

Bacteria in the milk of sheep with or without mastitis- mini Review

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SUMMARY

From a nutritional point of view, sheep milk is more valuable than cow and goat milk and the interest for sheep milk is increasing in many countries. However, sheep milk is easily contaminated during milking, handling, and transport and it is an ideal medium for bacterial propagation. Consequently, sheep milk spoils quite quickly. The proper, clean handling of milk is not only of sanitarian interest, but it also serves the farmers' interests, because contaminated milk may not be distributed, and is unsuitable for producing good quality products. Following this technological trend, this review addresses the bacterial composition of sheep milk with and without mastitis. Even though sheep milk contains a lot of bacteria, this review article highlighted total plate count, Enterobacteriaceae, coliform, Staphylococcus aureus, Escherichia coli, Listeria monocytogenes, Campylobacter, Salmonella spp. and Streptococcus spp. Mastitis in sheep is a vital cause of mortality, reduction in milk production and early culling. The reported risk factors for mastitis in sheep were age, a case of mastitis, breed, husbandry systems, and location. The main priority should be implementation of programs to minimize human pathogenic bacteria and mastitis in raw ewe milk.

Keywords: Mastitis, microorganisms, sheep milk

INTRODUCTION

According to Tsakalidou and Odos (2012), ewe milk is seldom consumed but 1.4% of global milk production is ewe milk as it is regularly used all over Europe in the manufacturing of cheese. Milk production by sheep has grown over the years and is now in search of new consumer markets (Selvaggi et al., 2014). The study from Greece by Fotou et al. (2011) conveyed that lactic acid bacteria dominated the microbial composition of sheep milk, with bacteria that grows best in moderate temperature representing 10^2 - 10^6 cfu/ml, whereas bacteria that grow at low temperature correspond to 10^2 - 10^4 cfu/ml. Different studies evaluated the effect of storage of ewe milk at refrigerator confirmed increases in not only in bacteria proliferating at low temperature but also in bacteria which grows best at medium temperature. However, the bacteria that is capable of surviving high temperature did not increase. Bacteria that were discovered occasionally can include microorganisms of concern from a milk safety outlook, comprising *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *Bacillus*, and *Clostridium perfringens* (Fotou et al., 2011).

Bacterial composition of ewe milk can be affected by not only the nutritional composition of milk but also the location where sheep farm is found (Yabrir et al., 2013). Many studies noted that ewe milk with a high-fat content resulted in greater counts of lactic acid bacteria, coliforms, and molds. In more acidic milk and milk with a lower level of nutrients, there were higher and lower populations of streptococci and *S. aureus* (Yabrir et al., 2013). Nevertheless, sheep milk production is booming, it does not have as high a

production yield as another animal's milk due to seasonal dependency (Albenzio et al., 2016).

According to the finding of Mennane et al. (2007), sheep milk and its derivatives constitute a conducive environment to the growth of microorganisms, especially disease-causing bacteria and fungi; this is why they have always been considered as one of the main causes of food poisoning. Sheep milk quality and shelf life can negatively be impacted by bacteria; for instance, bacteria that can grow at low temperature can reproduce during refrigeration and, via the production of extracellular lipases and proteases, resulting in putrefaction (Hantsis-Zacharov and Halpern, 2007). The report of Oliver et al. (2009) indicated that the microorganisms in sheep milk can have well-being linked indications in the consumption of raw ewe milk with disease-causing microorganisms which can lead to in some cases severe illness. Therefore, this review was aimed with the emphasis on sheep milk bacterial composition and prevalence of mastitis in sheep.

