica* (Oliver et al., 2009). These pathogens may also be present as the animals' normal flora and thus may also contaminate meat and milk products while slaughtering. Some of them may also be present in soil through improperly processed fertilizer (Oliver et al., 2009).

As discussed before, raw or unpasteurised milk presents itself as an abundant source of pathogenic agents which may cause very severe disease in people. Each risk factor will be discussed individually below accordingly.

*Mycobacterium* spp

Tuberculosis is one of the leading causes of deaths caused by infectious diseases in the world, with an estimated 1.7 billion people being infected, which is about a third of the human population, a number which is seemingly on the rise (Di Pinto et al., 2006). The members of the tuberculosis complex which cause disease in humans are known to be *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium microti, Mycobacterium africanum* and *Mycobacterium leprae.* They are characterised as being obligate aerobic bacteria which are facultatively intracellular preferring to replicate in macrophages, and have a tendency to infect oxygen rich tissues. They are split into Obligate pathogenic and facultatively pathogenic/saprophitic, the former are sensitive to heat and drying out and cannot reproduce outside the cell (Bolanos et al., 2017). TB as a disease is generally a chronic disease that takes place over a few weeks to several months and involves the formation of a primary complex which generally involves an organ system and its corresponding lymph nodes. Bovine TB is caused by *Mycobacterium bovis* and is transferrable to humans through unpasteurized milk, contaminated meat and aerosol infection (Bolanos et al., 2017). Although generally it is the obligate pathogenic *Mycobacterium* species that are relevant to raw milk, in recent years the facultative species have also come to significance especially in people who are immunocompromised or vulnerable to such infection.

In a public health context, the most important are *Mycobacterium bovis* and *tuberculosis*.

According to Bolanos et al.: “in Brazil, about 20-30% of cow’s milk is marketed without health inspection.” and goes on to emphasize the role of raw milk which creates potential risk of infection. *Mycobacteria* can remain viable in raw milk products as well, for instance they may remain viable in raw milk cheese and yoghurt for around 14 days (Bolanos et al., 2017). Extrapulmonary infections have also been noted in direct infections of *M. bovis* to humans (Bolanos et al., 2017).

People at highest risk of contracting bovine TB are generally farm personnel and veterinarians, especially in episodes of clinical TB, closed areas such as barns and keeping areas predispose this niche greatly (Di Pinto et al., 2006)

Diagnosing TB is generally done using a skin test and a purified protein derivate (Tuberculin) and the skin fold measured. This was noted as a slow process however, as is cultivating the bacteria on a medium due to their fastidious nature (Di Pinto et al., 2006). In a study done by Di Pinto et al. In 2006, an experiment was conducted to see if there was a quick and reliable way of detecting and identifying *Mycobacterium* species using a PCR technique used for food samples such as bovine milk. The study found that the PCR used in clinical sample application would not be adequate for food samples due to inhibitory products which would interfere with *Taq* polymerase reaction. This will allow for a better way of ensuring prophylactice measures in TB eradication programmes and monitoring non-EU derived milk.

NTM also serve a potential threat to immunocompromised individuals, especially those suffering from HIV/AIDS (Di Pinto et al., 2006). In Turkey, a study was done by Konuk et al. On 35 milk samples to isolate and identify mycobacterial strains present. The study found 15 acid fast rods which were taken from 28 of these samples (Konuk et al., 2007). The species were identified using PCR – PRA, and they were *Mycobacterium terrae, Mycobacterium kansassii, Mycobacterium hemophilum* and *Mycobacterium agri* (Konuk et al., 2007).

In Nigeria another study was conducted by Cadmus et al., which took 400 raw milk samples and identified, using isolation and molecular techniques, that *Mycobacterium bovis* and *Mycobacterium africanum* were present in 10% of the milk samples collected. This is significant as certain regions of Nigeria are still poverty stricken and as a result are still consuming raw milk, as bovine TB is endemic to Nigeria some prophalyctic measures need to be placed in order to better control the disease (Cadmus et al., 2010).

As TB is known more as a disease of the developing world, the consumption of raw milk still poses a severe risk and predisposition to TB disease caused by *Mycobacterium tuberculosis*

complex made of not only *M. bovis* but by NTM as well.

*Coxiella burnetti*

*Coxiella burnetti* is the causative agent of coxiellosis in animals and Q-fever in humans (Petruzzelli et al., 2013). It is an obligate intracellular bacterium that can be asymptomatic in humans, and can lead to abortions and periparturient problems in women (Guatteo et al., 2007). Although it has been found to be endemic in ruminants, and causes mostly reproductive disorders in them, it has also been found to be of limited public health impact. Q-fever can result in atypical pneumonia, hepatitis and retinal vasculitis, a more chronic form manifesting as endocarditis (Petruzzelli et al., 2013). Since *C. burnetti* can be found in ruminants as its reservoir, these animals can go one without clinical manifestations, and can contaminate milk from loci of infection during parturition or lactation, shedding is, however, periodic in these animals through milk. The major source of infection, however, is inhalation of aerosols (Petruzzelli et al., 2013).

In a study done in Italy, 2013, Petruzzelli et al. Tried to assess the prevalence of *C. burnett* using PCR techniques. The study found 21 positive samples out of 130 bulk milk tank samples, indicating a prevalence of 27%.

