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INFLUENCE OF MILK AND RAW MILK AS CARRIERS IN DISEASE TRANSMISSION BETWEEN HUMANS AND ANIMALS

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Abstract:
In many parts of the world, especially in underdeveloped and developing countries, the sale of raw milk is common and a large part of society consumes raw milk or its products. Even in developed countries, raw milk is consumed by numerous people even if its sale is prohibited by law. Raw milk is the unpasteurised milk of cows, sheep, goats and other animals. Advocates of raw milk consumption cite high nutritional value, good taste, demand for natural and unprocessed foods and freedom of choice as reasons for raw milk consumption. Milk consumption is thought to be beneficial for health. However, raw milk has long been known to be an important source of pathogens that can cause diseases in humans. For this reason, many public health organisations in different countries worldwide recommend that milk be pasteurised and are against raw milk consumption due to the potential risks of contamination by food pathogens. Pasteurisation is a process wherein raw milk is heated for a short period of time to eliminate the potential pathogens present in milk. Supporters of raw milk claim that milk pasteurisation has numerous adverse effects, most of which have not been proven. Raw or pasteurised milk consumption has been debated for decades. However, the rising demand for raw milk has stoked the raw milk debate. The debate continues to re-examine some of the above issues and explores the potential threats that raw milk consumption poses to consumers. In general, raw milk consumption is a real threat to human health and general risk. Therefore, the authors and other researchers do not recommend raw milk consumption given the risks of contamination by pathogens. Additional scientific studies are needed to evaluate the quality of raw milk and identify the benefits of its consumption as well as its beneficial factors. However, until these studies are conducted, the sure way to prevent diseases associated with raw milk is to avoid consuming raw milk.

Keywords: Raw Milk, Carriers, Disease Transmission, Humans, Animals.

Introduction

The consumption and sale of unpasteurised raw milk are permitted in many parts of the world, whether in developed, developing, or underdeveloped countries. However, they are banned in other countries, such as Canada. More than 75% of the milk on the market in many developing countries is sold raw through unofficial means (Mahfuz & Swapnil, 2022). For example, in East Africa, most of the milk is produced by smallholders (86% in Kenya and 92% in Uganda) and sold in the form of raw milk or unpasteurised milk products through unofficial means. Informal milk markets are booming because of their social and economic benefits, including high purchase prices from the farm itself provision to smallholder producers, employees of small markets and consumers; creation of employment; and competitive prices for consumers (Blackmore et al., 2022). In the United States, although the sale of packaged raw milk for interstate consumption violates federal law, the intrastate sale of raw milk is legal in many states. According to a recent poll by the National Association of the Department of Agriculture, 29 states allow the sale of raw milk in some way. In areas wherein raw milk is prohibited and illegal, sharing or renting cows and selling raw milk as pet food are the ways through which consumers obtain raw milk (Lampe & Sharp, 2019). Raw milk consumption in the United States is difficult to estimate. It has always been common amongst 35%–60% of farmer families and farm workers likely because it is a traditional method and obtaining milk from a bulk tank is cheaper than buying pasteurised milk from retail stores (Deneke et al., 2022). Estimating raw milk consumption by the urban community is difficult. In an epidemiological study on the prevalence of foodborne diseases related to raw milk in the United States from 1973 to 1992, O'Callaghan et al, (2019) demonstrated that raw milk accounted for less than 1% of all milk sales in states wherein raw milk is legal. They conducted another study to determine the extent of raw milk consumption in California, which, at the time of the study, was the largest legal raw milk–producing state in the United States. Amongst the 3999 people who responded to the survey, 2.3% reported drinking raw milk in the past year. Raw milk consumers have the following demographic and behavioural characteristics: under 40 years of age, male, Latino and had a sub diploma education (Merlino et al., 2023). A total of 3.5% of people polled in 2002 consumed raw milk within the 7-day period before the survey. If we take the results of this survey and those of the report of Udovicki et al. (2019) to represent the United States population, more than 10.5 million people in the United States regularly consume raw milk, perhaps even on a daily basis. According to data from Mangla et al., (2021), this estimate may even be considerably lower than the estimate given by the Price Foundation, a nonprofit educational foundation that promotes the consumption of clean raw milk from healthy cows fed with grass, showing that the demand for raw milk is growing rapidly. Some estimates show that the demand for natural and unprocessed foods based on the concept of production sales and purchase of premises has risen by 40% amongst consumers. This growth has likely led to an increase in interest in raw milk. Raw milk seems to be consumed for various reasons based on its offered benefits, such as its good quality and taste, added nutritional value and health benefits. The demand for natural and unprocessed foods by consumers interested in sustainable agriculture and ultimately freedom of choice has led to the support of producers who use environmentally friendly methods. Supporters of raw milk believe that pasteurised milk is lower in quality than raw milk consumed directly. Somatic cell count (SCC), the number of somatic cells in milk, is used worldwide as an indicator of milk quality. High-quality milk has low SCC. In the United States, pasteurised milk laws stipulate that SCC in milk should be 750 000/mL (Alhomoch, 2021). Various groups and organisations demand that the limit of SCC in milk in the United States be reduced from the current value of 750 000/mL to 400 000/mL or less to increase competition with the European Union and countries with lower SCC limits. A summary of the SCC data for all United States herds that were included in the 2008 Dairy Herd Breeding Pilot Programme showed that the national average SCC in 2008 was 262000 cells/mL milk, which is 14000 cells/mL or 3% lower than that in 2007 and approximately 10.6% lower than that in 2019 (Mulakala, 2019)). Supporters of raw milk believe that milk pasteurisation is associated with lactose intolerance, increased allergic reactions and reduced milk nutritional value. Moreover, they believe that milk pasteurisation increases pathogens; destroys antibodies and other protective bioactive agents, proteins, polypeptides and beneficial bacteria in milk; and contributes to arthritis and autism. Pasteurisation is the most effective method for increasing the microbiological safety of milk and its products. In pasteurisation, harmful bacteria are killed by heating milk to a certain temperature for a certain duration. The approved and common pasteurisation criteria in the United States and similar criteria and/or equivalents used around the world are summarised in Table 1.

Table 1: Time and temperature for liquid milk pasteurization

Temperature	**Time**
60°c (145°F)	30 Second
70°C (160°F)	15 Second
90°C (190°F)	1 Second
95°C (200°F)	0.5 Second
97°C (205°F)	0.1 Second
100°C (215°F)	0.05 Second

