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MASTITIS IN EWES: SOMATIC CELL COUNTS, PATHOGENS AND ANTIBIOTIC RESISTANCE

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ABSTRACT

The aim of paper review is to describe the influence of somatic cell count (SCC) in the milk of ewes on the composition and milk production associated with the presence of mastitis pathogens as well as the assessment of the effects of non-infectious factors such as breed, number of lambs, order and stage of lactation, age, oestrus, livestock management, the impact of the month, the season to change the SCC in the milk of ewes. It also points to the possible physiological value of SCC in sheep milk for udder health assessment as it is generally accepted for dairy cows. The important part of this paper is to show antimicrobial resistance of mastitis pathogens. The more complex results obtained in Slovakia under experimental and practical conditions are discussed. In conclusion, taking into account all mentioned factors and studies, SCC could play important role in management of dairy ewes breeding to get acceptable milk performances and good udder health at comparable physiological levels as in dairy cows. Regular milk recording could significantly contribute to effort of farmers in mastitis control program.

Keywords: mastitis, somatic cell counts, pathogens, non-infectious factors, milk performance

INTRODUCTION

Sheep breeding has a rich tradition, history and indispensable position in the structure of animal production in Slovakia. Long-term research into milk production was performed in our conditions in the past (**Masár, 1968; Mikuš, 1973; Margetín *et al.*, 1995, 1996; Milerski *et al.*, 2006**). In recent years, the importance of research due to increasing demand of dairy for ewe's milk is increased. Thus these requirements force the animal practice to increase the milk production and its quality which reflect in the research aimed to study environmental influences and animal factors involved in milk production efficiency not only in our country (**Oravcova *et al.*, 2015; Mačuhová *et al.*, 2017; Baranovič *et al.*, 2018**) but also in abroad this is key view (**Gelasakis *et al.*, 2015**). Additionally, in 2017, a new breed of Slovak dairy sheep was approved by Ministry of agriculture and rural development of Slovak republic (based on the Improved Valachian, Tsigai as native breeds, Merino and dairy breeds of the Lacaune and East Friesian sheep milk to improve milk production) (detailed description in **Margetín *et al.*, 2017**, review). Increasing milk production in dairy practice improves the economics of breeding, but on the other hand, puts increased demands on management of breeding, new approaches to milking, feeding, health etc.
To ensure the profitability of dairy sheep breeding the good health status of ewes, mammary gland especially, is very important. The health status of the mammary gland is considered to be one of the decisive factors influencing milk production and its nutritional, hygienic and technological quality (**Gelasakis *et al.*, 2015**). The most frequent disease of mammary gland is mastitis with large negative economic impact on viability of farms and welfare of animals (**Mørk *et al.*, 2007**). Therefore, early diagnosis of the disease associated with effective treatment and further prevention of relapse, the emergence of new cases and the subsequent spread of mastitis in the herd is very necessary (**Blagitz *et al.*, 2014; Addis *et al.*, 2016; Persson *et al.*, 2017**). An irreplaceable place in mastitis prevention has regular monitoring of the udder's health status (test day, in Slovakia only a few farms measure SCC) and the identification of the risks of breeding systems (milking setting, machine cleaning and milking routine deserve attention in our country) associated with the mentioned disease. Appropriate preventative measures and breeding systems are also based on the identification of the causes of the disease and the immune response of the affected individual. From 2016 a new project started (APVV-15-0072) to more complex analyse the health status of dairy ewes raised in Slovakia and to study or found out the cause of high somatic cell counts in relation to presence of microorganisms. The genetics, molecular and immune aspects are involved as well. As it was mentioned by **Mørk *et al.* (2007)** differences in climate, production forms, and management practices may give rise to differences in epidemiology, bacteriology, and manifestations of mastitis. We would like to contribute to this world knowledge by more intensive research in our country.
The aim of the review is to summarize the knowledge and results of the health status of the mammary gland of ewes in the breeding conditions of Slovakian farming following by the research in this field in the world. The aim also includes prospects and activities for further research aimed at improving the health status of the mammary gland of dairy ewes.

MASTITIS

A serious health and economic problem in dairy ewes breeding is mastitis, which causes economic and breeding losses. Mastitis is inflammatory disease of mammary gland. Economical negative impact of mastitis in dairy sheep represent reduced milk production and its quality, increased cure costs (**Riggio and Portolano, 2015**). Mastitis contributes to increased costs of animal treatment, presence of pathogens and enterotoxins in milk which increases the risk for of human health (**Pilipčincová *et al.*, 2010**). Intramammary infections are important also in meat production flocks, as reduced milk yield of ewes has been shown to lead to below-average growth of their lambs and higher mortality (**Clements *et al.*, 2003; Moroni *et al.*, 2007; Gelasakis *et al.*, 2015; Bramis *et al.*, 2016**). According to the form, this disease can be divided into clinical and subclinical mastitis (**Bergonier and Berthelot, 2003; Mørk *et al.*, 2007; Bramis *et al.*, 2016**). Clinical mastitis is accompanied by symptoms such as swelling of the udder, fever, changes consistency and colour of milk, anorexia and significant decline in milk yield (**Giadinis *et al.*, 2012; Balabánová *et al.*, 2014; Olechnowicz and Jaskowski, 2014**). Clinical mastitis can lead to mortality or culling of up to 90% of mastitic ewes in the herd (**Olechnowicz and Jaskowski, 2014**). It is reported that the incidence of clinical mastitis in the ewes is lower than 5% but the occurrence of subclinical mastitis is much higher (**Bergonier and Berthelot, 2003; Moroni *et al.*, 2007; Kern *et al.*, 2013; Olechnowicz and Jaskowski, 2014**).

The subclinical mastitis isn't accompanied by symptoms and visual changes in milk. For diagnose of subclinical mastitis can be used SCC. The most critical level of SCC considered as possible subclinical mastitis occurrence is SCC over 1000×10^3 cells/ml (**Berthelot *et al.*, 2006**). Our preliminary results obtained in breeding practice based on SCC indicate that the incidence of subclinical mastitis according above mentioned threshold was 8.73% with the high variability among the farms (from 2 to 40%) though the highest SCC was found out in farm with Lacaune breed (**Tančin *et al.*, 2017a**). In another our study with Lacaune breed, only the percentage of subclinical mastitis was higher 19.21% with also high influence of farm (from 8 to 34%) (**Tančin *et al.*, 2017b**). **Zigo *et al.* (2017)** found out 5.9% incidence of subclinical mastitis in their research farms in eastern Slovakia. Thus under these results the breed could play role in the occurrence of subclinical mastitis but the most important factor is management of the farm. From the abroad study, the conclusion could be similar because of the prevalence of subclinical mastitis is variable and ranges from less than 9 to 50% (**Albenzio *et al.*, 2002; McDougall *et al.*, 2002; Hall and Rycroft, 2007; Olechnowicz and Jaskowski, 2014; Gelasakis *et al.*, 2015**). **Moroni *et al.* (2007)** found out that the incidence of subclinical intramammary infection was 51.2% in Bergamasca meat sheep. The prevalence of subclinical mastitis in ewes varied from 7.5% to 39% (**Rahman *et al.*, 2016**) and in another study subclinical mastitis was detected in 26% of ewes (**Vasileiou *et al.*, 2018a**). According **Bergonier *et al.* (2003)** and **Contreras *et al.* (2007)** the prevalence of subclinical mastitis ranged from 5 to 30%.