A study conducted by Guatteo et al. Tried to establish the prevalence of *C. burnetti* in bulk milk tank milk using PCR techniques in relation to level of shedding present per cow. 14 dairy herds were studied participating in a "Q-fever control programme" in france which had at least one positive PCR sample from vaginal mucous or bulk milk tank samples, and a total of 1522 cows participated with a total sample size of 37 bulk milk tank samples (Guatteo et al., 2007). The results yielded 6 negative samples, whilst the "mean within-herd prevalence of milk shedder cows" was found to be 18.5%, whilst heavy milk shedder mean was 32.3%.

The studies shown above clearly indicated a high presence of *C. burnetti* present in raw milk collected and provides yet another source indicative that consuming raw milk, even in developed areas of the world is still a dangerous practice with possibly devastating repercussions to the consumer.

*Escherichia coli*

This bacterium has been accepted worldwide as an indicator for fecal contamination as it is a member of the normal flora in the intestines of endothermic animals (Oksuz et al., 2004). The bacterium itself is responsible for many diseases, due to their vast serotypic variation, they are capable of enterhemorrhagic diseases caused by EHEC and VTEC of the serotypes O157:H7 (Okzuz et al., 2004).

Since dairy cattle present as asymptomatic carriers, the routes of infection may be yielded through contamination at the slaughterhouse and on-site contamination in the farm from raw milk and bulk tank milk (Oksuz et al.,2004). Hence *E. coli* remains a very important threat of undercooked meat as well.

Another disease caused by *E. coli* in humans is hemolytic uremic syndrome (HUS). This disease is caused by serotype O26, and has been identified as 2 different H antigens (Allerberger et al., 2003).

A study was done in 2005 by Arimi et al. To study the risks associated with *E. coli* serotype O157:H7 and drinking unpasteurized milk in consumer and milk market agents in Nairobi city and Nakuru town, Kenya. The study sampled consumers within 120 census clusters, to which "30 clusters were randomly selected (Arimi et al., 2005). Within each selected cluster, 7 households were randomly chosen for a total of 210 households and milk samples obtained". Market agencies were sampled according to location and type, each having milking as an important activity in four districts (Arimi et al., 2005). All dairy related retailers and traders were sampled in a selected area up to 30. The milk samples collected from each area was then cultured and purified to collect the bacteria, and then run through a modified technique of PCR to identify verotoxin production (Arimi et al., 2005).

The prevalence of the bacteria was also taken into account between consumers and market agents in dry and wet seasons, in order to obtain a better and more accurate result, and not leave it to specific season (Arimi et al., 2005).

According to the results of the study, many households did boil the milk before drinking it with their tea, however the study found that a certain area in Nakuru rural did in fact consume it raw (Arimi et al., 2005).

The study found that the prevalence of the *E. coli* strain was 0.8%, this is quite low considering the other risks related to consuming raw milk but the real problem lies in its

virulence, which the study mentions the level of infection can be very high, as is the pathogenicity, and can lead to some serious damage such as kidney failure in individuals (Arimi et al., 2005).

Another study done by Allerberger et al. In 2003 follows two cases of an 11-month-old boy and 28-month-old girl, both of whom were confirmed to have consumed raw milk. The study used the stool samples of the children, the bacteria were then cultured and isolated, and using a technique called Pulse Field Gel Electrophoresis (PFGE), the strain was identified (Allerberger et al., 2003). The researchers then collected samples from the cows which produced the milk and tested for the bacteria and It was found that the strains were indistinguishable (Allerberger et al., 2003). The study also inquired into the epidemiology of the case and found the children had been staying in a hotel in Austria were raw milk was being served at the buffet, and the children had consumed it without the knowledge of their parents. As stated in the study, Austrian law does not allow raw milk to be served unless it has been boiled (Allerberger et al., 2003). This case is a clear example of how dangerous a situation can get without prior treatment of the milk.

In another study done by Oksuz et al., 100 raw milk samples and 50 pickled cheese samples were collected randomly in a village in Turkey. The researchers took the samples and cultured for *E. coli*, isolated using special agar and determined strains using serological methods. It was found that only one sample out of 100 of raw milk was positive for *E. coli* O157 (Oksuz et al.,2004). This does not mean a reduced risk however, as cattle are still reservoirs and may spread the bacterium, and it takes a long time for it to disappear from communal waters (Oksuz et al.,2004).

In 2011 a study was done in Lazio, Italy, by Marozzi et al. To check the prevalence of which strains were present in the region. The researchers cultured 161 samples specifically for *E. coli* and using molecular techniques to identify the strain. Only two O157 strains were identified and none were shiga toxin producing, and a 15.1% prevalence of shiga 1 and shiga 2 toxin producing strains (Marozzi et al., 2011).

The studies listed above show that there is a great need for surveillance and treatment of milk and milk products, along with a suitable control programme and educating farmers and consumers about the risks involved.