Pasteurisation at 72 °C–94 °C for 15 s has no effect on microbiological shelf life (Dash et al., 2022). More than 25 years ago, Claeys et al (2013) claimed that there were 'no significant differences in nutritional value between pasteurised and unpasteurised milk' and that other purported benefits for raw milk have not been proven. From a nutritional perspective, pasteurisation lacks a considerable effect on the main components of milk, such as lactose, casein and most milk proteins (van Lieshout et al., 2020). Heating milk can lead to the breakdown of lactose into lactulose and epilactose (de Oliveira Neves & de Oliveira, 2020). Dairy is a main source of high-quality protein in the human diet. However, pasteurisation may cause protein denaturation and accumulation and chemical changes in amino acids, which may affect protein quality. This systematic review covers the changes in milk protein as a result of heating and protein digestion and their physiological effect. Laboratory and animal studies have shown that glycosylation reduces protein digestibility and inhibits the availability of amino acids, especially lysine. Other chemical modifications, including oxidation, dephosphorylation and cross-linking, have been studied less than glycosylation but may also affect protein digestion and lead to reduced bioavailability and amino acid function. Although protein denaturation does not affect overall digestibility, it can facilitate the hydrolysis of proteins, especially β-lactoglobulin, in the stomach. Protein denaturation can also alter stomach protein depletion, thereby affecting digestive kinetics and eventually leading to the secretion of plasma amino acids after meals (van Lieshout et al., 2020). Large amounts of indigestible carbohydrates, such as lactulose, can cause digestive disorders in people who have difficulty digesting lactose. Milk is an important source of high-quality protein in the human diet. The high nutritional quality of milk proteins originates from their high levels of essential amino acids and bioavailability. Milk proteins have higher bioavailability than plant proteins due to their high digestibility, which can be partly attributed to their lack of antinutritional agents and different processing methods. The main protein changes that occur during processing are protein denaturation and accumulation and chemical changes in amino acids. These protein changes may alter the digestion and overall physiological effect of proteins when consumed. The most important physiological consequences of heat treatment are changes in the digestibility and bioavailability of proteins. Protein changes may also cause alterations in the digestive tract (e.g. microbiota, epithelial physiology and immune responses) and have other physiological consequences that can be localised or systemic (Dieterich, Schink & Zopf, 2018). Industrial dairy processing can alter the structure of milk proteins in different ways depending on processing conditions. However, pasteurisation generally does not result in remarkably high levels of lactulose in pasteurised milk. Pasteurisation also eliminates lactase-producing bacteria that may be beneficial for lactose-intolerant individuals (Morelli et al., 2019). Whey proteins, such as lactoferrin and immunoglobulins, retain their biological activity except when pasteurisation is conducted at excessively high temperatures (Kontopodi et al., 2022). Although pasteurisation reduces some cow enzymes in milk, humans do not have time to digest most of these enzymes. Other enzymes present at low concentrations in cow's milk, such as lactoperoxidase, lysozymes and xanthine oxidase, remain active after pasteurisation (Silva et al., 2021). The United States Food and Drug Administration (FDA) and Centre for Food Safety and Functional Nutrition stated that pasteurisation has negligible effects on vitamins E, D, A and K but partially reduces vitamin C. Interest in unprocessed and natural foods, including fresh milk (unpasteurised) and dairy products, is growing worldwide. Raw milk consumption is increasing in many countries, and scientific evidence showing that raw milk can reduce asthma, allergies and atopic eczema is growing. In addition, the intake of raw cow milk early in life has been shown to reduce the risk of overt respiratory infections and fever by approximately 30%. The consumption of boiled raw farm milk increased incidences of asthma, hay fever and atopic diseases even in farm children, who are the best-protected group of children worldwide (Berge & Baars, 2020). Recent statements have linked raw milk consumption to improvements in autism in children. Liquid milk is a highly nutritious beverage whose consumption has been reduced in recent years. Milk is an excellent source of fats, proteins and mineral foods, such as calcium and magnesium, for growing children. Debate about the potential benefits associated with consuming raw milk over its processed counterpart is ongoing. Physiological and environmental factors can influence the composition and quality of milk, and cow feeding systems have been identified as important factors that can change the nutritional status of milk. However, raw milk consumption poses a very real and serious health risk through the potential ingestion of pathogenic bacteria. The argument against refined milk has focused on the reduction in the nutritional quality of milk as a result of heat processing. However, these claims lack a scientific basis, and experts have widely agreed that the risk of exposure to pathogenic bacteria in raw milk is considerably greater than the potential benefits of raw milk consumption (Alegbeleye et al., 2018). Some studies published mainly in the European Union have shown that children raised in agricultural environments have fewer allergic conditions (including asthma, hay fever and eczema) than those who were not and raw milk consumption was a protective factor associated with allergy reduction (Loss et al., 2011; Sozańska, 2019). Other factors, such as exposure to grain stores and contact with animals, were also involved in allergy reduction. Given the potential health risks due to food pathogens in raw milk, the authors believe that raw milk consumption for preventing allergies cannot be recommended. The National Research Council stated that good nutrition involves a balanced diet that includes the required amount of essential nutrients and energy. Considering that the USDA recommends two to three servings of dairy products a day, the nutritional importance of dairy products cannot be called exaggerated. Milk and its products, which originate mainly from cows, blue buffalos, sheep, goats and other species, constitute an important part of the human diet. The inclusion of dairy in the diet helps prevent diseases, such as obesity, hypertension and diabetes. Dairy products are also a source of calcium, which is important for bone growth and osteoporosis prevention. In addition, dairy products are an important food source of protein, vitamins and other minerals. The consumption of milk products is related to the overall quality of the diet and proper absorption of numerous nutrients, including calcium, potassium, magnesium, zinc, iron, riboflavin, vitamin A, folate vitamin D and protein (Górska-Warsewicz et al., 2019). Microbes that may be present in milk can include pathogens, spoilage organisms, conditionally beneficial organisms (such as lactic acid bacteria [LAB]) and organisms that have not been associated with beneficial or harmful effects on product quality or human health. However, milk can also contain a full range of organisms that are classified as microbes (i.e., bacteria, viruses, fungi and protozoa), with a few exceptions (e.g., phages that influence fermentation; fungal spoilage organisms; and, to some extent, protozoan pathogens). Dairy microbiology has mainly focused on bacteria. Along with pasteurisation, other strategies for reducing microbial contamination across the dairy chain (e.g., improved dairy herd health, raw milk tests and clean-in-place technologies) play an important role in improving the microbial quality and safety of milk. Despite its tremendous progress in reducing food safety risks and spoilage issues, the dairy industry continues to face remarkable challenges, including the need for science-based strategies to improve the safety of raw milk cheeses and control postprocessing pollution, spore formation, pathogens and food spoilage organisms. Public debate about the real risks and possible benefits of the direct human consumption of raw milk has raged over the last few decades. From a scientific perspective, the naturalness of food does not directly imply its health, taste and safety. In fact, in the European Union, 27 outbreaks of milk-borne diseases that were claimed to be linked to raw milk consumption occurred in 2007–2012. The European Food Safety Authority has recently been asked to provide a scientific opinion on the public health risks associated with raw milk consumption. The risks associated with raw milk are also provided on the websites of reputable institutions, such as the FDA and Centre for Disease Control and Prevention. In European Union law, raw milk is defined as milk produced from the secretion of the mammary glands of farmed animals that has not been heated to temperatures above 40 °C or undergone any treatment with an equivalent effect. Raw milk intended for human consumption must be free of pathogens in accordance with the food safety requirements of the General Food Regulation (EC) No. 178/2002. Milk is sterile in healthy breast cells and does not contain bacteria in the mammary gland at the site of its production unless a systemic intramammary infection is present in the producing animal. Native gore mainly contains the genera *Streptococcus*, *Staphylococcus* and *Micrococcus*, which represent more than 50% of the total flora of raw milk (Voidarou et al., 2020). However, upon excretion, milk is immediately colonised by a complex microbiota that is composed of a considerable population of microorganisms and naturally resides in the nipple skin and epithelial lining of the nipple canal. In particular, the surface of the bovine nipple is colonised at lower levels by the branched bacterium *Bacteroides* (1.3%), Cyanobacteria, Verrucomicrobia, Planctomycetes and Chloroflexi (Lopez Franco, 2019). Milking equipment (Li et al., 2018), animal storage area (Du et al., 2020), feeding area (Albonico et al., 2020), substrate material (Oliveira et al., 2019) and lactation stage (Chen et al., 2018) also affect the microbiota of raw milk. The biodiversity of microbiota is influenced by the biochemical composition and almost-neutral pH of raw milk (Hahne et al., 2019). High water activity may also help microbes grow (Tapia, Alzamora & Chirife, 2020). The microbiota of raw milk can be classified mainly into two main groups: spoilage microorganisms (Table 1) and pathogens (Table 2). Both of these groups are undesirable in raw milk. Spoilage microorganisms can grow rapidly in milk, altering traits, such as food quality. Pathogens in raw milk pose a threat to immunity and are the main causes of human infections Table 2.