THE MOST COMMON MASTITIS PATHOGENS

Intramammary infection is generally caused by microbial agent attack, mainly by bacteria, viruses, fungi and algae (**Spuria *et al.*, 2017**). According to the origin of the organisms the mastitis could divided into contagious and environmental mastitis (**Quinn *et al.*, 2011**). Thus the mastitis pathogens could be classified as contagious (*Staphylococcus (S.) aureus*, *Streptococcus (Str.) agalactiae* and *Mycoplasma* sp.) and environmental pathogens (*Escherichia coli (E.)*, *Mannheimia (M.) haemolytica*, *Pseudomonas aeruginosa*, *Streptococcus uberis*, *Enterococcus faecalis and* coagulase-negative staphylococci (CNS)) (**Albenzio *et al.*, 2002; Contreras and Rodriguez 2011**). According **Quinn *et al.* (2011)** CNS can be classified as minor contagious pathogens because are considered to be part of the normal flora of animals and *S. aureus*, *Str. agalactiae* and *Mycoplasma* sp. are classified as major contagious pathogens.

The most frequent pathogens isolated from the milk samples were CNS as well documented by many researchers (**Moroni and Cuccuru, 2001; Boettcher *et al.*, 2005; Guaraná *et al.*, 2011; Santana *et al.*, 2013; Zafalon *et al.*, 2018**). The etiological agents with highest occurrence in milk samples of ewes were CNS (**Zafalon *et al.*, 2016**). **Zafalon *et al.* (2016)** found out the incidence of CNS in 56.8% of bacteriology positive samples. CNS were detected in 59.7% of cases of subclinical mastitis in study of **Vasileiou *et al.* (2018a)**. The most common CNS that cause intramammary subclinical infection of sheep and goats is *S. epidermidis* (**Leitner et al., 2004b; Moroni *et al.*, 2005a, 2005b; Pilipčincová *et al.*, 2010; Kunz *et al.*, 2011; Queiroga *et al.*, 2018;**) followed by *S. chromogenes, S. simulans, S. xylosus, S. caprae* (**Bergonier *et al.*, 2003; Queiroga, 2017**). In another study also CNS represented 44.9% of infected milk samples where *S. epidermidis* was again the most frequently isolated species, followed by *S. xylosus* and *S. simulans* (**Moroni *et al.*, 2007**). In Brazil conditions the most of the analysed samples were bacteriologically negative (76 %), and among the positive samples, most of the isolates (93%) were *Staphylococcus* sp., followed by *Streptococcus* sp. (5%) (**Blagitz *et al.*, 2014**). According **Rahman *et al.* (2016)** CNS formed 88.80% isolates of *Staphylococcus* sp. CNS were the most prevalent pathogens (58%) in milk samples with intramammary infection (**Person *et al.*, 2017**). **Zigo *et al.* (2014)** in their study reported that CNS were identified in 65.4% of all positive isolates. As compared with above mentioned results **Vasileiou *et al.* (2018a)** identified *S. chromogenes* (23.1%) as the most prevalent CNS followed by *S. epidermidis* (19.8%) a *S. simulans* (17.6%) in cases of subclinical mastitis.

Under Slovak dairy practice **Pilipčincová *et al.* (2010)** investigated the frequency of individual species of CNS in farms of eastern Slovakia and found occurrence of *S. epidermidis* (36.3%), *S. caprae* (21.3%), *S. hominis* (6.6%), *S. chromogenes* (6.3%), *S. xylosus* (5.8%), *S. warneri* (5.0%), *S. capitis* (4.6%). Also in another study in Slovakia the most frequent pathogens in milk samples from ewes were CNS *S. epidermidis* (16.0%), *S. chromogenes* (11.9%), *S. simulans* (7.0%), *S. schleiferi* (6.4%) (**Zigo *et al.*, 2014**). However, in other study **Zigo *et al.* (2011)** from 204 individual positive samples identified *S. schleiferi* (43.1%), *S. caprae* (16.2%), *S. chromogenes* (10.3%), *S. epidermidis* (8.3%). Recently **Zigo *et al.* (2017)** reported that *S. chromogenes* (14.3%) was the most frequent CNS, followed by *S. schleiferi* (12.2%) and *S. epidermidis* (10.2%) in farms of eastern Slovakia. In milk samples positive on *Staphylococcus* sp. were the most frequent *S. epidermidis* (24.3%), *S. schleiferi* (16.6%) and *S. chromogenes* (15.3%) (**Vasiľ *et al.*, 2018**). In our previous partial results of the study carried out in Slovakia (116 half udder samples), the most prevalent pathogens were CNS represented by *S. chromogenes* (33.33%), *S. xylosus* (21.67%), *S. epidermidis* (5%) (**Tančin *et al.*, 2017c**). **Holko *et al.* (2018)** found out that CNS were the most represented species in ewes milk.

S. aureus starts to be emerging problems for public health especially antibiotic resistant strains as pointed out in review of **Gelasakis *et al.* (2015)**. *S. aureus* is the most frequent pathogen causing clinical mastitis in dairy ewes (**Ariznabarreta *et al.*, 2002; Bergonier *et al.*, 2003; Mavrogianni *et al.*, 2011; Dore *et al.*, 2016**) with around 40% of cases of clinical mastitis in suckling ewes and 80% of cases in dairy ewes (**Mørk *et al.*, 2007; Koop *et al.*, 2010; Mavrogianni *et al.*, 2011**). *S. aureus* was isolated in 8.36% of infected milk samples in meat sheep (**Moroni *et al.*, 2007**). **Ergűn *et al.* (2009)** showed 3.1% incidence of *S. aureus* in samples without clinical sings. **Kern *et al.* (2013)** detected *S. aureus* in 5.5% of isolated bacteria. *S. aureus* was identified in 8.1% of bacteriology positive milk samples (**Zafalon *et al.*, 2016**). The occurrence *S. aureus* was 9% in samples where intramammary infection was identified (**Person *et al.*, 2017**). **Vasileiou *et al.* (2018a)** in their study determined *S. aureus* in 10.1% of cases of subclinical mastitis. Under our practical conditions *S. aureus* was identified in 9.3% (6.4% in clinical cases) from 204 individual positive samples (**Zigo *et al.*, 2011**). In another study **Zigo *et al.* (2014)** determined the presence of *S. aureus* in 8.3% of individual milk samples. **Vasiľ *et al.* (2018)** found out that *S. aureus* was isolated in 26.4% samples positive on *Staphylococcus* sp. during their research on farms of eastern Slovakia. From our study in dairy practice we found out 3.33% presence of *S. aureus* only (subclinical cases) (116 milk samples from half udders – 56 animals) (**Tančin *et al.*, 2017c**). In another study in our conditions **Holko *et al*. (2018)** detected the incidence of *S. aureus* in 6.9% of microbial isolates.

Other pathogens causing sheep mastitis included *Streptococcus* sp. Increase in the occurrence of mastitis caused by bacteria *Streptococcus* sp. is usually caused by inappropriate hygiene of milking and housing (**Marogna *et al.*, 2010; Dore *et al.*, 2016**). Intramammary infection caused by *Str. agalactiae* is connected with high SCC (**Ariznabarreta *et al.*, 2002**). **Moroni *et al.* (2007)** found out that *Streptococcus* sp. were the second most frequent pathogens of infected milk samples. In another study *Streptococcus* sp. were also the second most prevalent pathogen group isolated from milk samples of ewes, the most frequent was *Str. uberis* (6.1%) followed by *Str. dysgalactiae* (2%) and *Str. agalactiae* (2%) which was isolated only from 1 flock (**Ergűn *et al.*, 2009**). From positive milk samples **Marogna *et al.* (2010)** isolated the most frequently 3 pathogens, *Str. uberis*, followed by *S. epidermidis* and *S. aureus*. **Zafalon *et al.* (2016)** detected *Streptococcus* sp. in 11% of cases with subclinical mastitis. *Streptococcus* sp. were determined in 4.7% of positive bacteriology milk samples (**Vasileiou *et al.*, 2018a**). **Tančin *et al.* (2017c)** observed in their studies 23.3% occurrence of *Str. agalactiae*, but *Str. uberis, Str. parauberis, Enterococcus faecalis* only in 1.67% samples for each pathogen. In another study in Slovak dairy practice were detected the occurrence of *Str. agalactiae* in 10.7%, *Str. dysgalactiae* in 4.6% and *Enterococcus faecium* in 1.5% of positive isolates (**Holko *et al.*, 2018**).