*Listeria monocytogenes*

*Listeria* is an important pathogen associated with meningitis, meningoencephalitis and abortion found typically in the soil and in the gut of farm animals (Dalzini et al., 2016). It is also found as a contaminant in bulk milk tanks and raw milk vending machines. It is a resistant bacterium capable of surviving refrigeration, high salt environments and soil environments (Ryser, 2007). The bacterium also makes up some of the contaminants present in food processing plants, which makes it much more difficult to eradicate (Oliver et al., 2005). *Listeria* is also part of the microbiological criteria for foodstuffs, which includes criteria for *Listeria* in ready to eat foods (Regulation (EC) No. 2073/2005), which means foods already present at retailers must not exceed 100 cfu/g, along with producers having to demonstrate that there is no bacteria present in 25g of product before it has departed from the producing facility (Castro et al., 2017).

That being said, looking at a study by Castro et al. Compiled in 2017 in Finland, the researchers studied the *Listeria* growth rate and content present in packaged raw milk. According to the study, consumers seem to prefer raw milk due to the sensory difference to pasteurised milk, and to support local farming practices (Castro et al., 2017). The samples were collected over the period of November 2013 and September 2015, and these included: raw milk bottles, bulk tank milk, in-line milk socks and the contents of the environment present on the packaging area on which the research was done (in order to compare results). The samples included 105 bottles (1 L from a retail store) of raw cow milk, 115 (of 50 ml) bulk tank milk, 23 in line milk socks and 50 environmental samples from the packaging area. The bacterium was then isolated, identified using PFGE and then serotypes identified by PCR, the researchers then isolated the bacteria at different temperatures from different samples (Castro et al., 2017).

The researchers found that the occurrence in bottled raw milk was 6.7%, for bulk tank milk 3.5%, for in line milk socks 57% and 8.0% for environmental samples (Castro et al., 2017). The study thus concluded that the bottled raw milk and bulk milk tank was the major source of contamination, where the initial low levels of the bacterium in packaging which was then stored at consumer temperature was the catalyst for the growth of *Listeria*.

In 2015 a study was done by Dalzini et al. In northern Italy to survey the prevalence and seasonal variability of *Listeria*. The researchers took 8716 cows from 942 farms in the period

of January 2010 to September 2013. This included 5897 bulk tank milk samples intended for cheese making and 2819 samples intended for retailing use. A prevalence of 1.66% was detected in the bulk milk samples, but this was due to tank contamination rather than raw milk intended for vending machine, according to the researchers (Dalzini et al., 2015). The prevalence for raw milk in vending machines was around 0.50%. The researchers concluded that the prevalence being so low could indicate good farm hygiene practices, and these studies can be used as a quantitative assessment of listeriosis connected with raw milk and its products (Dalzini et al., 2015).

Although the prevalence was shown to be low in both articles, the threat of *Listeria* still remains significant, especially to vulnerable individuals such as pregnant women, children and the elderly, and thus the need for good farm hygiene and food hygiene practices are imperative to the prevention and eradication of the disease (Dalzini et al., 2015; Oliver et al., 2005).

*Campylobacter* spp.

*Campylobacter* spp. Are typically isolated from dairy cattle as they act as the reservoir for the bacterium (Del collo et al., 2017). It is a microaerophilic, spiral shaped, gram negative bacterium and is one of the leading causes for food borne diarrhea in the USA (Del collo et al., 2017). However, it is a self-limiting infection and can resolve within weeks, rarely needing antibiotic treatment, but may result in permanent damage in Guillain Barre syndrome (Del collo et al., 2017). *Campylobacter* infections may be typically caused by *Campylobacter jejuni*, *lari* and *coli*, and are very commonly associated with raw poultry meat, but have commonly been isolated from bulk milk tank samples (Del collo et al., 2017). Although dairy cattle may be regarded as intermittent shedders, a high concentration of bacteria may be shed during these times (Del collo et al., 2017). *Campylobacter* have also been implicated in mastitis, however this is not the major source of raw milk contamination, rather it is from fecal contamination that results in the presence of the bacterium in bulk milk tanks (Oliver et al., 2005). Campylobacteriosis includes clinical signs such as watery diarrhea, abdominal pain, fever and malaise, which could result in a severe neurological disease known as Guillain Barre syndrome, which causes a flaccid type paralysis (El-Zamkan and Hameed, 2016).

A study was done recently on the prevalence of *Campylobacter* spp. In bulk milk tank and

filters in the USA by Del collo et al. And the study was split into 17 states, which represent around 80.5% of dairy herds and 81.5% of dairy cows in the USA (Del collo et al., 2017), classified into small, medium and large herds accordingly (Del collo et al., 2017). The samples collected were from bulk tank milk and milk filters, collected as hygienically as possible, and was ensured that each sample represented one milking cycle (Del collo et al., 2017). the samples were: 234 from bulk tank milk, 231 from filters from the 17 states, and 231 paired samples from dairy operations were obtained (Del collo et al., 2017) The samples were then enriched, DNA extracted from the enriched samples and placed through real time PCR to identify prevalence or isolated on selective agar and then extracted DNA was placed through real time PCR to identify *Campylobacter*, and multiplex PCR to identify species, which was then run through and antibiotic susceptibility test and through PFGE. The antibiotic resistance was done using an automated microdilution pattern to test along a pattern of 9 antibiotics (Del collo et al., 2017). The results obtained from the PCR from the bulk tank milk and filters yielded an average prevalence of 24.9%. The study found that the amount of *Campylobacter* spp. Was more frequent in larger farms than in smaller dairy operations (Del collo et al., 2017). The study concluded that the consumption of raw milk exposes the consumer to a risk "150 times greater than the incidence due to pasteurized milk" (Langer et al., 2012).