Table 2: Show raw milk spoilage bacteria

Mushroom	**Streptococcus**	**Lactobacillus**	**Elkonostok**	**Propionibacterium**	**Nitrox**	**Gram-positive**	**Gram negative**	**Yeast**	**Mushroom**
Lactococcus Lactis cremoris	Streptococcus Agalactiae	Lactobacillus acidophilus	Lactococcus mesenteroides	Pseudomesenteroide s acidipropionici	Enterococcus	Arthrobacte	Achromobac ter	C.sake.C Parapsilosis Inconspicua	Aspergillus
Lactococcus Lactis	Streptococcus bovis	Lactobacillus brevis	Lactococcuspseudo mesenteroides	Pseudomesenteroide s freudenreichii	Enterococcus faecalis	Bacillus	Acinetobacte r	Carnescens victoriae	Fusarium
Lactococcus piscium	streptococcus dysgalactiae	Lactobacillus buchneri		Pseudomesenteroide s Jensenii	Enterococcus faecium	Bifidobacterium	Aeromonas	Debaryornyces Hanseni	Geotrichum
Lactococcus raffinolactis	Streptococcus macedonicus	Lactobacillus acsei		Pseudomesenteroide s thoenii	Enterococcus italicus	Brevibacterium	Alcaligenes	Geotrichcm Candidum. G. catenulate	Mucor
	Streptococcus thermophilus	Lactobacillus crispatus			Enterococcus mundtii	Clostridium	Chryseobact erium	Kluyveromyces Marxianus K.lactis	Penicillium
	Streptococcus uberis	Lactobacillus curvatus				Corynebacteriu m	Enterobacter	pichia	Rhizomucor

The class of spoilage microorganisms consists of different groups, the most important of which are LAB. The population of psychotropic bacteria is Gram-negative and Gram-positive and can include coliforms in milk stored at ≤6°C. Fungal populations include yeasts and moulds. LAB is an integral part of the raw milk microbiota (Biolcati et al., 2022). Their biodiversity in milk depends on the type of milk and other external parameters during milking (Oikonomou et al., 2020). In raw ewe milk, LAB often include enterococci (40%), lactococci (14%–20%), leuconostocs (8%–18%) and lactobacilli (10%–30%). Lactobacilli are dominant in raw goat milk (Islam et al., 2021). Lactococci and lactobacilli are usually the most common LABs, amongst which *Lactococcus lactis*, *Lactobacillus brevis* and *Lactobacillus fermentum* are the most common species (Bintsis, 2018). *Lactobacillus* spp. also have proteolytic activity and can produce aromatic compounds and exopolysaccharides. Fresh milk collected from the breast often does not contain a detectable population of psychotropic bacteria (Velázquez-Ordoñez et al., 2019). However, psychotropic bacteria grow throughout the cold chain after milk collection. Although these microorganisms have optimal growth temperatures of 5 °C and 20 °C, they can also grow at low temperatures, such as 2 °C–7 °C. This situation indicates that over time, psychotropic populations can develop in raw milk stored in the cold, and their presence in the microbiota of raw milk can cause concern. The drawback of psychotropic in milk is their ability to produce extracellular enzymes, mainly proteases and lipases, which are responsible for the spoilage of milk and dairy products, because extracellular enzymes can resist pasteurisation and even processing at very high temperatures (Yuan et al., 2018). Therefore, the rapid application of a cooling operation after milking and cold temperatures for storage, which is a common method for controlling the microbiological quality and safety of raw milk, is ineffective for decelerating the growth of psychotropic bacteria. The number of psychotropic bacteria that develop after milk collection depends on storage temperature, time and hygiene conditions. For example, under unsanitary conditions, more than 75% of all microflora are psychotrophs, whereas under sanitary conditions, less than 10% of all microflora are psychotropic microorganisms (Zhang, 2020). Multiple genera of psychotropic bacteria have been isolated from raw milk. They mainly include the Gram-negative species *Aeromonas pseudomonas*, *Flavobacterium*, *Chryseobacterium*, *Enterobacter*, *Achromobacter*, *Alcaligenes Acinetobacter* and *Serratia*. *Pseudomonas* and *Enterobacter* spp. are the most abundant species in cold-stored raw milk, with Gram-negative microflora accounting for more than 90% of the total psychotrophic microflora in raw milk (Zhang, 2020). Although the Gram-positive genera *Micrococcus*, *Microbacterium*, *Corynebacterium*, *Clostridium*, *Bacillus*, *Staphylococcus*, *Streptococcus* and *Lactobacillus* are also commonly found in raw milk, they account for only a small fraction of the psychotrophic microflora in raw milk (Coelho, Malcata & Silva, 2022). *Bacillus* spp., including *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus megaterium*, are the most common spore-forming bacteria. *B. cereus* is the most common contaminant in raw milk given that it has the highest rates of isolation amongst bacteria (Elisashvili, Kachlishvili & Chikindas, 2019). However, *B. subtilis* and *B. licheniformis* are more heat-resistant than *B. cereus*, spoiling sterilised and UHT milks (Dash et al., 2022). The Gram-positive bacterium *Arterobacter* enters milk at dairy plants. *Corynebacterium* spp. is found on the nipple surface and field environment (Martin, Boor & Wiedmann, 2018). Psychotrophic microflora in raw milk also includes pathogens, such as *Aeromonas hydrophila*, which is Gram-negative; *Yersinia enterocolitica* and *Listeria monocytogenes*, which are Gram-positive; and toxin-producing *B. cereus* strains, whose spores can survive thermal treatment even at 75°C–76 °C.

Table 3: The main pathogens of raw milk and related zoonoses

Pathogen	Taxnmamy	Morphology	Disease	Transmiselon Route	System Potentially Allected					
					Cardio Vascular	Cutaneous	Gastro Intestinal	Neuroogcal	Ocular	Pulmonary
Brucella spp B.abortus B. melitensis	Bacteria coccobacilli	Gram (-)	Brucellosis	Cutaneous Ingestion Inhalation	x	x	x	x	x	x
Campsisibacter spp. C.fetus c. jejun	Bacteria corkscrew	Gram (-)	Campylobacteriosis	Ingestion	x		x	x		
C.burnetii	Bacteria cocoabacilli	Gram (-)	Q fever	Ingestion Inhalation	x		x	x		x
E.coli	Bacteria bacilli	Gram (-)	Hemolytic uremic syndroma Hemorrhagiccolitis	Ingestion Inhalation		x	x	x		
L.monocytogenes	Bacteria bacilli	Gram+	Listeriosis	Ingestion Cutaneous	x	x	x	x		x
Mycobacterion spp. M. tuberculosis M.bovis	Bacteria bacilli	No Gram	Tubercolosis classification	Cutaneous Ingestion Inhalation		x	x	x		x
Salmonella spp.	Bacteria bacilli	Gram (-)	Salmonellosis	Ingestion			x			
Shigella spp.	Bacteria bacilli	Gram (-)	Shigellosis	Ingestion		x	x	x		
Staphylococcus	Bacteria staphylococci	Gram+	Staphyloccal disease	Cutaneous Ingestion Inhalation	x	x	x	x		x
Staphylococcus	Bacteria staphylococci	Gram+	toxic shock syndrome	Cutaneous Ingestion Inhalation	x	x	x	x		x
Yersinia spp. Y.pseudotubercolosis	Bacteria bacilli	Gram (-)	Yersiniosis	Cutaneous Inhalation	x	x	x	x		x