M. haemolytica cause intramammary infection in meat producing herds (**Omaleki *et al.*, 2010; Mavrogianni *et al.*, 2011; Gelasakis *et al.*, 2015**). Only 11% of cases of intramammary infections caused by *M. haemolytica* were found in milk of dairy breeds (**Mavrogianni *et al.*, 2007**). **Persson *et al.* (2017)** found out that the occurrence of *M. haemolytica* was in 6% of subclinical mastitis cases. The incidence of *M. haemolytica* was detected in 2.5% of cases of subclinical mastitis in ewes (**Vasileiou *et al.*, 2018a**).

Intramammary infection can be also caused by *Bacillus* sp., *Clostridium* sp., *Corynebacterium* sp., *Enterococcus* sp., *Listeria monocytogenes*, *Micrococcus* sp., *Mycobacterium* sp. a *Trueperella pyogenes* (**Ariznabarreta *et al.*, 2002; Marogna *et al.*, 2010**). **Moroni *et al.* (2007)** detected *Bacillus* sp. in 14.3% of infected samples. *Bacillus* sp., *Micrococcus* sp. and *Corynebacterium* sp. were identified in 2%, 2% and 1% isolated pathogens in Awassi breed, resp. (**Ergűn *et al.*, 2009**). **Zafalon *et al.* (2016)** showed 3.4% of cases with subclinical mastitis were caused by *Micrococcus* sp. **Vasileiou *et al.* (2018a)** found out that the occurrence of *Corynebacterium* sp., *Micrococcus* sp., *Trueperella pyogenes*, *Bacillus* sp. and *Enterococcus* sp. were 3.6%, 2.6%, 2.5%, 1.6%, 1.2%, respectively.

Citrobacter sp., *E. coli*, *Enterobacter* sp., *Klebsiella* sp., *Pasteurella multoocida*, *Proteus* sp., *Pseudomonas aeruginosa*, *Salmonella* sp., *Serretia* sp., *Yersinia pseudotuberculosis* are environmental mastitis pathogens causing mastitis (**Contreras and Rodríguez, 2011**). These pathogens presented 3% isolated pathogens from milk samples in ewes (**Bergonier *et al.*, 2003**). **Ergűn *et al.* (2009)** determined *Pseudomonas* sp. in 2% and *E. coli* in 2% isolated pathogens in Awassi breed. *E. coli* was detected in 5.5% of isolated pathogens from milk samples in study of **Kern *et al.* (2013)**. **Vasileiou *et al.* (2018a)** found out that the incidence of *E. coli* was determined in 3.4% of milk samples with subclinical mastitis. **Zigo *et al.* (2017)** determited *E. coli* in 1.2% from all testing samples. In our conditions was detected the occurrence of *Klebsiella* sp. below 1% (**Holko *et al*., 2018)**.

ANTIMICROBIAL RESISTANCE

The antimicrobial resistance is one of the most important challenge in the mastitis treatment. Therefore, research in this topic deserve high priority. Of 1284 strains *S. aureus* from ovine mastitis cases were evaluated for antibiotic susceptibility and were found increased resistance to streptomycin (48-87%) and lower resistance were found to penicillin and ampicillin (2-12% and 0-12%, respectively) (**Lollai *et al.*, 2008**). **Attili *et al.* (2016)** determined resistance *S. aureus* to enrofloxacin

(2.5 mg/kg and 5mg/kg). They found out that both treatments were effective, but treated with 5 mg/kg enrofloxacin was more effective for reduction clinical mastitis caused by *S. aureus*. The highest value of resistance of *S. aureus* were found on novobiocin 14.5%, erythromycin 12.8%, lincomycin 7.69% and penicillin 7.69% (**Vasiľ *et al.*, 2018**).

CNS were tested on susceptibility to antibiotics with rifampicin (5 µg), linezolid (30 µg), vancomycin (30 µg), clindamycin (2 µg), erythromycin (15 µg), penicillin (10 IU), tetracycline (30 µg), gentamicin (10 µg), ciprofloxacin (5 µg) and cotrimoxazole (25 µg). Antibiotics were effective, but higher resistance were found for penicillin (17%) and tetracycline (10.7%) (**Martins *et al.*, 2017**). **Vasiľ *et al.* (2018)** detected susceptibility of *S. epidermidis* on 14 antibiotics, resistance was found in 11.1% to novobiocin and 8.3% to erythromycin. **Holko *et al.* (2019)** observed the highest resistance of CNS to lincomycin (57.65%) and neomycin (36.4%) and the lowest resistance to trimethoprim-sulfamethoxazole (0%) and enrofloxacin (3.0%). From our preliminary study in farm with Lacaune breed there were tested CNS susceptibility to following antibiotics: amoxicillin-clavulanic acid (AMC) (20 µg-10 µg), tetracycline (TE) (30 µg), enrofloxacin (ENR) (5 µg), trimethoprim-sulfamethoxazole (SXT) (1.25 µg-23.5 µg), neomycin (N) (30 µg), lincomycin (MY) (2 µg). We found out that the most effective antibiotics were amoxicillin-clavulanic acid and trimethoprim-sulfamethoxazole (tab.1) (**Tvarožková *et al.*, unpublished**).

Table 1 Antimicrobial resistance of CNS (73 isolates) isolated from Lacaune ewes in Slovakia (**Tvarožková *et al.*, unpublished**)

	AMC	TE	MY	ENR	SXT	N
Susceptible	90.91%	78.79%	54.55%	84.85%	90.91%	36.36%
Intermediate	3.03%	3.03%	9.09%	12.12%	6.06%	45.45%
Resistant	6.06%	18.18%	36.36%	3.03%	3.03%	18.18%

AMC- amoxicillin-clavulanic acid, TE- tetracycline, MY- lincomycin, ENR- enrofloxacin, SXT- trimethoprim-sulfamethoxazole, N- neomycin.

Ergűn *et al.* (2009) evaluated antimicrobial susceptibility of 78 *Staphylococcus* sp. isolates. They found out resistance on tetracycline (24.4%), ampicillin (42.3%), penicillin (56.4%) and the most effective antibiotics were cephalothin (97.4%), trimethoprim – sulphamethoxazol (97.4%), amoxicillin-clavulanic acid (97.4%), enrofloxacin (94.9%), gentamycin (92.3%), and erythromycin (84.6%). **Űnal *et al.* (2012)** tested 46 CNS and 21 *S. aureus* isolates against antimicrobial agents. All *Staphylococcus* sp. were sensitive to cephalothin, trimethoprim-sulphamethoxazole, rifampin, cefoxitin, vancomycin and linezolide. 21 *S. aureus* and 46 CNS strains were resistant to penicillin G 19.0% and 30.4%, to tetracycline 4.8% and 8.7%, to erythromycin 4.8% and 6.5%, to gentamicin 4.8% and 0.5%, to enrofloxacin 0.0% and 0.5%, respectively (**Űnal *et al.*, 2012**). Rahman *et al*. (2016) found out that 85.18% of isolates were resistant to penicillin, to ampicillin were resistant 48.14% of isolates. The lowest resistance of drugs was observed in chloramphenicol, ciprofloxacin, neomycin and streptomycin (**Rahman *et al.*, 2016**). **Vasiľ *et al.* (2016)** determined the highest value of resistance to penicillin (21.0%) neomycin (10.5%) and novobiocin (9.7%). The lowest values of resistance were found to cefoxitin (0.8%), lincomycin (2.4%), erythromycin and streptomycin (in both 3.2%). **Zigo *et al.* (2017)** determined medium value of antibiotic resistance to penicillin (11.7%), cloxacillin (11.7%), ampicillin (10.7%) and oxacillin (10.7%). Other studies have nevertheless grouped staphylococcal strains independently of coagulase-production type and detected increased resistance to penicillin G (up to 31%) and ampicillin (up to 30%) (**Corrente *et al.*, 2003; Onni *et al.*, 2011**).