BACTERIAL COMPOSITION OF SHEEP MILK

Hygienic indicators in sheep milk

The hygienic standards for the production and distribution of ewe milk are less strict, although there is increasing demand by consumers. The Regulation (EC) 853/2004 of the European Parliament and of the Council for raw milk hygiene has been implemented in European Union countries, which is basically focused in cow milk hygiene (EC, 2004). According to the Regulation (EC) 853/2004 of the European Parliament and of the Council (EC, 2004), for milk from animal species other than cows which will undergo pasteurization must not exceed total plate count (TPC) of 1.5×10^6 cfu/ml and which is intended for processing

without heat treatment must not exceed TPC of 5×10^5 cfu/ml.

Sheep raw milk potentially contains a diverse bacterial population. The occurrence of bacteria in ewe milk could be from various sources, mainly the environment of the farm and from the body of the animal to the milk (Quigley et al., 2013). Bacteria enter milk from these sources and, once in milk, can play a number of roles, such as facilitating dairy fermentations, causing spoilage or causing disease. Bogdanovičova et al. (2016) from the Czech Republic reported that sheep milk bacteriological quality can be determined using different parameters, such as the number of *Enterobacteriaceae* and the total bacterial count (TBC).

The study from Vermont by D'Amico and Donnelly (2010) pointed out that there was no significant difference in the TBC results of raw milk from sheep, goat, and cows. The TBC results of sheep milk differ according to the month in which the animal is milked, the milking system, the frequency of milking and the size of the herd (Alexopoulos et al., 2011). According to Alexopoulos et al. (2011) study, the results showed

as an average, TBC was 5.48 lg cfu/ml, somatic cell count (SCC) was 6.05 lg cells/ml, coliforms: 4.49 lg cfu/ml and environmental *Streptococcus* counts was 4.95 lg cfu/ml. Similarly, the study in Switzerland revealed that the TBC from bulk-tank of ewe was 4.78 lg cfu/ml; *Enterobacteriaceae* were detected in 45 (71.4%) ewe's milk samples (Muehlherr et al., 2003).

Somatic cell count is also a good indicator of the quality of sheep milk. Somatic cell count is generally considered as an indicator of udder health. High number of SCC in ewe milk is an indication that we are dealing with unsanitary milk and weakened ewe health (Bytyqi et al., 2013). However, there is no accepted threshold that can permit to differentiate between healthy and infected udders in dairy ewes, Berthelot et al. (2006) reported the udder as healthy if individual SCC is lower than 0.5×10^6 cells/ml, and infected if at least two individual SCC were higher than 1 or 1.2×10^6 cells/ml, while at the flock level, if SCC exceeded 0.65×10^6 cells/ml, they indicated up to 15% occurrence of mastitis. An overview of recent studies regarding the bacteriological quality and SCC of raw milk of sheep is shown in *Table 1*.

Table 1

Bacteriological quality and somatic cell count of sheep milk in different countries

Sample type	Country	N ^o of sample	Findings	References
REM	Egypt	35	The mean of TBC was 2.04×10^6 cfu/ml, <i>Enterobacteriaceae</i> count (EBC) was 1.67×10^5 cfu/ml and coliforms was 1.66×10^5 cfu/ml.	Ombarak and Elbagory, 2017
REM	Morocco	44	Total bacterial count ranged from 4.5×10^3 to 9.4×10^7 cfu/ml, 1.2×10^5 cfu/ml of total coliforms and 2×10^4 cfu/ml of fecal coliforms.	Bouazza et al., 2015
REM	Egypt	50	The results revealed that the presence of coliforms, yeast, and mold with mean values of $8.8 \times 10^3 \pm 5.3 \times 10^3$, $3.8 \times 10^3 \pm 0.9 \times 10^3$ and $1.6 \times 10^3 \pm 0.2 \times 10^3$ cfu/ml, respectively.	El-Makarem, 2016
BTM	Switzerland	63	Ewe's milk samples were tested for standard plate count and it was 4.79 lg cfu/ml.	Zweifel et al., 2005
REM	Algeria	105	The result revealed that TBC was 90.2×10^3 cfu/ml and EBC was 10.8×10^2 cfu/ml.	Fatima et al., 2013
REM	Greece	240	Total mesophilic flora ranged from 6.4×10^2 to 6.4×10^6 cfu/ml and total number of psychrotrophic bacteria was from 5×10^2 to 5.1×10^4 cfu/ml.	Fotou et al., 2011
REM	Hungary	24	The mean values of TPC were 3.25, 3.60 and 3.81 lg cfu/ml in milk samples from Dorper, Merino, and Cigaja, respectively. EBC was less than 10 cfu/ml.	Tonamo et al., 2018
REM	Slovakia	1192	The negative effect of high SCC on milk yield was observed in all farms and milk samples had SCC below 0.6×10^6 cells/ml.	Tančin et al., 2017a
REM	Slovakia	2159	82.03% of the tested samples were below 4.0×10^5 cells/ml and only 8.89% over 1.0×10^6 cells/ml indicating a good status of udder health in tested animals.	Tančin et al., 2017b