Another study conducted in Italy by Serraino et al. In 2013 followed research as to the presence of *Campylobacter* in herds of dairy cattle and in a water buffalo farm. The sampling was done over 13 dairy cattle farms which were authorised to sell raw cow milk and one water buffalo farm with 90 lactating animals (Serraino et al., 2013). A total of 196 in line milk filters were collected by the researchers, and were collected specifically after milking. The samples were then enriched, DNA extracted and isolated for *Campylobacter*. The study found a prevalence of 35.7% of the farms sampled being positive for *Campylobacter* with 8.1% of samples being positive and the species were *C. jejuni, C. hyointestinalis ssp hyointestinalis, C. concisus* and *C. fetus ssp fetus* (Serraino et al., 2013).

Another study done in Italy was conducted for the presence of *Campylobacter* present in vending machines, assessing quantitative risk associated with consuming such milk, which was conducted by Giacometti et al. In 2015. The study was done to update a risk assessment model using several variables over a 4-year period, including exposure, prevalence of the bacterium in the vending machines (the study found at least one positive sample for *C. jejuni*

in each vending machine), time and temperature of the vending machine milk, doses of the bacterium and dose response. The study concluded that consumer behaviour requires further monitoring but with quantitative risk assessment can give a good indication of trends in epidemiological data (Giacommeti et al., 2015).

*Brucella*

Brucellosis is a worldwide disease which causes abortion and reproductive problems in animals and may be transmitted to humans via consumption of raw milk and its products (Ning et al., 2012). It is known to be easily transmitted among animals, however human to human transmission is not typical, rather contact with infected animal fluids is the norm (Ning et al., 2012). The bacterium is normally identified via cultivation, however this requires a large amount of time to finish and predisposes its users to occupational risks of being infected (Ning et al., 2012).

In some countries, for example Turkey, Brucellosis is endemic (Kaynak-Onurdag et al., 2016), and can be treated as a preventative measure using a live-attenuated vaccine, the problem presented with this is that PCR methods cannot distinguish between vaccine and actual bacteria (Kaynak-Onurdag et al., 2016).

In one study conducted in Edirne, Turkey, which is a pilot region for vaccines in the country, carried out by Kaynak-Onurdag et al., the research was done to check the raw milk of the region for *Brucella* by comparing isolation methods and molecular methods. The sampling was done on 99 cows from 12 different farms in 5 villages in the area, collecting the samples straight after milking (Kaynak-Onurdag et al., 2016). Then the samples were cultured for *Brucella* and then suspect colonies subjected to biochemical tests in order to confirm the bacterium. Real time quantitative PCR was then used and compared with the bacteriological analysis, the positive PCR samples were then subjected to special Primers which the researchers had designed in order to identify the vaccine from the virulent strain (Kaynak-Onurdag et al., 2016). However, the bacterium was only isolated in two of the samples and the amount of PCR positives were considered mostly to be false positives, thus the study concluded that both the virulent and vaccine strains still somehow need to be distinguished from each other (Kaynak-Onurdag et al., 2016).

In another study done by Ning et al. The research was carried out as PCR on milk samples

rather than blood serum (Ning et al., 2012). 816 cows were used for the study and each one was separated into 3 different groups according to their *Brucella* infection status (Ning et al., 2012). A questionnaire was also issued to the farmers to see how far *Brucella* had impacted the farms in question as to abortions, vaccinations and herd size.

The study had concluded its results were less accurate than one done by Serum Agglutination Test for several reasons: The bacterium is shed intermittently in the milk, differences in infection stages and PCR sensitivity differences (Ning et al., 2012). However, despite these considerations, the researchers still insist that milk PCR be used as a screening method alternative to SAT, since it is cheaper and quicker (Ning et al., 2012).

With that being said, Brucellosis is a very unpredictable disease, and seeing as it is very highly associated from zoonotic risk, along with still fluctuating diagnostic screening methods, makes it a very high pathogenic risk from unpasteurized milk.

*Staphylococcus aureus*

*Staphylococcus aureus* is characterised as being a gram-positive bacterium, and is one of the top foodborne disease-causing bacteria in the world (Jamali et al., 2015). This bacterium is typically associated with mastitis and is the leading cause of intramammary bacterial infections which lead to major economic loss (Hein et al., 2005). *S. aureus* is also a major pathogen involved in subclinical mastitis, and may be able to produce a heat stable enterotoxin which is toxic to humans, causing vomiting, diarrhea and abdominal pain (Mehmeti et al., 2015). The presence of *S. aureus* is typically indicative of good quality farm hygiene and milking technique, especially involved with bulk milk collection (Mehmeti et al., 2015). Unfortunately, due to repeated use of penicillins, this bacterium has become immune to the antimicrobial effect caused by this drug, this is thanks to an enzyme it produces called beta lactamase (Jamali et al., 2015).