DISCUSSED LIMITS FOR THE PHYSIOLOGICAL LEVEL OF THE SOMATIC CELL COUNTS

Somatic cells in milk represent epithelial cells of alveoli and ducts and leukocytes (**Paschino *et al.*, 2019**). SCC is considered from many aspects as an indicator of udder health and generally is used for detection of subclinical mastitis in ewes (**Gonzalo *et al.*, 1994; Gonzáles-Rodríguez *et al.*, 1995; Margetín *et al.*, 1996; Pengov, 2001; Olechnowicz *et al.*, 2005**). However, there is still a big discussion among scientists about the physiological level of SCC in udder of ewes for detection of udder health (**Persson *et al.*, 2017**). From the research study of excellent laboratories in the world the discussed and proposed recommendations for physiological level of SCC in milk are systematically decreased during the last years.

Already in the 90's it was considered at the physiological and pathophysiological level that the SCC limit ranged from 250 to 1000 × 10^3 cells/ml (**Gonzalo and Gaudioso Lacasa, 1985**), and the authors proposed SCC at level 500 × 10^3 cells/ml for healthy udders. In the work of **Hariharan *et al.* (2004)** the limit for high SCC as a possible detection of mastitis was over 1000 × 10^3 cells/ml. **Pengov (2001)** determined physiological level of SCC in ewe's milk at 250 × 10^3 cells/ml. Values of SCC less than 500 × 10^3 cells/ml for healthy ewes and for infected ewes SCC more than 1000 × 10^3 cells/ml were reported by **Berthelot *et al.* (2006)** and if SCC was in flock over 650 × 10^3 cells/ml it showed 15% incidence of subclinical mastitis. Ewes with mammary glands without clinical abnormalities, with bacteriologically positive milk and with a SCC of ≥ 500 × 10^3 cells/ml, were considered to have subclinical mastitis (**Kiossis *et al.*, 2007**). **Maurer and Schaeren (2007)** derived a critical limit at level 500 × 10^3 cells/ml as indicator of problems with udder health. **Leitner *et al.* (2008)** established a limit for uninfected milk samples at 250 × 10^3 cells/ml for Latxa sheep and for other sheep breeds. **Nunes *et al.* (2008)** followed Santa Inês sheep throughout lactation and reported that ideal value SCC for the diagnosis of mastitis was 500 × 10^3 cells/ml. **Ozenc *et al.* (2011)** specified a value for detection of subclinical mastitis at 374 × 10^3 cells/ml for Pirlak sheep. As a limit value in determining the relationship to milk yield **Arias *et al.* (2012)** determined 300 × 10^3 cells/ml. **Kern *et al.* (2013)** indicated threshold of SCC at 400 × 10^3 cells/ml in meat breeds of sheep, 300 × 10^3 cells/ml in dairy breeds and 100 × 10^3 cells/ml in extensive breeds as right value in detecting problems with udder health. Limit for detection subclinical mastitis was determined by **Hussein *et al.* (2015)** as value of SCC ≥ 400 × 10^3 cells/ml in Ossimi sheep. **Swiderek *et al.* (2016)** determined as the limit for detection of subclinical mastitis SCC per 200 × 10^3 cells/ml. The value of SCC > 400 × 10^3 cells/ml can be used for diagnostic of subclinical mastitis in flocks in which the predominant infectious aetiology is CNS (**Zafalon *et al.*, 2016**). **Caboni *et al.* (2017)** reported similar threshold of SCC for diagnosis of mastitis in Sarda sheep, and in their study was determined the threshold of SCC at 265 × 10^3cells/ml. In a study with meat and pelt producing ewes the possible limit for SCC as the health of udder indicator **Persson *et al.* (2017)** proposed less than 400 - 500 × 10^3 cells/ml. **Sutera *et al.* (2018)** in their study showed value SCC below 500 × 10^3 cells/ml as a possible limit in relation to milk quality. The value of SCC 545 × 10^3 cells/ml can be proposed as limit of SCC for quality milk and cheese yield (**Paschino *et al.*, 2019**). From above mentioned results it is clear that research deals very intensively to prove and establish the physiological level of SCC in milk for detection of mastitis as it is well accepted in dairy cows.

In our conditions **Margetín *et al.* (2013)** determined in breeding practise that only 6.3% milk samples had SCC over 1000 × 10^3 cells/ml. In the study of **Idriss *et al.* (2015)** there was reported that 78% of the samples were below 600 × 10^3 cells/ml. In group of samples below 100 × 10^3cells/ml there were the highest percentage of sheep Tsigai breed and Improved Valachian as compared with Lacaune pure or crossbreed. Similarly, **Vršková *et al.* (2015a)** found out that 76% of Tsigai had SCC below 300 × 10^3 cells/ml. **Tančin *et al.* (2017a)** in their research detected that 82.03% individual milk samples were below 400 × 10^3 cells/ml, 71.79% milk samples were below 200 × 10^3 cells/ml and only 8.89% milk samples were over 1000 × 10^3 cells/ml. In other research **Tančin *et al.* (2017b)** determined that 53.36% milk samples were below 200 × 10^3 cells/ml in Lacaune breed indicating a good udder health in high producing breed too. **Baranovič *et al.* (2018)** found out that in SCC group below 400 × 10^3 cells/ml were 67.7% ewes in May and even 87.9% in July. In another study 60% of samples had SCC less than or equal to 200 × 10^3 cells/ml (**Oravcová *et al.*, 2018**). **Tvarožková *et al.* (2018)** observed the most occurrence of ewes in SCC groups below 400 × 10^3 cells/ml (78.89% in 2016 and 83.33% in 2017). Though above mentioned work significantly documented low physiological value for SCC level in ewe's milk, but in recently published article the limits for SCC were again much higher (**Sutera *et al.*, 2018**). Thus the results of the scientific studies emphasise the need to specify the physiological values of SCC in raw sheep's milk in relation to the reliable detection of mastitis in ewes.

The importance of monitoring SCC in sheep milk showed **Spanu *et al.* (2011)** who found out that in ewes that were 3 or more months during lactation in SCC group above 400 × 10^3 cells/ml were 5.6 to 7.5-fold higher probability of a microbiologically positive samples compared to samples of milk from the ewes, which were below 400 × 10^3 cells/ml. Similarly, **Ozenc *et al.* (2011)** found out significantly higher SCC in contaminated samples as compared with uncontaminated ones. In our preliminary study we have also found out significantly higher SCC values in contaminated samples (log 5.28 ± 0.09 cells/ml) compared to uncontaminated (log 4.73 ± 0.06 cells/ml, P <0.001) (**Tančin *et al.*, 2017c**). Similarly, **Bagnicka *et al.* (2011)** also confirmed that SCC was higher in contaminated goat milk. A significant correlation between bacteriologically positive milk samples and CMT and SCC was found in another study (**Blagitz *et al.*, 2014**). In our last mentioned study in SCC category below 400 × 10^3 cells/ml were only 16.67% of contaminated samples (**Tančin *et al.*, 2017c**). On the other hand, **Hariharan *et al.* (2004)** did not found out the differences in SCC between contaminated and free of pathogen ewes milk. In study with Pirlak ewes 13.2% contaminated samples had SCC below 500× 10^3 cells/ml SCC and 25% uncontaminated samples had over last mentioned limit. As conclusion of above mentioned works the positive relationship between SCC in milk and presence of pathogens was not so clear.