REM: raw ewe milk, BTM: bulk tank milk, TBC: total bacterial count, EBC: *Enterobacteriaceae* count, SCC: somatic cell count

Pathogens in sheep milk

This review confirmed that many literature indicated that most of the disease outbreaks were related to the consumption of raw milk, which contains pathogenic microorganisms. The finding of Oliver et al. (2009) from the United States within six years confirmed that 12 disease outbreaks from raw milk samples that were caused by *Listeria monocytogenes* (1

outbreak), *Campylobacter* spp. (5 outbreaks), *Salmonella* spp. (4 outbreaks), and verotoxigenic *Escherichia coli* (2 outbreaks). According to the report by the European Food Safety Authority (EFSA) in 2017 (EFSA-ECDC, 2018), out of 33 outbreaks, 18 (54.5%) strong-evidence outbreaks were caused by *Campylobacter* spp. originated from raw milk. Raw milk is a significant source of human infection due to



Campylobacter (EFSA-ECDC, 2018). *Campylobacter* spp. was also reported in raw bulk tank milk from sheep in Scotland and the frequency of occurrence was 2.2% (Verraes et al., 2014).

Staphylococcus aureus and *Escherichia coli* were the two most important bacteria in case of ewe bulk tank milk (de Garnica et al., 2011). *S. aureus* is one of the highly prevalent pathogens in unpasteurized ewe milk (Marogna et al., 2012). The report from Switzerland indicated that from 63 milk samples from bulk tank examined, 33.3% were *S. aureus* positive (Muehlherr et al., 2003). According to the report by Alexopoulos et al. (2011) all the examined bulk tank milk samples were *S. aureus* positive in Greece. D'Amico and Donnelly (2010) found that in case of the examined 15 bulk tank milk samples, 47% of the samples were *S. aureus* positive in US. In addition, the research of Bogdanovičova et al. (2016) revealed that in case of the examined 23 bulk tank milk samples, 39.1% of the samples were *S. aureus* positive in Czech Republic. The research conducted by Normanno et al. (2007) in Italy indicated that *S. aureus* is a common pathogen that causes slight skin infection to more severe diseases such as pneumonia and septicemia.

Shiga toxin-producing *Escherichia coli* (STEC) is one of the emerging pathogen related to the consumption of raw milk (Meng et al., 2013). The study by Solomakos et al. (2009) pointed out that gastrointestinal diseases, food poisoning, and even death in some cases make this microorganism an insidious risk to human health. The European Union summary report on trends and sources of zoonosis, zoonotic agents and food-borne outbreaks showed as the most commonly reported STEC serotype in 2017 was O157 (31.93%) followed by O26, O103 and O91

serotypes. Serotype group O157 was the most frequently reported cause of haemolytic-uraemic syndrome cases in 2017 (EFSA-ECDC, 2018). From Table 2 below, *E. coli* O157 was detected in Greece, 0.84% (Solomakos et al., 2009), Czech Republic 4.4% (Bogdanovičova et al., 2016) and Spain 10.1% (Rey et al., 2006). Another study from Switzerland and United States reported also 12.1% (Muehlherr et al., 2003) and 0.75% (D'Amico and Donnelly, 2008), respectively.