Coliforms are commonly found in raw milk at different levels (Wanjala, Nduko & Mwende, 2018). They have various sources. such as water, equipment, soil and faeces. High levels of coliforms (e.g. 1000 CFU/mL) generally indicate unsanitary practices on farms as well as inappropriate management practices, such as milking machine wash failures; losses in the numbers of milking units can also contribute to contamination (Mogotu, 2021). Efforts have failed to discover a link between coliform bacterial levels and the possible public health risks posed by raw milk. A recent review in the United States found that the number of coliforms can be considered to be an indicator of the presence of *L. monocytogenes*, *E. coli* 2,0157:17, *B. cereus* and *Salmonella* strains (Fusco et al., 2023). Therefore, testing coliforms in raw milk for human consumption cannot be used as a reliable tool for screening public health risks, and additional research is needed on this topic (Fusco et al., 2020). Yeasts and moulds form an important population of raw milk microorganisms. They usually originate from contaminated areas in dairy farms and/or processing plants and can also be due to physiological, nutritional and climatic conditions (Thompson & Darwish, 2019). The most common yeasts identified in raw milk include *Debaryomyces*, *Cryptococcus*, *Candida*, *Debaryomyces hansenii*, *Trichosporon*, *Rhodotorula*, *Pichia*, *Kluyveromyces* and *Geotrichum*. *Kluyveromyces marxianus* var. marianus and lactis have also received special attention. Moulds are less common in raw milk than yeasts and mostly include *Penicillium*, *Geotrichum*, *Aspergillus*, *Mucor*, *Rhizomococcus*, *Rhizopus* and *Fusarium* (Perin et al., 2019). Raw milk can also contain a large number of pathogens (Table 2) even when produced by healthy animals. Therefore, it can pose a serious threat to human health. Pathogens that can be found in feed and drinking water include *Toxoplasma gondii*; those found in the environment of dairy farms include *E. coli* and *L. monocytogenes*; and those found in mammary glands include toxin-producing *Salmonella* spp., *Shigella*, *Campylobacter jejuni*, *Y. enterocolitica* and *Clostridium* spp. (Alegbeleye et al., 2018). *Salmonella*, *Listeria*, *E. coli*, *Campylobacter*, *Brucella*, *Clostridium* and/or *Shigella* spp. are the most common milk-borne pathogens and main cause of foodborne microbial diseases, milk poisoning and milk-borne toxic infections (van den Brom et al., 2020). In general, the usual symptoms induced by drinking raw milk contaminated with the above pathogens include fever, nausea, vomiting, diarrhoea and abdominal pain. However, these pathogens can potentially affect the cardiovascular, skin, neurological, ophthalmic and pulmonary systems and cause death in some cases, as is the case for *Listeria* spp. (30%–35%) and *Streptococcus* strains (29%) (Balaji, JebaMercy & Balamurugan, 2019). *Salmonella* spp. are natural inhabitants of the animal gastrointestinal tract. Milk contamination by these species generally occurs during milking. In rare cases, they cause subclinical mastitis, which in turn causes milk-borne diseases. *Salmonella* spp. are mesophilic microorganisms with optimal growth temperatures of 35 °C–37 °C (Dubey, Raj & Kumar, 2022). However, they can also be found growing over the wide temperature range of 5 °C–46 °C. The digestive form of nontyphoid salmonellosis is also often associated with raw milk consumption. *Salmonella* species, however, have poor thermal tolerance and are therefore sensitive to pasteurisation. *L. monocytogenes* is another food pathogen that is likely to contaminate raw milk. It causes widespread outbreaks of listeriosis in humans; dangerously aggressive abortions in pregnant women; and meningitis, encephalitis and septicaemia in infants and immunocompromised adults (Schlech, 2019). *L. monocytogenes* poses the above threats because it can grow and reproduce at low temperatures (0 °C–4 °C) during raw milk storage. Therefore, even using a correct cold chain method cannot completely destroy this microorganism. *E. coli* is a faecal pollution index. The most pathogenic strains of *E. coli* are verocytotoxigenic *E. coli* (VTEC) and enterohemorrhagic *E. coli* (EHEC), which is also known as *E. coli* serotype O157:H7. Cow stool is the main reservoir of EHEC, which usually contaminates milk in bulk tanks. Therefore, milk contamination is the result of direct exposure to environmental pollution. Raw milk is a dangerous source of VTEC, and several outbreaks of this pathogen have occurred recently (Abebe, Gugsa & Ahmed, 2020). A total of 3% of 860 raw milk samples tested positive for VTEC in Europe in 2003 (Giacometti et al., 2012). The CDC stated that *Shigella* and *E. coli* caused 17% of the outbreaks that occurred in the United States between 2007 and 2012. VTEC serotypes have also been identified in milk from cows with mastitis, suggesting that an additional infection pathway may lead to other infections. Most strains are resistant to heat and removed by pasteurisation. *Campylobacter* is a member of the Campylobacteraceae family and causes human gastroenteritis. *C. jejuni* is a strain identified in raw milk. It is sensitive to acid and heat and is therefore destroyed by pasteurisation. Outbreaks of campylobacterosis after raw milk consumption have been reported in the United States, the Netherlands and Hungary (Authority, 2019). Aslett et al. reported that raw milk consumption accounted for a high number of campylobacter outbreaks. Strong evidence for 32 outbreaks was found in 2012. Cases of infections with *Campylobacter* spp. were reported in the European Union (9% in 2013 and 20% in 2012) (Tumbarski, 2019). *Brucella* spp. is the main cause of zoonotic and bacterial brucellosis. They are highly infectious organisms that can cause diseases in animals and humans. *Brucella abortus* and *Brucella melitensis* are the most pathogenic strains that have been associated with diseases in humans. *B. abortus* is associated with cattle, whereas *B. melitensis* is particularly associated with sheep and goats. Most cases of food-borne brucellosis in humans are caused by the consumption of raw milk and its derivatives. Amongst the pathogens transmitted from milk, *Brucella* spp. can survive and multiply at cold storage temperatures along with *L. monocytogenes* and *Y. enterocolitica*. These microoganisms are not particularly resistant to heat and can be sufficiently removed by standard pasteurisation. However, they also survive and multiply in milk after pasteurisation (Alegbeleye et al., 2018). *Staphylococcus aureus* is a Gram-positive bacterium that causes mastitis in cows and other dairy ruminants. It may contaminate milk through the nipple channel when the zodiac gland is infected or through the environment as a result of poor hygiene habits during or after the procedure, such as not washing hands or touching milk storage equipment (Gad et al., 2021). *S. aureus* may cause diseases by producing heat-stable enterotoxins. They are highly resistant to heat and pasteurisation. Although boiling for an hour may reduce the amount of toxins in milk, autoclaving at 15 psi for 20 min appears to be the main treatment that can completely eliminate toxins (Choudhary, Sharma & Gaur, 2024). Two other microorganisms in milk cause concern: paratuberculosis (MAP) and *Mycobacterium*. MAP causes tuberculosis (TB) that mainly affects domestic animals but is survivable. It reproduces in the intestinal mucosa. Recent studies have provided evidence on the relationship between MAP and Crohn's disease in humans (Mintz & Lukin, 2023). However, this association remains controversial. Current studies indicate that MAP is highly prevalent in raw milk and relatively heat-resistant. It may survive the pasteurisation of dairy products at 72 °C for 15 s, and experiments on its resistance to heat have so far reported controversial results (Lindsay et al., 2021). In 2002, researchers at Cole University of Belfast sieved 567 samples of commercial pasteurised milk and found that 1.8% of the samples were contaminated with *Mycobacterium avium* subsp. This microorganism can survive HTST and can be found in pasteurised milk due to postprocessing contamination (Martin, Boor & Wiedmann, 2018). *Mycobacterium bovis* is the cause of bovine TB in animals and can also spread to humans through raw milk consumption, causing zoonotic TB that is indistinguishable from human TB. In addition, countries, such as the Netherlands, have official bovine TB–free status, potentially overseeing the removal of pathogens from the food chain (Ramanujam & Palaniyandi, 2023). *Y. enterocolitica* is an acute gastroenteritis agent whose symptoms are abdominal pain, diarrhoea and fever. The similarity of its symptoms with those of appendicitis can sometimes lead to misdiagnosis. Pasteurisation can kill this bacterium. However, sometimes heat treatment is insufficient or recontamination may occur. This bacterial species can also multiply at cold-storage temperatures (Wei et al., 2019). However, *Y* *enterocolitica* has low incidence in raw milk and dairy products, and only a few positive results have been reported recently in the European Union (Bonardi et al., 2018). *Coxilla burnetti* is the cause of Q fever, which can infect several animal species, such as cattle, sheep and goats. Animal cases of Q fever are far more severe than human infections. *C. burnetti* infections appear with flu-like symptoms that lead to endocardia and hepatitis; although *C. burnetti* is relatively heat-resistant, it is eliminated by regular pasteurisation methods (Alegbeleye et al., 2018). Ensuring the safety of raw milk can be difficult. Storage temperature control can maintain the microbiological stability and shelf life of milk because some bacteria in raw milk require high temperatures. Nevertheless, bacterial proliferation is not limited even when milk is properly cooled and stored at 4 °C, and growth limits are inapplicable to psychotrophic bacterial pathogens that may multiply at these temperatures (De Silvestri et al., 2018). Given that the scientific community claims that raw milk is associated with public health risks, the increasing consumption of heat-treated and raw milk is considered a risk-based approach. Diseases associated with drinking raw milk include campylobacterosis (Burakoffet al., 2018; Kenyon et al., 2020), listeriosis (McLauchlin et al., 