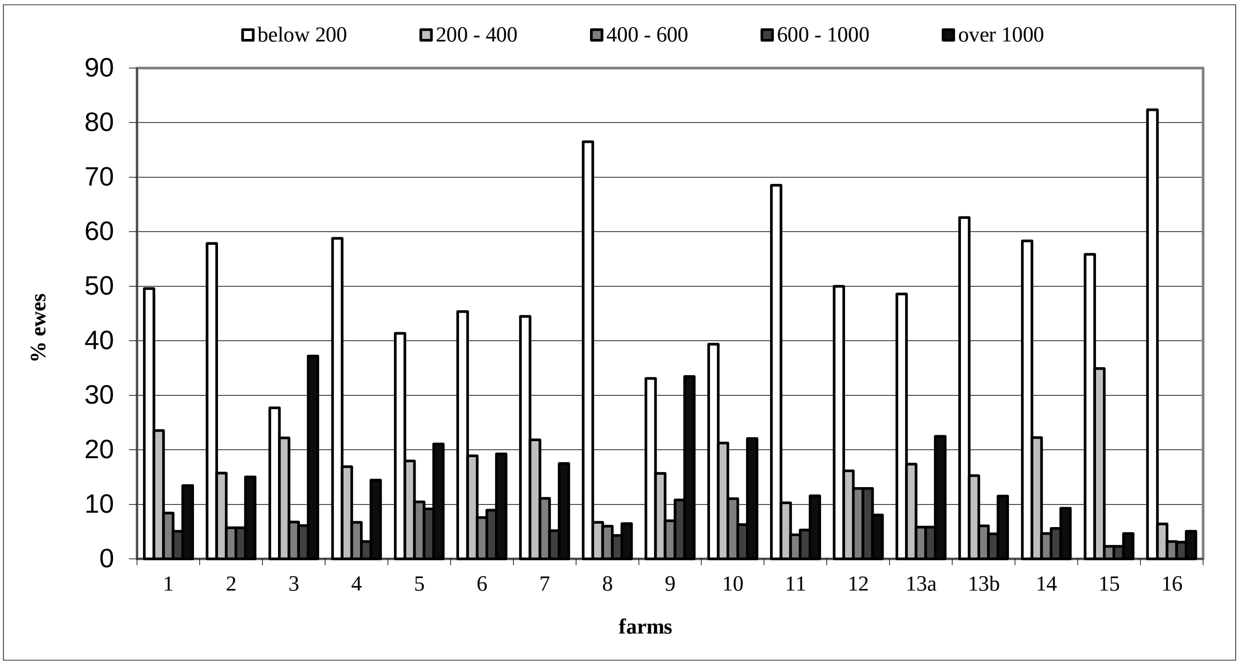

Figure 1 Frequency of occurrence ewes in five SCC groups (×10^3 cells/ml) in year 2016 (**Baranovič *et al.*, 2017**).
Farms: 1, 2, 3, 4, 5, 6, 7, 8- Tsigai, 9, 10, 11, 12, 13a- Lacaune (LC), 13b, 14- Slovak dairy sheep, 15- Improved Valachian (IV), 16- crossbreed IV/LC

IMPACT SCC ON MILK YIELD

Research related to the relationship between SCC and milk yield in goat and sheep confirm decrease milk production with increase of SCC in milk. Such, subclinical mastitis is considered the most important cause of reduced milk yield (**Leitner *et al.*, 2004c; Raynal-Ljutovac *et al.*, 2007; Leitner *et al.*, 2008; Koop *et al.*, 2010; Le Maréchal *et al.*, 2011**). Decline milk yield was from 3 to 10% in depending on species of pathogen and unilateral or bilateral infection (**El- Saied *et al.*, 1999; Gonzalo *et al.*, 1994; Gonzalo *et al.*, 2002**). **Gonzalo *et al.* (2002)** showed that the highest milk yield was by healthy ewes, followed by ewes infected with minor pathogens and the lowest milk yield was found in ewes infected with major pathogens. **Othomane *et al.* (2002)** observed a decrease in milk production in relation to the increase SCC in milk of Churra breed. **Nudda *et al.* (2003)** showed decreased milk yield of Sarda ewes with SCC over 1000 × 10^3 cells/ml compared with ewes with SCC below 1000 × 10^3 cells/ml (1015 and 1104 g/d, respectively). Throughout subclinical mastitis the milk yield of the infected halves was significantly lower than milk yield of the uninfected ones (0.36 and 0.76 kg/milking) (**Leitner *et al.*, 2004c**). **Silanikove *et al.* (2005)** also documented decreased milk yield due to subclinical mastitis. According to **Leitner *et al.* (2008)**, decline milk yield due to subclinical mastitis can reach 12.2% in herd with 75% incidence of intramammary infection. Ewes characterized during lactation by the number of somatic cells in milk from both udder halves up to 250 × 10^3 cells/ml produced more milk (1092.44 ml) than ewes in which SCC in milk from one or both udder halves exceeded 250 × 10^3 cells/ml (918.83 and 762.34 ml) (P<0.01). Milk yield of ewes where SCC from both udder halves exceeded 250 × 10^3 cells/ml was significantly lower than for ewes which SCC from one halves exceeded 250 × 10^3 cells/ml (P<0.01) (**Olechnowicz *et al.*, 2009**). **Olechnowicz *et al.* (2009)** showed that SCC had a significant effect on daily milk production, established that if SCC from one or two half udder is over 250 × 10^3 cells/ml milk yield decreased by 15.89% and 30.22% respectively. According to **Cuccuru *et al.* (2011)** in subclinical mastitis increase SCC and decrease milk yield of up to 25%, while decline milk yield is more pronounced in staphylococcal infections than in intramammary streptococcal infections. **Arias *et al.* (2012)** showed that ewes with SCC below 300 × 10^3 cells/ml had higher milk yield in compare with ewes with SCC over 300 × 10^3 cells/ml (1199 ± 641 ml/d and 1073 ± 615 ml/d). The estimated losses in milk yield according levels of SCC were approximately 16% (**Sutera *et al.*, 2018**).
In our breeding practise **Margetín *et al.* (1996)** found out that higher SCC was related to lower milk yield in Tsigai and Improved Valachian ewes. **Vršková *et al.* (2015a, 2015b)** and **Tančin *et al.* (2017a,** table 2**)** observed a decrease in milk production in sheep with high SCC in milk, which was also confirmed in our latest study (**Oravcová *et al.*, 2018,** table 3). Thus taking into account both abroad and our results we could conclude that though SCC is still do not considered as parameter for evaluation of milk quality in dairy ewes on milk trade, one has to point out that SCC should be considered in management of dairy farms if they want to produce milk more efficiently.