This review noted that most of the studies from bulk milk tank samples in Europe indicated that the frequencies of occurrence of *L. monocytogenes* ranged from 4.9 to 6.1% (Ruusunen et al., 2013). The study from farm tank milk in Czech Republic revealed that 4.4% prevalence of *L. monocytogenes* (Bogdanovičova et al., 2016). On the other hand, a recent report from Estonia confirmed that 29% prevalence of *L. monocytogenes* in the bulk tank milk samples (Kalmus et al., 2015). The review of Verraes et al. (2014) confirmed that the frequency of *L. monocytogenes* in raw sheep milk is below 4%.

Salmonella spp. have been detected in raw milk from sheep and the frequency of occurrence was low and was 5% (Fotou et al., 2011). However, different pieces of literature indicated the total absence of *Salmonella* spp. in raw milk of sheep. *Streptococcus* spp. have been detected in raw milk from sheep (Table 2). The study by Alexopoulos et al. (2011) revealed the mean value of *Streptococcus* spp. was 4.95 lg cfu/ml. However, the studies did not always mention whether the strains involved were able to produce toxins. An overview of recent studies regarding frequency of occurrence of *Salmonella* spp. and *Streptococcus* spp. in raw milk of sheep is presented in Table 2.

Table 2

Frequency of occurrence of pathogenic microorganisms in raw milk of sheep

Microorganisms	Type of sample	Country	N ^o of sample	Frequency of occurrence (%)	References
<i>Salmonella</i> spp.	Bulk tank milk	Greece	240	5	Fotou et al., 2011
	Bulk tank milk	Greece	595	0.84	Solomakos et al., 2009
Human pathogenic <i>E. coli</i>	Bulk tank milk	Czech Republic	23	4.4	Bogdanovičova et al., 2016
	Bulk tank milk and fresh cheese curds	Spain	287	10.1	Rey et al., 2006
<i>Listeria monocytogenes</i>	Bulk tank milk	Czech Republic	23	4.4	Bogdanovičova et al., 2016
	Bulk tank milk	Switzerland	63	33.3	Muehlherr et al., 2003
<i>Staphylococcus aureus</i>	Farm tank milk	Czech Republic	23	39.1	Bogdanovičova et al., 2016
	Bulk tank milk	US	15	47	D'Amico and Donnelly, 2010
	Bulk tank milk	Greece	71	100	Alexopoulos et al., 2011
<i>Streptococcus</i> spp.	Bulk tank milk	Greece	65	100	Alexopoulos et al., 2011
<i>Mycobacterium avium</i>	Bulk tank milk	Switzerland	63	23.8	Muehlherr et al., 2003



Mastitis in sheep and its risk factors

Khan and Khan (2006) defined mastitis as swelling of the mammary gland usually caused by bacterial infection. According to Marogna et al. (2012), mastitis in sheep reduces the economic value of sheep for milk production due to reduced lifespan of ewes. The review of Bergonier et al. (2003) indicated that most of the reports on subclinical mastitis are from dairy sheep, where the prevalence ranges from 10 to 50%. As indicated in *Table 3* below, the prevalence of mastitis was highest in Netherland with 94% (Koop et al., 2010) followed by 85% in Norway (Mørk et al., 2007). Only 2.5% prevalence was recorded in UK as reported by Claire et al. (2016). This review underlined that mastitis in dairy ewe results in a reduction in milk yield, ewe and lamb death, culling of chronically diseased ewes (Conington et al., 2008), and decreased live- weight gain in lambs (Huntley et al., 2012).