2020; Nichols et al., 2020) haemolytic uremic syndrome (Bruyand et al., 2018; Joseph et al., 2020), salmonellosis (Castañeda-Salazar et al., 2021; Ung et al., 2019); and staphylococcal diseases, as reported in Table 3 (Kou et al., 2021; Regasa, Mengistu & Abraha, 2019). Milk is rich in macronutrients, including amino acids; lipids; sugars; and micronutrients, such as vitamins and minerals. Given that it is rich in nutrients, it is a fertile environment for the growth of microorganisms that may cause spoilage. In addition, some enzymes in milk help initiate undesirable changes during storage. Hence, milk is usually industrially processed to be safe for human consumption and prolong its shelf life. Heat treatment is the most common way to preserve and immunise milk. Its main objectives are to (1) kill pathogenic microorganisms, (b) inactivate more than 95% of spoilage organisms and (3) inactivate enzymes inherent in milk or excreted by microorganisms responsible for reducing milk quality (Pathot, 2019). *S. aureus* does not survive pasteurisation but may produce heat-stable enterotoxins. It is highly resistant to heat and pasteurisation. Specifically, enterotoxin A can remain active for 28 min after heat treatment at 121 °C. Rathod et al (2021) screened samples of raw and pasteurised milk for *S. aureus* and found it in 70.4% of raw milk samples, eight pasteurised milk samples before expiry and 11 expired samples. The effect of pasteurisation on MAP is also controversial, and MAP can survive 15 s of HTST pasteurisation at 27 °C and persist as a postprocessing contaminant (Chiozzi, Agriopoulou & Varzakas, 2022). However, *M. bovis* is killed by pasteurisation despite being the most resistant nonsporulating pathogen in milk (Mullan, 2019). Therefore, milk that has undergone correct pasteurisation is unlikely to become pathogenic (Alegbeleye et al., 2018). However, if inadequate heat treatment is applied, milk and dairy products may be recontaminated by *Salmonella* spp., *B. cereus* STEC, *Y. enterocolitica*, *C. jejuni*, *L. monocytogenes*, *Ecobacterium*, *S. aureus*, or botulinum (Bezie, 2019). In the case of spoilage microorganisms, thermogenic psychotrophic agents are destroyed by pasteurisation, but postprocess contamination or thermal resistance may occur. For example, pasteurised milk may be contaminated by Gram-negative psychotrophs. However, the presence and number of psychotrophs in pasteurised milk depends on the initial count of the microbes before heat treatment. *Pseudomonas* spp. are heat-sensitive and able to survive pasteurisation. New analytical methods have shown that pasteurisation decreases, rather than removes, the population of *Pseudomonas* (Reichler et al., 2018). This situation indicates that cells are damaged but remain potentially metabolically active after heat treatment. Hence, they are the predominant microorganisms in pasteurised milk, along with *Flavobacterium*, which is present at low levels. *Pseudomonas fluorescens* is the main cause of undesirable flavours in milk, e.g. musty, sour, cheesy and bitter tastes (Saha et al., 2024). *Lactobacillus* spp. are rarely found in pasteurised milk. Milk contains almost all the nutrients necessary to sustain life and has a high nutritional value. Heat treatment also affects the nutritional profile of milk, which contains casein and whey protein (or serum). Casein accounts for 80% of milk proteins and contains calcium- and phosphorus-containing precursors of bioactive compounds with antimicrobial activity. In contrast to whey proteins, casein is insensitive to heat and does not experience thermal denaturation. Under intense heat treatment at low pH, whey may hydrolyse, causing its aggregation and coagulation. Its activity in milk may cause clotting (Huppertz & Chia, 2021). Whey proteins include α-lactalbumin, β-lactoglobulin, serum immunoglobulins and bioactive peptides and have important physiological properties. Heat treatment causes their denaturation into serine phosphate, glycosylated cysteine and cysteine. These compounds may undergo β-elimination and form dehydroalanine, which can react with several amino acids and produce proteins that are not hydrolysed by the intestinal tract, thus reducing the nutritional value of milk (Aguilera-Toro et al., 2022). In general, pasteurisation affects casein structure negligibly and causes slight changes in whey structure (Edwards, & Jameson, 2020). Animal and human studies have failed to find considerable changes in the nutritional quality of milk protein due to pasteurisation (Bakar et al., 2021). Lysine is the main amino acid in milk, and heat treatment causes lysine losses of 1%–4% but has negligible effects on other amino acids (Li, Ye & Singh, 2021). Lysine losses are caused by a widespread Maillard reaction that occurs during heat treatment, particularly sterilisation in bottles. Nevertheless, the loss of this amino acid is not severe given the excess amounts of lysine present in protein (Estévez & Xiong, 2019). Heat treatment can usually denature several other enzymes in milk. Therefore, the activity of enzyme systems is used as an indicator of the thermal treatments of milk. Alkaline phosphatase activity is used to monitor pasteurisation efficiency. Enzyme inactivation ensures that all nonsporulating pathogens are killed. Lactoperoxidase activity is also used as an indicator for heat treatments that are more intense than pasteurisation. γ-Glutamyl transferase is applied to evaluate milk treatment methods involving temperatures above 77 °C (Deeth, 2021). The fat content of milk on the market is standardised by removing cream or adding whole, semifat, or skim milks. Ajmal et al. (2018) investigated the effect of pasteurisation and UHT treatments on milk lipids and found no changes in lipid levels or fatty acid profiles. Lactose is the main carbohydrate in milk. It has prebiotic properties and induces calcium and magnesium absorption. Thermal treatments above 100 °C determine the rate of the degradation of lactose into acids, especially formic and lactic acids, and hence result in an increase in titrable acidity. Lactose may also participate in the Maillard reaction that determines the formation of products, browning and flavours. Pasteurisation has no effect on lactose. Usually, lactulose forms from lactose through the Lolary de Bruyn–Alberda van Ekestein conversion when heated under slightly alkaline conditions. Given that lactulose is not detectable in raw milk, it is used as an indicator of thermal load and then as an indicator of the intensity of milk heat treatment. Thermal treatments above 100 °C change the ratio of lactose to acids, especially formic and lactic acids, and therefore increase titrable acidity. Raw milk has been claimed to have higher nutritional value than pasteurised milk because it supplies more vitamins. Heat treatment conditions, in addition to packaging type and storage conditions, may also affect the vitamin content of milk products on the market (Bezie, 2019). McDonald et al. conducted a systematic review to assess the effect of pasteurisation on vitamins in raw milk. Their review included 40 studies evaluating the effects of pasteurisation on vitamin content. They found that vitamin B12, vitamin E, folate and riboflavin (vitamin B2) decreased after pasteurisation. By contrast, pasteurisation increased vitamin A levels and did not remarkably affect vitamin B6 levels. Although heat treatment destroys some vitamins (such as vitamin C and folate), the contribution of vitamin content in milk to the recommended daily intake (RDI) should be considered to compare the nutritional value of raw and heat-treated milks. For example, 20 L of raw milk per day must be consumed to meet the RDI of vitamin C. Hence, degradation due to heat treatment is not a problem. The same situation applies to vitamins B12 and E. Therefore, the effects of pasteurisation on the daily intake of these vitamins by adults is not of concern because milk is not the primary source of these vitamins (Brett et al., 2011). However, vitamin C protects folic acid against oxidation, and its breakdown is linked to vitamin B12. The consumption of 250 mL of raw milk contributes more than 80% of the RDI of vitamin B12 (Gille & Schmid, 2015). Milk is a good source of some minerals, especially calcium and phosphorus, and the calcium and phosphorus contents of raw and heat-treated milks do not considerably differ. Furthermore, heat treatment has no effect on the bioavailability of these nutrients (Broersen, 2020). Good-quality milk is slightly sweet, has a subtle smell and feels smooth and thick in the mouth. It is characterised by a white and glossy taste. The heat treatment applied to achieve milk safety may affect the organoleptic properties, nutrient content, taste and colour of milk depending on its thermal load. Each heat treatment creates a distinct profile. Heat treatment creates some flavours, whereas it degrades others (caused by microorganisms or enzymes). The typical 'bovine' taste of fresh milk can be obscured by the formation of flavour compounds, such as ketone flavours, and reduction in sterile caramel flavours, during UHT. A cooked taste is mainly contributed by sulphur compounds resulting from the denaturation of whey protein. The denaturation of whey protein exposes sulfhydryl groups that may form rilic sulphide and dimethyl sulphide (Zhang et al., 2021). The consumption of raw milk has been linked to benefits for human health, such as high nutritional value and protection against lactose intolerance, asthma and allergies. Conversely, heat treatment has been reported to have harmful effects. Lactose is the main carbohydrate in milk and its products. The inability to digest lactose is called lactose intolerance. It is due to lactase deficiency. The main symptoms, include bloating, diarrhoea and abdominal pain, and incidence of lactose intolerance increase with age and vary by community and ethnic group (Sharp et al., 2021). The consumption of raw milk has been claimed to reduce lactose intolerance, and raw milk has been claimed to contain natural lactase enzymes that are not found in heated milk because they are destroyed by heat. However, scientific evidence supporting this claim is lacking. Claeys et al. reported that lactase is absent from raw and heated milks and its production by LAB in raw milk is limited because raw milk must be refrigerated for safety reasons (Clawin-Rädecker et al., 2021).