IMPACT SCC ON MILK COMPOSITION

Higher SCC, as mentioned above, has negative impact not only on milk production but the milk composition is affected as well which has negative influence on milk processing. One of the most important milk component is protein. Sheep milk with a high SCC contains more total protein than milk with low SCC (**Nudda *et al.*, 2003; Albenzio *et al.*, 2004; Bianchi *et al.*, 2004**). **Albenzio *et al.* (2004)** investigated the percentages of protein in Comisana ewes in two groups with different SCC, in ewes with high SCC over 1000 × 10^3 cells/ml a higher percentage of total protein was recorded in early and late lactation (5.86 and 6.27%) than in ewes from group with low SCC below 500 × 10^3 cells/ml (5.40 and 5.99%). During mastitis **Albenzio *et al.* (2004)** and **Nudda *et al.* (2003)** observed an increase in the concentration of soluble whey proteins as serumalbumin, immunoglobulins. **Leitner *et al.* (2004c)** detected in their study that the concentrations of total whey proteins and albumin were significantly higher in the infected than in the uninfected halves and concentrations of casein was significantly lower in the infected halves. **Le Maréchal *et al.* (2011)** reported in review an increase in the concentration of protein as result of the inflammatory and immune response and a decrease in endogenous milk protein such as casein throughout intramammary infection. Decrease of casein content was observed in infected Sarda dairy sheep compared to healthy ewes and also was found significantly higher content of total protein in infected sheep (**Bianchi *et al.*, 2004**). **Silanikove *et al.* (2006)** detected a decrease content of casein during subclinical mastitis. **Martí De Olives *et al.* (2013)** found out decrease of casein content during subclinical mastitis and throughout the postinfection period also. Content of protein was higher in milk samples with high somatic cell count (5.48 g/100 ml) in compared with content of protein and fat in milk samples with low somatic cell count (5.23 g/100 ml) (**Paschino *et al.*, 2019**).
Lactose is another important component of milk representing the source of energy and osmotic regulation of milk volume in udder (**Shamay *et al.*, 2000**). Reducing content of lactose in milk could indicate some problems in udder like mastitis (**Leitner *et al.*, 2003b**). Analysis of milk sample for ewes of Sarda breed with low (<500 × 10^3 cells/ml), middle (500-1000 × 10^3 cells/ml) and high (1000-2000 × 10^3 cells/ml) SCC showed significantly more lactose (4.74 g/100 g milk) in milk with a low SCC than in milk from the other two groups, 4.54 and 4.38 g/100 g milk, respectively (P<0.01) (**Pirisi *et al.*, 2000**). Decrease in lactose content (from 4.55 to 4.14%) with higher SCC reported **Nudda *et al.* (2001)** for Sarda ewes in their study. **Nudda *et al.* (2003)** found out decrease in concentration of lactose in sheep milk with increase SCC. The lactose content in milk from group with high SCC in early, middle and late lactation was significantly lower (4.47, 4.08 and 3.70%, respectively) than in milk of ewes from group with low SCC (4.81, 4.59 and 4.36%) (P<0.05) (**Albenzio *et al.*, 2004**). **Bianchi *et al.* (2004)** found out a decrease of lactose content in infected Sarda dairy sheep compared to healthy ewes. **Leitner *et al.* (2004c)** detected in their study that concentrations of lactose were significantly lower in the infected halves. During subclinical mastitis a decrease content of lactose was observed (**Silanikove *et al.*, 2006**). **Vivar-Quintana *et al.* (2006)** observed in Assaf × Churra and Castelana crossbred ewes

a significant decrease of lactose content with higher SCC (P<0.05). **Olechnowicz *et al.* (2010)** showed that SCC in milk below and above 250 × 10^3 cells/ml had a significant effect on content of lactose. Also **Martí De Olives *et al.* (2013)** found in their study decrease of lactose content due to subclinical mastitis and this decrease remained throughout the postinfection period. **Paschino *et al.* (2019)** observed reduction of lactose content in milk with high somatic cell count in compared with content of lactose in milk with low somatic cell count (4.66 and 4.87 g/100 ml, resp.).

Results concerning fat content are unclear even **(Raynal- Ljutovac *et al.*, 2007). Leitner *et al.* (2003b)** detected lower fat percentage in uninfected than in infected halves of udder (4.68 ± 0.08 and 5.29 ± 0.14, resp.). Fat content in milk decreased significantly in infected udder halves (**Bianchi *et al.*, 2004**). **Vivar-Quintana *et al.* (2006)** found out in Assaf × Churra and Castelana crossbred ewes that SCC did not significantly affect fat content of milk. Increase content of fat in milk with SCC over 250 × 10^3 cells/ml in one and both half of udder was observed in study by **Olechnowicz *et al.* (2010)**. Milk samples with high somatic cell count had higher content of fat (6.54 g/100 ml) in compared with content of fat in milk samples with low somatic cell count (6.28 g/100 ml) (**Paschino *et al.*, 2019**).

Despite of increase content of fat and protein, yield of cheese decreases because the individual fractions in fat and protein are change and the content of casein decreases (**Leitner *et al.*, 2004a; Caboni *et al.*, 2017**). The fatty acids profile in milk with high SCC had also been altered (**Caboni *et al.*, 2017**).

Under our conditions we have also studied relationship between SCC and milk composition. **Margetín *et al.* (1996)** found statistically significant relation between higher SCC and lower content of lactose (P < 0.001). Higher content of fat and protein were detected in milk samples of ewes with higher SCC (**Margetín *et al.*, 1996**). **Vršková *et al.* (2015a)** determined that with increase SCC in milk, increased content of protein (from 6.17 to 6.61%) and fat (from 7.20 to 8.04%), while content of lactose decreased (from 4.78 to 4.32%). The decrease of content of lactose were observed in study of **Vršková *et al.* (2015b)**. Also the reduced content of lactose in relation to high SCC was also reported **Tančin *et al.* (2017a,** table 2**). Baranovič *et al.* (2018)** recorded lower lactose content in milk samples with increase SCC (from 5.19 to 5.00% in May and from 4.88 to 4.72% in July). **Oravcová *et al.* (2018)** reported decrease in lactose content and increase fat and protein content with increasing SCC as show table 3.

Table 2 The effect of SCC groups on milk yield and milk composition (**Tančin *et al.*, 2017a**)

SCC groups	Low		Middle		Higher		High		Mastitis		
Variable	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	P
Milk yield, mL	503[a]	6.61	450[bc]	12.33	455[bc]	17.43	465[ab]	19.45	419[c]	13.22	<0.0001
Fat, %	6.32[a]	0.058	6.29[a]	0.093	5.95[b]	0.123	6.18[ab]	0.137	6.23[a]	0.099	0.0298
Protein, %	5.6[a]	0.028	5.61[a]	0.045	5.44[b]	0.06	5.56[ab]	0.066	5.68[a]	0.048	0.0117
Lactose, %	4.88[a]	0.015	4.84[ab]	0.024	4.76[c]	0.032	4.78[bc]	0.036	4.58[d]	0.026	<0.0001

[a-d] within row significantly different at P<0.05

Table 3 Least squares means and standard errors for milk traits by somatic cell count (SCC) class (**Oravcová *et al.*, 2018**)

Trait	SCC groups Low N=1763	Middle N=285	High N=575	Scheffe's test
Milk yield (ml)	526.8 ± 9.9	503.8 ± 12.4	486.8 ± 11.6	1:2[+], 3[++]
Fat content (%)	6.91 ± 0.05	6.93 ± 0.07	7.08 ± 0.07	1:3[+]; 2:3[+]
Protein content (%)	5.52 ± 0.03	5.58 ± 0.03	5.66 ± 0.03	1:2[+], 3[++]; 2:3[+]
Lactose content (%)	4.54 ± 0.01	4.50 ± 0.02	4.41 ± 0.02	1:2, 3[++]; 2:3[++]

SCC class: low – SCC under 300 × 10^3 cells/ ml, middle – SCC between 300 × 10^3 and 600 × 10^3 cells/ml, high – SCC above 600 × 10^3 cells/ml; N – number of observations; * – milk yield per milking; [++] – P ≤ 0.01, + – P ≤ 0.05

COMPOSITION OF SOMATIC CELLS DEPENDING ON THE PRESENCE OF THE PATHOGEN

SCC in milk increases during mastitis mainly as a result of increased migration of leukocytes from blood to mammary tissue (**Leitner *et al.*, 2003a; Le Roux *et al.*, 2003**). The analysis of the representation of individual types of leukocytes in milk can reveal changes in their numbers, which can provide information about inflammatory processes in the mammary gland (**Pilla *et al.*, 2012**) with possible implementation for mastitis diagnostic in dairy practice (**Damm *et al.*, 2017**) However, this research is mainly realised in dairy cows. One study analysed the effect of species (cow, goats and ewes), where the cellular immune response to CNS infection was similar for the three animal species, although the number of cells was different (**Leitner *et al.*, 2012**).