A study conducted in 2015 was done by Jamali et al. In a province of Iran to test for the prevalence and antimicrobial resistance of the bacterium. The samples were collected from retail sellers of raw milk, totalling 2650 samples split between raw cow milk, ovine milk, raw milk cheeses and “kashk” (Jamali et al., 2015). The samples were cultured for *S. aureus* and tested for antimicrobial susceptibility, along with PCR to detect the antimicrobial genes (Jamali et al. 2015). Of all the samples, 15.7% of raw cow milk, 10.9% ovine milk, 10.9%

cheese and 11.5% kashk were confirmed to be contaminated with *S. aureus,* along with this antimicrobial resistance was found to be such: 36.3% of samples were found to be resistant to one antibiotic, 46.6% were found to be resistant to 2 antibiotics and 12.8% were resistant to greater than 2 antibiotics (Jamali et al., 2015). These findings are quite disconcerting as antimicrobial resistance is on the rise and the development of these strains of bacteria make it harder and harder to combat disease, it is thus important to recognise the threats and treat raw milk accordingly.

Another study conducted in Kosovo by Mehmeti et al. Was done to monitor the prevalence of *S. aureus* present in raw milk, as farms are still on quite a small scale in the country and farmers still live off the raw milk, selling the rest off to dairy companies (Mehmeti et al., 2017). The samples were collected from the bulk tank milk (603) from 221 farms around the country, and were cultured and SPC was done, along with PCR, biochemical tests and DNA sequencing. The researchers had found that only 7% of the farms tested actually fit to the standards of the EU, and that it was the larger herds that were more hygienically viable (Mehmeti et al., 2017). The researchers had concluded that in order to fit into proper hygienic standards a drastic change must be done with cleaning practices on the farm and training veterinary professionals for more strict control.

In a study published in 2005, conducted by Hein et al. The researchers analysed samples of bovine and caprine raw milk in order to quantify *S. aureus* concentrations using Real Time PCR. 80 cow and 107 goat milk samples were collected in certain regions of Norway, these were then cultured and subjected to biochemical tests in order to identify the presence of the bacterium (Hein et al., 2005). The results presented a 95% occurrence in bovine milk with PCR, however many of these were false positives, thus the study concluded that Real Time PCR may still be used as a rapid alternative to microbiological methods.

The studies accounted for bring to light some problems present in the developing world as well as the developed world, where there is a serious need for more responsible antimicrobial use and surveillance along with mastitis control.

*Salmonella* spp.

Salmonellosis from cattle is typically caused by *Salmonella enterica* subspecies *enterica* serovar Dublin (Vignaud et al., 2017) and can be found worldwide as a major cause of

foodborne illness in humans. Cattle may also show clinical signs through diarrhea, pneumonia and high mortality in calves, and can be problematic due to its ability to resist environmental conditions, stays active in meat and milk and may remain in cattle as they act as carriers. (Vignaud et al., 2017). Cows tend to shed the bacterium especially in febrile episodes of the acute form of the disease, particularly post calving, and may contaminate any food products via fecal contamination (Poppe, 2011). In humans, particularly in regions where the practice of raw milk consumption along with its products, are susceptible to outbreaks of the disease which includes symptoms such as fever, vomiting, bloody diarrhea and abdominal cramps (Poppe, 2011). It is also important to take into consideration certain groups of people with higher risk of contracting the disease, such as those with underlying illnesses (HIV/AIDS for example), age related (the elderly and infants being more susceptible due to their fragile immune system), use of drugs and any previous treatment on the gastrointestinal tract.

A study published in 2017 by Vignaud et al. Was conducted in 2012 in order to investigate a suspicious rise in the amount of *S.* Dublin cases in France. The researchers located the source of the outbreak via epidemiological inquiry, which led them to associate the cases to consumption of raw milk cheeses of the brand "Saint Nectaire" (Vignaud et al., 2017). Then, by utilising a new molecular technique known as MLVA, the study was able to identify strains and epidemiologically characterise them accordingly in cases of outbreaks or sporadic occurrences (Vignaud et al., 2017). Since then the technique of MLVA had been in use and has helped studying the epidemiology of the disease more carefully (Vignaud et al., 2017).

Another study published in 2017 conducted by Ding et al. Goes into detail on multiplex Real Time PCR techniques with a pre-enrichment for *S. aureus, L. monocytogenes* and *Salmonella* in order to highlight some of the risks involved present in raw milk, however this section will focus on *Salmonella*. As some countries are very strict on their limits of bacterial detection, pre-enrichment is almost a must as there is almost no margin for error, hence this study would be useful in a diagnostic and screening sense as an enhanced pre-enrichment would allow for better detection of the bacteria (Ding et al., 2017). The farm environment as well as pasture environment was sampled, totalling 46 farms sampled (Ding et al., 2017). The pre-enrichment broth used was BHI, which allows for greater bacterial density (Ding et al., 2017). After pre-enrichment, DNA was extracted and run through multiplex Real Time PCR, the result of which, however, yielded no *Salmonella* positives (Ding et al., 2017). Nonetheless this method seems to be a useful tool in future screening and diagnostic methods in order to detect more

accurately pathogens associated with raw milk.

Control measures for *Salmonella* in milk has been greatly successful over the years, naturally pasteurisation is still the best way of getting rid of these pathogens, however people still believing in the nutritional benefits of raw milk along with farmers and farm personnel are still greatly at risk and must be informed and educated of the dangers associated with it (Poppe, 2011).

*Yersinia* spp.