General Conclusions and Suggestions

Raw milk consumption is a real threat to human health and a general hazard because raw milk can act as a vector of pathogens and spoilage microorganisms. Processing milk through heat treatment ensures the safety of milk but does not fully allow the primary organoleptic and nutritional characteristics of raw milk to be preserved. Good agricultural, hygiene and farm farming practices enable obtaining high-quality raw milk, which in turn allows for heat treatment with low intensity and thus raw milk preservation. The beneficial effects of raw milk consumption on human health include an inverse link between the consumption of raw milk in childhood and development of asthma, allergies and atopia. However, further research is needed to explain the purported protective effect of raw milk on the onset of asthma and allergy disorders in children.

References

1. Abebe, E., Gugsa, G., & Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of tropical medicine*, *2020*.
2. Aguilera-Toro, M., Poulsen, N. A., Akkerman, M., Rauh, V., Larsen, L. B., & Nielsen, S. D. H. (2022). Development in maillard reaction and dehydroalanine pathway markers during storage of uht milk representing differences in casein micelle size and sedimentation. *Foods*, *11*(10), 1525.
3. Ajmal, M., Nadeem, M., Imran, M., & Junaid, M. (2018). Lipid compositional changes and oxidation status of ultra-high temperature treated Milk. *Lipids in health and disease*, *17*(1), 1-11.
4. Albonico, F., Barelli, C., Albanese, D., Manica, M., Partel, E., Rosso, F., ... & Hauffe, H. C. (2020). Raw milk and fecal microbiota of commercial Alpine dairy cows varies with herd, fat content and diet. *Plos one*, *15*(8), e0237262.
5. Alegbeleye, O. O., Guimarães, J. T., Cruz, A. G., & Sant'Ana, A. S. (2018). Hazards of a 'healthy'trend? An appraisal of the risks of raw milk consumption and the potential of novel treatment technologies to serve as alternatives to pasteurization. *Trends in Food Science & Technology*, *82*, 148-166.
6. Alhomoch, F. (2021). Influence of different technical elements and settings in automatic milking systems on the quality of dairy cows' milk.
7. Authority, E. F. S. (2019). The European Union one health 2018 zoonoses report. *EFSA Journal*, *17*(12).

8. Bakar, S. Y. B. A., Salim, M., Clulow, A. J., Nicholas, K. R., & Boyd, B. J. (2021). Human milk composition and the effects of pasteurisation on the activity of its components. *Trends in Food Science & Technology*, *111*, 166-174.
9. Balaji, K., JebaMercy, G., & Balamurugan, K. (2019). Role of Bacteria in Blood Infections. In *Pocket Guide to Bacterial Infections* (pp. 375-390). CRC Press.
10. Berge, A. C., & Baars, T. (2020). Raw milk producers with high levels of hygiene and safety. *Epidemiology & Infection*, *148*, e14.
11. Bezie, A. (2019). The effect of different heat treatment on the nutritional value of milk and milk products and shelf-life of milk products. A review. *J. Dairy Vet. Sci*, *11*(5), 555822.
12. Bintsis, T. (2018). Lactic acid bacteria as starter cultures: An update in their metabolism and genetics. *AIMS microbiology*, *4*(4), 665.
13. Biolcati, F., Ferrocino, I., Bottero, M. T., & Dalmasso, A. (2022). The bacterial and fungal microbiota of "Robiola di Roccaverano" protected designation of origin raw milk cheese. *Frontiers in Microbiology*, *12*, 776862.
14. Blackmore, E., Guarin, A., Vorley, W., Alonso, S., & Grace, D. (2022). Kenya's informal milk markets and the regulation–reality gap. *Development Policy Review*, *40*(3), e12581.
15. Bonardi, S., Le Guern, A. S., Savin, C., Pupillo, G., Bolzoni, L., Cavalca, M., & Pongolini, S. (2018). Detection, virulence and antimicrobial resistance of Yersinia enterocolitica in bulk tank milk in Italy. *International dairy journal*, *84*, 46-53.
16. Brett, J., Kelton, D., Majowicz, S. E., Snedeker, K., & Sargeant, J. M. (2011). A systematic review and meta-analysis of the effects of pasteurization on milk vitamins, and evidence for raw milk consumption and other health-related outcomes. *Journal of food protection*, *74*(11), 1814-1832.
17. Broersen, K. (2020). Milk processing affects structure, bioavailability and immunogenicity of β-lactoglobulin. *Foods*, *9*(7), 874.
18. Bruyand, M., Mariani-Kurkdjian, P., Gouali, M., De Valk, H., King, L. A., Le Hello, S., ... & Loirat, C. (2018). Hemolytic uremic syndrome due to Shiga toxin-producing Escherichia coli infection. *Medecine et maladies infectieuses*, *48*(3), 167-174.
19. Burakoff, A., Brown, K., Knutsen, J., Hopewell, C., Rowe, S., Bennett, C., & Cronquist, A. (2018). Outbreak of fluoroquinolone-resistant Campylobacter jejuni infections associated with raw milk consumption from a herdshare dairy—Colorado, 2016. *Morbidity and Mortality Weekly Report*, *67*(5), 146.
20. Castañeda-Salazar, R., del Pilar Pulido-Villamarín, A., Ángel-Rodríguez, G. L., Zafra-Alba, C. A., & Oliver-Espinosa, O. J. (2021). Isolation and identification of Salmonella spp. in raw milk from dairy herds in Colombia. *Brazilian Journal of Veterinary Research and Animal Science*, *58*, e172805-e172805.
21. Chen, W., Mi, J., Lv, N., Gao, J., Cheng, J., Wu, R., ... & Liao, X. (2018). Lactation stage-dependency of the sow milk microbiota. *Frontiers in microbiology*, *9*, 945.
22. Chiozzi, V., Agriopoulou, S., & Varzakas, T. (2022). Advances, applications, and comparison of thermal (pasteurization, sterilization, and aseptic packaging) against non-

thermal (ultrasounds, UV radiation, ozonation, high hydrostatic pressure) technologies in food processing. *Applied Sciences*, *12*(4), 2202.