In milk samples of cows with the highest SCC were dominant types of leukocytes polymorphonuclear leukocytes (PMNL): neutrophils, basophils and eosinophils, followed by macrophages and lymphocytes (**Lindmark-Mansson *et al.*, 2006**). The increase PMNL in milk samples of cows was caused by infections with pathogens causing mastitis (**Leitner *et al.*, 2000**). **Albenzio and Caroprese (2011)** detected that PMNL represented the main population of leukocyte in ewe's milk with high SCC (over 1000 × 10^3 cells/ml). PMNL being the principal leukocytes that increase during pathogen invasion and they may be considered a good marker to evaluate udder health (**Albenzio and Caroprese, 2011**). In dairy ewes the milk samples with SCC >1000 × 10^3 cells/ml showed differences in leukocyte populations between uninfected and infected ewes, with higher percentages of PMNL and macrophages and lower percentages of lymphocytes in infected animals (**Albenzio *et al.*, 2012**). **Albenzio *et al.* (2012)** showed that nonviable PMNL levels were the highest in ewe milk samples with SCC below 300 × 10^3 cells/ml, in contrast from SCC over 500 × 10^3 cells/ml nonviable PMNL were higher in uninfected ewes than in infected ewes. **Leitner *et al.* (2003a)** reported that as chronic infection progressed the number of PMNL decreased and number of macrophages and lymphocytes increased. In the goat milk there was found out the changes of leucocyte population as a consequence of udder infection (**Bagnicka *et al.*, 2011**). In another study the effect of mastitis even more affected neutrophils in goat milk where in noncontaminated milk neutrophils constitute 45–74% of the total SCC, while in milk from infected separate mammary halves increased to 71–86% (**Paape *et al.*, 2007**). Similar results published **Boulaaba *et al.* (2011)**. **Blagitz *et al.* (2011)** in their study with goat's milk showed a significant positive correlation between the percentage of the viable neutrophils and milk SCC (r = 0.495, P = 0.008). **Della Libera *et al.* (2011)** found out that with higher California mastitis test (CMT) score which were in relation with higher SCC, they observed more neutrophils and less macrophages but lymphocytes count was without change. **Pilla *et al.* (2012)** used changes in the leukocyte ratio in milk to identify inflammatory processes in cows with low SCC to distinguish healthy quarters from those quarters that had an early and late inflammation, respectively. **Takano *et al.* (2018)** observed increase in neutrophil percentages in the udder from which mastitis causing pathogens were isolated.

Bagnicka *et al.* (2011) in their study observed that the percentage of neutrophils in noncontaminated samples constituted 15% in the total SCC. In milk samples with high numbers of minor and major pathogens, the neutrophils amounted to 21 and 32%, respectively. **Bagnicka *et al.* (2011)** observed the effect of pathogens where they established increased neutrophils and eosinophils in milk samples from goats containing major pathogens such as *S. aureus*, *S. intermedius*, *Str. agalactiae* and an increased number of monocytes in samples of high occurrence minor pathogens such as CNS, *Enterococcus* sp., *Corynebacterium* sp. The type of pathogen didn't have effect on percentage of lymphocytes in total SCC (**Bagnicka *et al.*, 2011**). **Bagnicka *et al.* (2011)** was shown that not only the neutrophils and monocytes, but also the eosinophils play a crucial defensive role against the pathogenic bacteria. Researches confirm the correlation between intramammary infection and the immune response of the organism in relation to different numbers of individual leukocyte types depending on the type of pathogen**.**

IMPACT OF NON-INFECTIOUS FACTORS ON SCC IN MILK

Breed, number of lambs, order and stage of lactation, age, oestrus, livestock management, the impact of the day, the month, the season are factors which can affect the SCC in the milk of ewes and goat (**Gonzalo *et al.*, 1994, 2002, 2005; Pappe *et al.*, 2001, 2007; Olechnowicz *et al.*, 2010; Arias *et al.*, 2012; Souza *et al.*, 2012**). However, non-infectious factors can cause changes in SCC of sheep milk from 40 × 10^3 to 100 × 10^3 cells/ml **Bergonier *et al.* (2003)** and **Gonzalo *et al.* (2002)**.

Milk composition in Comisana ewes was affected by the stage of lactation and lambing season (**Sevi *et al.*, 2004**). **Sevi *et al.* (2004)** observed that irrespective of the lambing season, a higher SCC was recorded in late-lactation, compared to early- and mid-lactation (6.16 vs. 5.93 and 5.87 log_{10}somatic cells/ml). The stage of lactation was statistically significant effect on SCC (P<0.01) (**Olechnowicz *et al.*, 2010**). **Bonelli *et al.* (2013)** found no significant differences between sampling from early till late stage of lactation although SCC trend seemed to increased. **Romero *et al.* (2017)** found out that the highest level SCC were in samples taken at 2nd week from lambing (276×10^3 cells/ml), level SCC gradually decreased, and the lowest SCC was reached at 12th week from lambing (95×10^3 cells/ml). In our recent study the effect of stage of lactation on SCC was not significant (**Oravcová *et al.*, 2018**) though there were tendency of changes throughout of lactation (**Tančin *et al.*, 2017b**, Table 4). Also the frequency of distribution of milk samples into different SCC groups was not clearly influenced by stage of lactation (**Idriss *et al.*, 2015**). Such results could contribute to the evidence that animals should be healthy during whole lactation.

Table 4 The effect of stage of lactation on milk yield, milk composition and SCC (**Tančin *et al.*, 2017b**)

		Milk composition (%)			
Stage of lactation	Milk yield (ml)	Fat	Protein	Lactose	SCC (cell/ml)
30–60. days	$962.09^a \pm 49.33$	$5.28^a \pm 0.18$	$5.25^a \pm 0.09$	$4.87^a \pm 0.04$	5.51 ± 0.10
60–90. days	$1038.39^a \pm 21.2$	$5.12^a \pm 0.08$	$5.44^a \pm 0.04$	$4.78^{ac} \pm 0.02$	5.54 ± 0.04
90–120. days	$844.82^b \pm 27.53$	$5.81^c \pm 0.11$	$5.72^b \pm 0.05$	$4.69^{bc} \pm 0.02$	5.47 ± 0.05
120–150. days	$637.08^d \pm 31.34$	$6.52^d \pm 0.12$	$6.00^c \pm 0.06$	$4.57^d \pm 0.03$	5.44 ± 0.06
150–180. days	$524.37^c \pm 23.63$	$7.35^b \pm 0.09$	$6.59^d \pm 0.04$	$4.43^{ef} \pm 0.02$	5.46 ± 0.05
180–210. days	$460.41^{cd} \pm 42.29$	$6.83^{bd} \pm 0.16$	$6.60^d \pm 0.08$	$4.49^{df} \pm 0.04$	5.56 ± 0.08
210< . days	$378.36^d \pm 54.98$	$7.3^{bd} \pm 0.21$	$6.63^d \pm 0.10$	$4.27^e \pm 0.05$	5.66 ± 0.11

Note: $^{a, b, c, d, e, f}$ LS means in the same column with different letters are different (P <0.05).