*Yersinia* spp. Are a group of gram negative bacteria of the family Enterbacteriaecae and are considered some of the most important food related disease causative agents (Jamali et al., 2015), most commonly known are *Yersinia pestis, Yersinia enterocolitica* and *Yersinia pseudotuberculosis* (Jamali et al., 2014). *Yersinia enterocolitica* is most relevant for human disease as it is transferred through consumption of raw pork, raw milk and infected water (Jamali et al., 2014). Whilst *Yersinia pseudotuberculosis* is more frequently encountered through fecal contamination of milk and meat, and can be sub clinical for 7-18 days (Parn et al., 2015. Typical symptoms may range from vomiting, diarrhea, abdominal pain, fever and in extereme cases "erythema nodosus and reactive arthritis" (Parn et al., 2015).

A study published in 2015 by Parn et al. Follows an outbreak of 55 individuals having been infected by *Yersinia pseudotuberculosis* between February and April 2014. The researchers notified public health doctors to watch out for Yersiniosis like signs in patients and to collect stool or detect antibodies (Parn et al., 2015). The outbreak was traced back to a farm that had sold raw milk to the infected individuals, and sampling was done on that farm for cow feces and milk samples (Parn et al., 2015). The bacterium was then cultured and then isolated and serotyping was done (Parn et al., 2015). Some of the patients were also asked questions, and 93 cases were identified as positives from different age ranges (Parn et al., 2015). The outbreak started on 10$^{th}$ of February and eventually reached a maximum in March and clinical signs lasted 14 days (Parn et al., 2015). Only 3 cases developed erythema nodosus, only 4 were sent to hospital, and the correlation between those who drank raw milk versus those who didnt and illness was very strongly skewed towards those who did (Parn et al., 2015). The investigation of the milk on farm concluded that packaging raw milk was being done and distribution started on the 10$^{th}$ of February, the same day the outbreak started (Parn et al.,

2015). However, the milk still had a warning label stating that it should be heated first as it contains many dangerous pathogens (Parn et al., 2015). PFGE results indicated that 8 samples had the same profile. The research concluded that the control measures for this retail of raw milk are not sufficient and that better surveillance methods need to be taken into consideration along with better education to individuals wishing to consume raw milk.

Another study by Jamali et al. Published in 2014 was done between 2008 and 2010 to monitor the prevalence and antimicrobial resistance of the bacterium in Iran. 446 bulk tank milk samples were taken from different species of lactating animals in a region in Iran (240 from cows, 165 sheep, and 41 goat) and then they were cultured, isolated and biotyped (Jamali et al., 2014). The virulence genes were then tested for, along with antibiotic susceptibility (Jamali et al., 2014). *Yersinia* spp was detected in 29 of the samples, 23 of them being from bovine, 5 from ovine and 1 from caprine animals, with 65.5% of the samples being identified as *Yersinia enterocolitica* (Jamali et al., 2014). The study also showed that the bacteria had the highest resistance to tetracyclines, which is a disconcerting notion as antibiotic resistance is a growing problem today (Jamali et al., 2014). These studies concluded that with the presence of such pathogenic bacteria in raw milk, and with the issue of outbreaks happening unknowingly to consumers, drinking raw milk presents itself as a very dangerous hazard and may result in very painful consequences.

Coliform bacteria

Coliforms are gram negative bacteria bacteria which can be found in fecal matter of animals and in the environment (Pantoja et al., 2011). Apart from the obvious pathogenic bacteria mentioned previously, there is also a need for identifying those bacteria responsible for contamination from the environment as well, which may cause problems with milk quality (Piepers et al., 2014). When milk is deemed satisfactory, it generally means the coliforms present are eradicated through pasteurisation, however when the process is done wrongly, the presence of coliforms could indicate this (Pantoja et al., 2011). Coliform bacteria include the likes of *Klebsiella* and *Citrobacter,* which may be pathogenic to humans and may grow rapidly in storage conditions (Pantoja et al., 2011).
A study published in 2011, done by Pantoja et al., was conducted in a 10-week period to

utilise a CC method in order to identify coliforms present in bulk milk tanks of farmers in Wisconsin, USA. The in-line milk drips were sampled for milk, and swabs of the milking equipment was taken, along with teat swabs of the cows themselves (Pantoja et al., 2011). Bacteriology using specific culture media for coliforms and SCC was done to observe milk hygiene (Pantoja et al., 2011). The average number of cows present split between farm samples was 1205, and each farm milked by parlors and taken straight to processing (Pantoja et al., 2011). Most farms were technologically advanced, and had milkings 3 times in a day, and most farms had their equipment inspected at least twice a year (Pantoja et al., 2011).

The resulting CC varied from farm to farm ranging from 5 to 1198 CFU/mL, and the study found most considerably that CC increased when washing failures occurred, rather than being affected by temperature or environmental problems.

In another study published in 2014 in Flanders, Belgium, done by Piepers et al., the research was conducted to identify the factors associated with manageable risk by using CC and BC methods. A questionnaire was first given to farmers on their general farm and hygiene practice, of which 254 responded and then the bulk tank milk of these farms were taken as samples (Piepers et al., 2014). The study had concluded that the BC of conventional parlours was actually lower than that of an automated milking system.