23. Choudhary, S., Sharma, P., & Gaur, A. (2024). Prevention and control of milk-borne zoonoses. In *The Microbiology, Pathogenesis and Zoonosis of Milk Borne Diseases* (pp. 305-331). Academic Press.
24. Claeys, W. L., Cardoen, S., Daube, G., De Block, J., Dewettinck, K., Dierick, K., ... & Herman, L. (2013). Raw or heated cow milk consumption: Review of risks and benefits. *Food control*, *31*(1), 251-262.
25. Coelho, M. C., Malcata, F. X., & Silva, C. C. (2022). Lactic acid bacteria in raw-milk cheeses: From starter cultures to probiotic functions. *Foods*, *11*(15), 2276.
26. Dash, K. K., Fayaz, U., Dar, A. H., Shams, R., Manzoor, S., Sundarsingh, A., ... & Khan, S. A. (2022). A comprehensive review on heat treatments and related impact on the quality and microbial safety of milk and milk-based products. *Food Chemistry Advances*, *1*, 100041.
27. de Oliveira Neves, L. N., & de Oliveira, M. A. L. (2020). Quantification of lactose and lactulose in hydrolysed-lactose UHT milk using capillary zone electrophoresis. *International Dairy Journal*, *106*, 104710.
28. De Silvestri, A., Ferrari, E., Gozzi, S., Marchi, F., & Foschino, R. (2018). Determination of temperature dependent growth parameters in psychrotrophic pathogen bacteria and tentative use of mean kinetic temperature for the microbiological control of food. *Frontiers in Microbiology*, *9*, 3023.
29. Deeth, H. C. (2021). Heat-induced inactivation of enzymes in milk and dairy products. A review. *International Dairy Journal*, *121*, 105104.
30. Deneke, T. T., Bekele, A., Moore, H. L., Mamo, T., Almaw, G., Mekonnen, G. A., ... & Berg, S. (2022). Milk and meat consumption patterns and the potential risk of zoonotic disease transmission among urban and peri-urban dairy farmers in Ethiopia. *BMC Public Health*, *22*(1), 222.
31. Dieterich, W., Schink, M., & Zopf, Y. (2018). Microbiota in the gastrointestinal tract. *Medical Sciences*, *6*(4), 116.
32. Du, B., Meng, L., Liu, H., Zheng, N., Zhang, Y., Guo, X., ... & Wang, J. (2020). Impacts of milking and housing environment on milk microbiota. *Animals*, *10*(12), 2339.
33. Dubey, K. K., Raj, T., & Kumar, P. (2022). Pathogenic microorganisms in milk: their source, hazardous role and identification. In *Advances in Dairy Microbial Products* (pp. 145-161). Woodhead Publishing.
34. Edwards, P. J., & Jameson, G. B. (2020). Structure and stability of whey proteins. In *Milk proteins* (pp. 251-291). Academic Press.
35. Elisashvili, V., Kachlishvili, E., & Chikindas, M. L. (2019). Recent advances in the physiology of spore formation for Bacillus probiotic production. *Probiotics and antimicrobial proteins*, *11*, 731-747.

36. Estévez, M., & Xiong, Y. (2019). Intake of oxidized proteins and amino acids and causative oxidative stress and disease: recent scientific evidences and hypotheses. *Journal of food science*, *84*(3), 387-396.
37. European Food Safety Authority (EFSA), Clawin-Rädecker, I., De Block, J., Egger, L., Willis, C., Da Silva Felicio, M. T., & Messens, W. (2021). The use of alkaline phosphatase and possible alternative testing to verify pasteurisation of raw milk, colostrum, dairy and colostrum-based products. *EFSA Journal*, *19*(4), e06576.
38. Fusco, V., Chieffi, D., Fanelli, F., Logrieco, A. F., Cho, G. S., Kabisch, J., ... & Franz, C. M. (2020). Microbial quality and safety of milk and milk products in the 21st century. *Comprehensive Reviews in Food Science and Food Safety*, *19*(4), 2013-2049.
39. Gad, S., Sheta, M. M., Al-Khalafawi, A. I., Abu El-Fadl, H. A., Anany, M., Sahmoud, S., & Amin, M. K. (2021). Expressed Breast Milk Contamination in Neonatal Intensive Care Unit. *Pediatric Health, Medicine and Therapeutics*, 307-313.
40. Gerrard, Z. E., Swift, B. M., Botsaris, G., Davidson, R. S., Hutchings, M. R., Huxley, J. N., & Rees, C. E. (2018). Survival of Mycobacterium avium subspecies paratuberculosis in retail pasteurised milk. *Food microbiology*, *74*, 57-63.
41. Giacometti, F., Serraino, A., Finazzi, G., Daminelli, P., Losio, M. N., Bonilauri, P., ... & Zanoni, R. G. (2012). Foodborne pathogens in in-line milk filters and associated on-farm risk factors in dairy farms authorized to produce and sell raw milk in northern Italy. *Journal of food protection*, *75*(7), 1263-1269.
42. Gille, D., & Schmid, A. (2015). Vitamin B12 in meat and dairy products. *Nutrition reviews*, *73*(2), 106-115.
43. Górska-Warsewicz, H., Rejman, K., Laskowski, W., & Czeczotko, M. (2019). Milk and dairy products and their nutritional contribution to the average polish diet. *Nutrients*, *11*(8), 1771.
44. Hahne, J., Isele, D., Berning, J., & Lipski, A. (2019). The contribution of fast growing, psychrotrophic microorganisms on biodiversity of refrigerated raw cow's milk with high bacterial counts and their food spoilage potential. *Food microbiology*, *79*, 11-19.
45. Huppertz, T., & Chia, L. W. (2021). Milk protein coagulation under gastric conditions: A review. *International Dairy Journal*, *113*, 104882.
46. Islam, M. Z., Uddin, M. E., Rahman, M. T., Islam, M. A., & Harun-ur-Rashid, M. (2021). Isolation and characterization of dominant lactic acid bacteria from raw goat milk: Assessment of probiotic potential and technological properties. *Small Ruminant Research*, *205*, 106532.
47. Joseph, A., Cointe, A., Mariani Kurkdjian, P., Rafat, C., & Hertig, A. (2020). Shiga toxin-associated hemolytic uremic syndrome: A narrative review. *Toxins*, *12*(2), 67.
48. Kenyon, J., Inns, T., Aird, H., Swift, C., Astbury, J., Forester, E., & Decraene, V. (2020). Campylobacter outbreak associated with raw drinking milk, North West England, 2016. *Epidemiology & Infection*, *148*, e13.
49. Kontopodi, E., Boeren, S., Stahl, B., van Goudoever, J. B., van Elburg, R. M., & Hettinga, K. (2022). High-temperature short-time preserves human milk's bioactive proteins and

their function better than pasteurization techniques with long processing times. *Frontiers in pediatrics*, *9*, 798609.
50. Kou, X., Cai, H., Huang, S., Ni, Y., Luo, B., Qian, H., ... & Wang, X. (2021). Prevalence and characteristics of Staphylococcus aureus isolated from retail raw milk in Northern Xinjiang, China. *Frontiers in Microbiology*, *12*, 705947.
51. Lampe, M., & Sharp, P. (2019). *A land of milk and butter: how elites created the modern Danish dairy industry*. University of Chicago Press.
52. Li, N., Wang, Y., You, C., Ren, J., Chen, W., Zheng, H., & Liu, Z. (2018). Variation in raw milk microbiota throughout 12 months and the impact of weather conditions. *Scientific reports*, *8*(1), 2371.
53. Li, S., Ye, A., & Singh, H. (2021). Impacts of heat-induced changes on milk protein digestibility: A review. *International Dairy Journal*, *123*, 105160.
54. Lindsay, D., Robertson, R., Fraser, R., Engstrom, S., & Jordan, K. (2021). Heat induced inactivation of microorganisms in milk and dairy products. *International Dairy Journal*, *121*, 105096.
55. Lopez Franco, J. (2019). Correlations between concentration of vitamin B12 in milk and the composition of the bovine microbiota.
56. Loss, G., Apprich, S., Waser, M., Kneifel, W., Genuneit, J., Büchele, G., ... & Gabriela Study Group. (2011). The protective effect of farm milk consumption on childhood asthma and atopy: the GABRIELA study. *Journal of Allergy and Clinical Immunology*, *128*(4), 766-773.
57. Mahfuz, C., & Swapnil, M. (2022). Value Chain Analysis Of Ghee In The Pabna District Of Bangladesh.
58. Mangla, S. K., Kazancoglu, Y., Ekinci, E., Liu, M., Özbiltekin, M., & Sezer, M. D. (2021). Using system dynamics to analyze the societal impacts of blockchain technology in milk supply chainsrefer. *Transportation Research Part E: Logistics and Transportation Review*, *149*, 102289.
59. Martin, N. H., Boor, K. J., & Wiedmann, M. (2018). Symposium review: Effect of post-pasteurization contamination on fluid milk quality. *Journal of Dairy Science*, *101*(1), 861-870.
60. McLauchlin, J., Aird, H., Elliott, A., Forester, E., Jørgensen, F., & Willis, C. (2020). Microbiological quality of raw drinking milk and unpasteurised dairy products: results from England 2013–2019. *Epidemiology & Infection*, *148*.
61. Merlino, V. M., Mosca, O., Blanc, S., Sparacino, A., Massaglia, S., Borra, D., ... & Fornara, F. (2023). The role of socio-demographic variables and buying habits in determining milk purchasers' preferences and choices. *Frontiers in Nutrition*, *10*, 1072208.
62. Mintz, M. J., & Lukin, D. J. (2023). Mycobacterium avium subspecies paratuberculosis (MAP) and Crohn's disease: the debate continues. *Translational Gastroenterology and Hepatology*, *8*.