This review confirmed that from several pathogenic microorganisms, *S. aureus* is the most common clinical mastitis causing bacteria in sheep (Mavrogianni et al., 2011) and the report by Mørk et al., (2007) indicated that coagulase-negative staphylococci (CNS) was the

most common cause of subclinical mastitis in dairy sheep. The review report by Bergonier et al. (2003) showed that the frequency of occurrence of clinical and sub-clinical mastitis in dairy ewes that is caused by *S. aureus* ranges from 20 to 60%. However, this review confirmed that it ranges from 2.5 to 94% (Mørk et al., 2007; Arsenault et al., 2008; Koop et al., 2010; Marogna et al., 2010; Ozenc et al., 2011; Spanu et al., 2011; Blagitz et al., 2014; Claire et al., 2016).

The study by Marogna et al. (2010) in Italian and German flocks revealed that the prevalence of mastitis caused by *Staphylococcus* spp. ranges from 20 to 30% and unsuitable housing conditions and unhygienic milking practices were reported risk factor. In addition to *S. aureus* and CNS, other bacteria that were related with the occurrence of mastitis in dairy ewe includes *Bacillus cereus*, *Clostridium* spp. (Fotou et al., 2011), *Corynebacterium* spp. (Spanu et al., 2011), *Enterococcus* spp. (Marogna et al., 2010), *Listeria monocytogenes* (Brugère-Picoux, 2008), *Mycobacterium* spp. (Nebbia et al., 2006). An overview of the frequency of occurrence of mastitis in dairy ewe is presented in *Table 3* below.

Table 3

Prevalence of mastitis in dairy sheep in different countries

Country	Sample number	Prevalence (%)	References
UK	90	2.5	Claire et al., 2016
USA	426	10	Spanu et al., 2011
Turkey	464	11	Ozenc et al., 2011
Canada	696	18	Arsenault et al., 2008
Brazil	550	24	Blagitz et al., 2014
Italy	2198	50	Marogna et al., 2010
Norway	547	85	Mørk et al., 2007
Netherland	31	94	Koop et al., 2010

Various factors associated with management of ewes have been reported as possible risk factors for mastitis. Of these, some can be related to specific causative bacterial agents. Our review, inveterate that as indicated by many studies, the litter size, age, case of mastitis, ewe breed, husbandry systems and location are considered to be risk factors for mastitis in dairy sheep (Onnasch et al., 2002; Huntley et al., 2012; Cooper et al., 2013 and Pereira et al., 2014). Increased incidence risk of clinical and subclinical mastitis in ewes with vitamin A and selenium deficiencies has been reported (Koutsoumpas et al., 2013). Recently, reduced feed-energy availability has been recognized as a risk factor for mastitis in ewes (Barbagianni et al., 2015). A genetic background to increased susceptibility in mastitis in dairy ewes has also been reported (Bramis et al., 2014). These authors have suggested that, in a selection of ewes for resistance to mastitis, the use of reduced log-transformed somatic cell score may be employed as an indirect trait.

CONCLUSION

In conclusion, consumption of raw sheep milk poses problems to health due to human pathogenic bacteria in raw milk. In this review, bacteria in sheep milk with and without mastitis were collated based on the frequencies of occurrence of pathogenic microorganisms and disease outbreaks connected to consumption of raw ewe milk. *Staphylococcus aureus*, *Escherichia coli* and *Listeria monocytogenes* are highly prevalent pathogenic bacteria in raw sheep milk. *Staphylococcus aureus* and coagulase-negative staphylococci were the most common clinical and subclinical mastitis causing bacteria in dairy sheep, respectively. Mastitis in ewe results in the financial problem, loss of ewe and lambs, reduction of milk yield and loss of live-weight. Therefore, the main priority should be implementation of programs to minimize human pathogenic bacteria and mastitis in raw ewe milk.

ACKNOWLEDGEMENT

The publication is supported by the EFOP-3.6.3-VEKOP-16-2017-00008 project. The project is co-

financed by the European Union and the European Social Fund.

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