These studies thus show the importance of milk hygiene, and how it contributes to the hazards of consuming unpasteurised milk.

## 4. Discussion

Milk is one of the more nutritious substances currently available and has a very diverse effect on culture, nutrition and health. That being said, with all the current techniques involved in screening and testing in milk, a better and wider awareness should be present about the risks involved in drinking raw milk. The number of pathogens which cause human illness, and may cause irreparable damage or even lead to high mortality, is a very real problem, despite some prevalences presented above being at a lower rate than one could imagine, even small amounts of certain pathogens may end up causing disease (Oliver et al., 2005).

Nowadays the dairy industry needs to take better measures at regulating such practices, considering the hygiene required to maintain a farm environment, maintain a good milk hygiene practice and keeping the animals healthy themselves should be imperative for not just farm practices, but food safety measures as well. This is because raw milk is proven to harbour pathogens and acts as a vector of sorts, causing several outbreaks around the world, along with farm staff and families known to consume it in general, along with a majority of the population consuming it (Oliver et al., 2005).

Another problem presented is that pasteurisation does not necessarily kill all pathogens (Oliver et al., 2005) and any fault in pasteurisation or any miss in the production line may result with catastrophic problems (Oliver et al., 2005).

Nevertheless, people are still free to choose what they eat or drink, and this has led to the latest interest in buying everything “natural”, which consequently has given rise to a larger demand for retail of such, advertising that raw milk is somehow superior in some way, despite there being evidence stating otherwise (Oliver et al., 2009).

Thus there is a great need for regulating food safety laws, as well as educating those who consume it and tighter veterinary rules with regards to hygiene (Oliver et al., 2005). As seen from the evidence given, the prevalence in bulk tanks remains quite low, as these are points which are easy to clean rather than in line filters and the rest of the milking equipment (Griffiths et al., 2010). It is also important to note that the number of milkings per day, collection and storage of milk and transport all contribute to significant changes to milk quality with regards to pathogens and may all lead to an increased growth of bacteria (Griffiths et al., 2010).

One other major problem presented by raw milk is also its products; soft cheeses, dried raw

milk, food for infants and the sort all contribute to the distribution to a great number of individuals present in particular risk groups to contract diseases associated with milkborne pathogens (Oliver et al., 2005).

When examining raw milk consumption, it is also important to understand the sociological and psychological processes that go through each individual. The notion of blindly accepting that anything labelled as "organic" or "natural" is ever growing in society, thus no critical appraisal to this is being given by the lay person, it is almost encouraged by healthy individuals wanting to live a more "wholesome" lifestyle (Enticott 2003). For instance: "In the case of organic foods, consumers can be seen to be drawing on a culture of nature emphasising the purity of nature. Organic foods are purified from 'industrial natures' such as fertilisers and pesticides and this action of purification justifies their claim to be 'natural' and 'healthy'" as stated in Enticott, 2003, which implies that people turn to the notion of 'if its natural it is good', including the pathogenic bacteria in the milk and this imposes a great health risk (Enticott, 2003).

The mentality of "rural is safe" is also important to mention, as individuals minimise the thought of risk by implying there is no risk accompanied from local produce, whilst in fact, scientifically speaking, it has no difference from commercially made food (Enticott, 2003).

Another mentality born out of this "Natural is good" psyche is the fact that all bacteria are good, when scientifically, they are not. In a paper published by Enticott, 2003, the study goes on to highlight some opinion based conversation about raw milk:

"Raw milk—its OK—you get some germs but you get a better resistance to all these diseases —you build up some antibodies. Milk never used to be sold in bottles—you'd just go down the farm and get it—you'd take a can and a jug and get it But that's all gone, killed—you can't do that now I do think it's a bit too clean now as its sold. It's all got to be date stamped and you can't eat it after that—well you didn't know what you was eating years ago did you. You didn't know how long they'd had it. There's too many rules and regulations."

This sentence implies that not only do they oppose the notion of pasteurisation, but also reject the idea of scientific expertise to replace it with "alternate" methodologies representing a misguided train of thought with no scientific backing (Enticott, 2003).

This mentality is again reinforced with the notion of purity, regarding the 'natural' product as an end all to health benefits, and an unreasonable super cure to diseases (Enticott, 2003).

From the literature reviewed we can also see a particular trend in Italy with raw milk vending machines (Tremonte et al., 2014; Giacommeti et al., 2015), which the practice has been going on since 2004, with the Italian government imposing strict laws about the storage of it (Tremonte et al., 2014). This will not make a difference with regards to destroying the pathogens and even more so with regards to transporting the milk from the vending machine to other places (Tremonte et al., 2014).

Moreover, the bacteria present in the milk may be able to produce certain enzymes capable of reducing the overall quality of the milk, thus rendering it possibly more dangerous due to secondary products (Tremonte et al., 2014).

It also worth noting that raw milk is also exposed from these vending machines to children, and already there has been outbreaks of hemolytic uremic syndrome caused by verotoxigenic *E. coli* in children (Tremonte et al., 2014). Tremonte et al., highlights that microwaving such milk would be very useful without compromising the nutrients within, however goes on to detail that there is not enough data on the container involved in microwaving and whether it would affect it at all.