Arias *et al.* (2012) found out that ewes with twins and more lambs had higher SCC in compare with ewes with one lamb. In milk of ewes with two and three lambs were higher SCC than in milk of ewes with one lamb (1659×10^3: 708×10^3 cells/ml, respect.) (**Prpic *et al.*, 2016**). This fact could be due to probably related to greater mechanic damage during lambing two and more lambs.

Moroni *et al.* (2007) observed that SCC had upward trend as parity increased. **Olechnowicz *et al.* (2010)** showed a significant effect of parity of ewes on SCC (log SCC) from udder halves below 250×10^3 cells/ml. The youngest ewes had the lowest SCC, while the oldest ewes showed in general the highest SCC and the lowest milk yield (**Arias *et al.*, 2012**). Subclinical mastitis occurred less frequently in primiparous ewes than those with two or more lactations significantly (P<0.05) and ewes in third lactation had the most cases of subclinical mastitis (**Sani *et al.*, 2015**). **Takano *et al.* (2018)** showed in their study that multiparous Lacaune ewes had a higher incidence of intramammary infections during early lactation than primiparous ewes. The increase of SCC in multiparous ewes could be attributed to potential intramammary infection in previous lactating period.

The breeds could also affect SCC in milk. The most samples over 1000×10^3 cells/ml had ewes of Lacaune compared to ewes of Tsigai, Improved Valachian × Lacaune and Slovak dairy sheep (**Tančin *et al.*, 2017a, Oravcová *et al.*, 2018**) (tables 5 and 6). Farms with Friesarta breed had the highest prevalence of subclinical mastitis and the smallest prevalence of subclinical mastitis was recorded in farms with Assaf breed (**Vasileiou *et al.*, 2018b**).

Table 5 Frequency of distribution of SCC in milk samples from different breeds and their crossbreds (**Idriss *et al.*, 2015**)

Breeds	**N**	**SCC groups (%)**				
		G_1	G_2	G_3	G_4	G_5
TS	211	47.39	29.38	9.00	5.21	9.00
TS × LC	814	41.03	23.10	12.41	6.63	16.83
LC	577	38.82	27.4	11.61	6.7	16.46
IV	54	66.67	24.7	5.56	3.70	0.00
IV × LC	976	38.63	28.38	9.73	7.7	16.19

SCC- Somatic cell count, TS- Tsigai, LC- Lacaune, IV- Improved Valachian, TS×LC- crossbreeds, IV×LC- crossbreeds. G1= Group1 of (SCC <100×10^3 cells/ml), G2= (SCC between 100-300 × 10^3 cells/ml), G3= (SCC between 300-600 × 10^3 cells/ml, G4= (SCC between 600-1000 × 10^3 cells/ml) and G5= (SCC >1000×10^3 cells/ml), N= The number of ewes.

Table 6 Least squares means and standard errors for milk traits and decadic logarithm of somatic cell count (log_{10}SCC) by genotype (**Oravcová *et al.*, 2018**)

Trait	**Genotype**					Scheffe's test
	TS (1) N=194	IV (2) N=49	LC (3) N=577	TS × LC (4) N=826	IV × LC (5) N=977	
Milk yield, ml	374.9 ± 21.0	438.7 ± 36.4	625.3 ± 12.3	516.9 ± 11.0	573.2 ± 10.5	$1:3,4,5^{++}$, $2:3,5^{++}$, $3:4^{++}$, 4.5^{++}
Fat content, %	6.93 ± 0.11	6.89 ± 0.20	6.87 ± 0.07	7.17 ± 0.06	7.02 ± 0.06	$3:4^{++}$,
Protein content, %	5.56 ± 0.06	5.81 ± 0.10	5.43 ± 0.03	5.69 ± 0.03	5.44 ± 0.03	$1:4^{+}$, $2:3,5^{+}$, $4:5^{++}$
Lactose content, %	4.46 ± 0.03	4.45 ± 0.05	4.54 ± 0.02	4.48 ± 0.01	4.49 ± 0.01	$3:4^{+}$
log_{10}SCC/ml	5.20 ± 0.07	4.71 ± 0.13	5.34 ± 0.04	5.31 ± 0.04	5.33 ± 0.04	$1:2^{+}$, $2:3,4,5^{++}$,

N – number of observations; TS – Tsigai, IV – Improved Valachian, LC – Lacaune; * – milk yield per milking; $^{++}$ – $P \leq 0.01$, + – $P \leq 0.05$

Season and temperatures during year could impact to SCC and occurrence of bacteria in udder of ewes. Ewes had the lowest SCC and highest milk yield in spring and highest SCC and lowest milk yield had in winter (**Arias *et al.*, 2012**). January, February and March had a significantly higher risk of being infected by mastitis pathogens than April and May (**Kern *et al.*, 2013**). During the winter months due to the higher transpiration and respiration of ewes in stables could higher damp stimulates bacterial growth. This higher microbial pressure could affect the higher SCC during the winter season.

The management of dairy ewes at farm levels is a critical factor that should be taking into account if SCC is evaluated. **Sevi *et al.* (2003)** mentioned that in intensively managed herds, where surface area smaller than 7 m^2 per ewe was available, there was an increased risk of clinical mastitis. Ewes allowed access to the outdoor area had lower SCC in their milk, whereas reduced space allowance led to an increase in SCC of milk (**Caroprese *et al.*, 2009**). The SCS (somatic cell score) was significantly lower in extensive (3.00) compared to dairy (4.41) and meat systems (4.72) (**Kern *et al.*, 2013**). **Vasileiou *et al.* (2018a)** detected prevalence of subclinical mastitis more frequently in farms managed semi-intensively (0.296) or intensively (0.254) and less frequently in farms managed semi-extensively (0.196) or extensively (0.178). We observed in our study significantly impact farm management on SCC (**Tvarožková *et al.*, 2019**). **Romero *et al.* (2017)** in their study found out, that SCC were higher in milk from evening milking (162×10^3 cells/ml) than morning milking (129×10^3 cells/ml).

Despite these non-infectious factors, intramammary infection is the main cause that leads to increase SCC in sheep milk (**Leitner *et al.* 2001; Pappe et al., 2001; 2007; Raynal- Ljutovac *et al.*, 2007; Souza *et al.*, 2012**). Thus non-infectious factors contribute only to the tendency of increasing or decreasing of SCC within possible physiological range and they could explain some risk factors determining the mastitis occurrence.

CONCLUSIONS

On the basis of available results from the world laboratory the physiological levels of SCC for diagnosis of subclinical mastitis of udder of dairy ewes significantly reduced during last years but this question is still open. Our results and obtained experiences coming from the frequency of distribution of milk samples among SCC groups could indicate that most of the samples were in SCC group below 400 × 10^3 cells/ml. Also we showed that the most of the milk samples obtained from half udder with high SCC were connected with the presence of microorganisms indicating the subclinical mastitis. Thus SCC in milk give reliable information about udder health and therefore low SCC indicate good udder health. Also the management (effect of farms) play important role as it was presented by different SCC among farms with the same breed of ewes. On the other side breeds with high yield were more affected by subclinical mastitis. Obtained results under Slovakian conditions also show how important regular milk recording including SCC analysis is for further improvement of udder health because there was demonstrated clear negative effect of high SCC on milk yield and its composition under practical conditions. Though there is negligible using antibiotics id mastitis treatment in Slovakian sheep farms the mentioned results about antibiotic resistance showed in some antibiotics difficulties in their using in dairy practice. Taking into account presented information in our review there is the high needs to extend the number of sheep farms involved in regular milk recording which increase the available data for farmers to manage their farms more effectively with emphasis on milk performance efficiency where udder health play crucial role.

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