63. Mogotu, M. W. (2021). *Investigating Hygienic Practices and Microbial Safety of Milk Supplied by Small-holder Farmers to Processors in Bomet, Nakuru and Nyeri Counties in Kenya* (Doctoral dissertation, Uon).
64. Morelli, L., Amrani, N., Goulet, O., & Lukito, W. (2019). Lactose intolerance: clinical symptoms, Dieterich, W., Schink, M., & Zopf, Y. (2018). Microbiota in the gastrointestinal tract. *Medical Sciences*, *6*(4), 116. diagnosis and treatment. *Global Diabetes Open Access Journal*, *1*(1), 1-10.
65. Mulakala, B. K. (2019). *Comparative Studies of Galectin Gene Expression and Secretion in Cow Milk and Blood* (Doctoral dissertation, North Carolina Agricultural and Technical State University).
66. Mullan, W. M. A. (2019). Are we closer to understanding why viable cells of Mycobacterium avium subsp. paratuberculosis are still being reported in pasteurised milk?. *International Journal of Dairy Technology*, *72*(3), 332-344.
67. Nichols, M., Conrad, A., Whitlock, L., Stroika, S., Strain, E., Weltman, A., ... & Williams, I. (2020). Multistate outbreak of Listeria monocytogenes infections retrospectively linked to unpasteurized milk using whole-genome sequencing. *Journal of Dairy Science*, *103*(1), 176-178.
68. O'Callaghan, T. F., Sugrue, I., Hill, C., Ross, R. P., & Stanton, C. (2019). Nutritional aspects of raw milk: A beneficial or hazardous food choice. In *Raw milk* (pp. 127-148). Academic Press.
69. Oikonomou, G., Addis, M. F., Chassard, C., Nader-Macias, M. E. F., Grant, I., Delbès, C., ... & Even, S. (2020). Milk microbiota: what are we exactly talking about?. *Frontiers in microbiology*, *11*, 60.
70. Oliveira, G. S., Lopes, D. R. G., Andre, C., Silva, C. C., Baglinière, F., & Vanetti, M. C. D. (2019). Multispecies biofilm formation by the contaminating microbiota in raw milk. *Biofouling*, *35*(8), 819-831.
71. Pathot, Y. D. (2019). Hygienic practices and bacteriological quality of milk: A review. *Int. J. Res. Granthaalayah*, *7*, 341-356.
72. Perin, L. M., Pereira, J. G., Bersot, L. S., & Nero, L. A. (2019). The microbiology of raw milk. In *Raw milk* (pp. 45-64). Academic Press.
73. Ramanujam, H., & Palaniyandi, K. (2023). Bovine tuberculosis in India: the need for One Health approach and the way forward. *One Health*, 100495.
74. Rathod, N. B., Kahar, S. P., Ranveer, R. C., & Annapure, U. S. (2021). Cold plasma an emerging nonthermal technology for milk and milk products: A review. *International Journal of dairy technology*, *74*(4), 615-626.
75. Regasa, S., Mengistu, S., & Abraha, A. (2019). Milk safety assessment, isolation, and antimicrobial susceptibility profile of Staphylococcus aureus in selected dairy farms of Mukaturi and Sululta town, Oromia region, Ethiopia. *Veterinary medicine international*, *2019*.
76. Reichler, S. J., Trmčić, A., Martin, N. H., Boor, K. J., & Wiedmann, M. (2018). Pseudomonas fluorescens group bacterial strains are responsible for repeat and sporadic

postpasteurization contamination and reduced fluid milk shelf life. *Journal of Dairy Science*, *101*(9), 7780-7800.

77. Saha, S., Majumder, R., Rout, P., & Hossain, S. (2024). Unveiling the significance of psychrotrophic bacteria in milk and milk product spoilage-A review. *The Microbe*, 100034.
78. Schlech III, W. F. (2019). Epidemiology and clinical manifestations of Listeria monocytogenes infection. *Microbiology Spectrum*, *7*(3), 7-3.
79. Sharp, E., D'Cunha, N. M., Ranadheera, C. S., Vasiljevic, T., Panagiotakos, D. B., & Naumovski, N. (2021). Effects of lactose-free and low-lactose dairy on symptoms of gastrointestinal health: A systematic review. *International Dairy Journal*, *114*, 104936.
80. Silva, E., Oliveira, J., Silva, Y., Urbano, S., Sales, D., Moraes, E., ... & Anaya, K. (2021). Lactoperoxidase system in the dairy industry: challenges and opportunities.
81. Sozańska, B. (2019). Raw cow's milk and its protective effect on allergies and asthma. *Nutrients*, *11*(2), 469.
82. Tapia, M. S., Alzamora, S. M., & Chirife, J. (2020). Effects of water activity (aw) on microbial stability as a hurdle in food preservation. *Water activity in foods: Fundamentals and applications*, 323-355.
83. Thompson, L. A., & Darwish, W. S. (2019). Environmental chemical contaminants in food: review of a global problem. *Journal of toxicology*, *2019*.
84. Tumbarski, Y. (2019). Epidemiology and prevalence of Campylobacter infections in the European Union and Bulgaria between 2010 and 2017 (A Review). *Bulgarian J Vet Med*, *22*(1), 160-5.
85. Udovicki, B., Djekic, I., Kalogianni, E. P., & Rajkovic, A. (2019). Exposure assessment and risk characterization of aflatoxin M1 intake through consumption of milk and yoghurt by student population in Serbia and Greece. *Toxins*, *11*(4), 205.
86. Ung, A., Baidjoe, A. Y., Van Cauteren, D., Fawal, N., Fabre, L., Guerrisi, C., ... & Le Hello, S. (2019). Disentangling a complex nationwide Salmonella Dublin outbreak associated with raw-milk cheese consumption, France, 2015 to 2016. *Eurosurveillance*, *24*(3), 1700703.
87. van den Brom, R., de Jong, A., van Engelen, E., Heuvelink, A., & Vellema, P. (2020). Zoonotic risks of pathogens from sheep and their milk borne transmission. *Small ruminant research*, *189*, 106123.
88. van Lieshout, G. A., Lambers, T. T., Bragt, M. C., & Hettinga, K. A. (2020). How processing may affect milk protein digestion and overall physiological outcomes: A systematic review. *Critical Reviews in Food Science and Nutrition*, *60*(14), 2422-2445.
89. Velázquez-Ordoñez, V., Valladares-Carranza, B., Tenorio-Borroto, E., Talavera-Rojas, M., Varela-Guerrero, J. A., Acosta-Dibarrat, J., ... & Pareja, L. (2019). Microbial contamination in milk quality and health risk of the consumers of raw milk and dairy products. *Nutrition in Health and disease-our challenges Now and Forthcoming time*.

90. Voidarou, C., Antoniadou, M., Rozos, G., Tzora, A., Skoufos, I., Varzakas, T., ... & Bezirtzoglou, E. (2020). Fermentative foods: Microbiology, biochemistry, potential human health benefits and public health issues. *Foods*, *10*(1), 69.
91. Wanjala, W. N., Nduko, J. M., & Mwende, M. C. (2018). Coliforms contamination and hygienic status of milk chain in emerging economies. *Journal of Food Quality & Hazards Control*, *5*(1).
92. Wei, Q., Wang, X., Sun, D. W., & Pu, H. (2019). Rapid detection and control of psychrotrophic microorganisms in cold storage foods: A review. *Trends in Food Science & Technology*, *86*, 453-464.
93. Yuan, L., Sadiq, F. A., Burmølle, M., Liu, T., & He, G. (2018). Insights into bacterial milk spoilage with particular emphasis on the roles of heat-stable enzymes, biofilms, and quorum sensing. *Journal of Food Protection*, *81*(10), 1651-1660.
94. Zhang, D. (2020). *The effect of psychrotrophic bacteria on the quality of UHT milk: a thesis presented in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Food Microbiology, The School of Food and Advanced Technology, Massey University, Palmerston North, New Zealand* (Doctoral dissertation, Massey University).
95. Zhang, L., Zhou, R., Zhang, J., & Zhou, P. (2021). Heat-induced denaturation and bioactivity changes of whey proteins. *International Dairy Journal*, *123*, 105175.