Another problem presented with raw milk is the products associated with it, Verraes et al., goes on to describe some outbreaks that have occurred in 2013, and describes that the greater part of this outbreak was caused by *Salmonella* and VTEC strain O157 along with *Listeria, Brucella* and *C. jejuni* in the USA. There have also been cases mentioned on *Streptococcus* spp and Tick borne Encephalitis Virus present in consumption of raw milk cheeses from Mexico in 1983 and in 1997 – 2008 in the Czech Republic via raw sheep cheese (Verraes et al., 2015).

It is also worth mentioning the effect on growth rate with regards to additives, particularly in raw milk cheeses. Salting may be an effective way of reducing water content, thus inhibiting bacterial activity, as well as manipulating pH, temperature and dry matter content, and competing microbes have been shown to have an effect on hazardous bacteria (Verraes et al., 2015).

Different methods of packaging may also make a difference in bacterial populations within raw milk cheeses such as vacuum packing may prevent growth of certain microbial aerobes such as fungi (Verraes et al., 2015).

## 5. Conclusion

It is thus safe to conclude the hazards associated with raw milk consumption are numbered in large amounts and the on-going research associated with it needs further support in order to ensure a safer and better monitoring system of dealing with the pathogens present.

With all that has been said above, the microbial diversity present in raw milk has been demonstrated to be quite rich, encompassing a great number of pathogenic bacteria responsible for milk borne disease outbreaks all over the world, capable of surviving harsh environments and even pasteurisation and cold temperatures. It can also be seen that farm and animal hygiene along with good milk hygiene practice is imperative to ensuring that the risks are brought down to a minimum, as complete sterility at this point is quite unlikely given the unpredictability of bacterial adaptation.

From the studies listed it can also be seen that antimicrobial resistance is a growing problem, and efforts within the realm of prevention is the main aim to trying to prevent this, so vaccination, milk treatment and food safety regulation is imperative for prophylactic measures in both human and animal populations.

It is also worth noting from the studies that the current diagnostic and screening tests for milk still have drawbacks, such as culture time, fastidious bacteria, criteria associated with the animal's immune system and changes in bacterial genome, which draws attention to the need for better monitoring of diseases and the development of more strict eradication programmes.

Educating the population on the risks of raw milk is also very important, as the lay person wanting to improve their quality of life with misguided help, communication in science and awareness of the present every day retailing of raw milk and the hazards it carries (regardless of whether it is 'natural' or not) is key to implementing a more preventative mentality rather than a curative one.

There is still much to be found with regards to scientific literature and communication on certain aspects regarding milk products, In cheese *Salmonella, Listeria, Campylobacter, Brucella* and VTEC are quite prevalent, even causing disease at very low counts (Verraes et al., 2015). However when it comes to buttermilk, butter and cream products scientific literature is lacking slightly (Verraes et al., 2015).

Pasteurisation is thus a very important tool in dealing with vegetative pathogens, however there is still an important risk that comes in after the process is finished. It is at this stage that a good Hazard Analysis Critical Control Point practice is necessary to identify which step of the way to alter (Verraes et al., 2015).

Culturing techniques also need to be reviewed, as more sensitive and specific methods are needed in order to screen for bacteria being overlooked which may be able to thrive despite pasteurisation conditions.

Further studies on social behaviour would be needed, however, on such issues as the interactions between the public and such controversial issues are never simple, as individuals seek to return to their 'natural' habits, end up overlooking scientific information for myths, resulting in possibly far worse consequences.

In conclusion, the ideal world would carry with it a safe and informative meat and milk industry with an aim to better quality of life, health and well-being with knowledge present in the safety of our foods and an acknowledgement that it can always improve.

## 6. Summary

Milk is a nutritious substance which is important both in health and culture in today's world, but also presents certain food safety risks if not treated properly, or hygiene standards are not done properly. In today's emerging trends on healthy habits raw milk has been put on the spotlight as being an unaltered substance with great benefits, however, there is no solid scientific backing towards this mentality, and thus is putting a lot of people at risk since raw milk is a very good medium to convey dangerous zoonotic diseases.

Nowadays even raw milk vending machines are being placed all over the world, showing there is a demand for the substance and some sort of retailing, but the question is how is this being regulated? With all the scientific literature available currently on the hazards present it can be clearly seen unpasteurized milk cannot be controlled correctly as the level of zoonotic pathogens dangerous to human health is unacceptable, especially when considering certain high risk groups like those infected with HIV/AIDS or immunocompromised individuals. It is also a major problem with farmers, farm staff, families living on farm with no access to heating material and possibly even veterinarians as an occupational hazard since drinking unpasteurized milk from the cow is common practice among this certain group of individuals be it for convenience sake or just to save on additional expenidtures on farm.

Several papers reviewed here bring to light the presence of many dangerous disease causing pathogens such as *Mycobacteria, Salmonella, Listeria, Brucella* etc. and the various techniques to screen for them in raw milk and test for several other factors such as antibiotic resistance.

This literature review is meant to highlight some of the important pathogens present in raw milk, the dangers associated with drinking raw milk, why pasteurisation or heat treatment is important in preventing human disease, bring to attention and debunk the myths associated with consumption of raw milk, the diverse number of ways on how to screen for the pathogens involved in milkborne disease and certain shortcomings of some diagnostic methods and certain sociological and psychological reasonings as to why individuals consume it.

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