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Symbiotic effect on some microbiological species and physicochemical properties in milk in subclinical mastitis of dairy cows

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Abstract. *Subclinical mastitis (SCM) is the most common form of mastitis and the greatest cause for concern in dairy cows. The overuse of antibiotics for the treatment of mastitis leads to the development of resistance, resulting in the use of symbiotics. The study was carried out between February and May 2017 at a pilot dairy cattle farm in the Tipaza region (north-central Algeria) aiming to investigate the effect of a symbiotic on SCM. California Mastitis test (CMT) was used to diagnose SCM in a total of 240 dairy cows. A number of 58/240 (24.16%) cows were found to have SCM. These mastitis cows were then divided into two lots; an experimental lot of 37 cows and a control lot of 21 cows. A symbiotic was administered to the experimental lot once a month for three months. Cell count, microbiological analysis and analysis of certain physicochemical parameters of the milk were applied before and after each administration of the symbiotic. The results revealed that the average somatic cells count (SCC) in cows from the control lot was higher than that of cows from the experimental lot throughout the study period (p<0.0001). Staphylococci were isolated from 51/58 (87.93%) of the mastitis cows, of which 21 (36.20%) were infected with Staphylococcus aureus and 30 (51.72%) with coagulase-negative Staphylococcus. Enterobacteriaceae were isolated from 36/58 (62.07%) of the mastitis cows, of which 21 (36.20%) were due to Escherichia coli strain and 15 (25.86%) to other strains of Enterobacteriaceae. After administration of the symbiotic, the prevalence of S. aureus and E. coli decreased significantly in the experimental lot compared to the control lot (p<0.001). The physicochemical characteristics of the milk were not altered by the administration of the symbiotic. All these results show that the symbiotic constitutes an adequate solution to replace antibiotics in the treatment of SCM.*

Keywords: bacteriological control, enzymes, medicinal plants, plant extracts, probiotics, somatic cells

Introduction

Mastitis is an infection of the mammary gland, usually caused by bacteria. It is characterized by pathological changes in the glandular tissue of the mammary gland and physical, chemical and bacteriological changes in the milk (Sharma et al., 2007). Mastitis is classified as clinical or subclinical depending on the signs presented by the animal (Kehrli and Shuster, 1994). Subclinical mastitis (SCM) is the most common form of mastitis and is the most serious form (Roy et al., 2009; Salvador et al., 2012). Bovine mastitis causes economic losses; decreased milk production, increased treatment and slaughter costs (Dhakal and Thapa, 2002; Singh and Bansal, 2004).

The main indicator used for the detection of SCM is the somatic cell count (SCC) in milk (Sharma et al., 2011). Somatic cells are composed of leukocytes and mammary gland epithelial cells (Santos and Fonseca 2007). Cows with a SCC greater than 200,000 cells/ml are considered to have SCM (Lukas et al., 2005; Chebel, 2007).

Clinical mastitis is characterized primarily by fever and inflammation of the mammary gland, a large increase in SCC and a decrease in milk production and quality. Gram-negative (G-) bacteria such as *Escherichia coli* mainly induce short-term acute clinical mastitis (Shuster et al., 1991). Subclinical mastitis, which can persist for several months, is caused mainly by gram-positive (G+) bacteria such as *Staphylococcus aureus* and coagulase-negative staphylococci (Djabri et al., 2002; Schukken et al., 2003). However, some strains of *E. coli* have been shown to induce subclinical mastitis as well (Blum et al., 2014). Subclinical mastitis is often a chronic disease

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characterized by a moderate increase in SCC with no apparent signs of local inflammation or systemic infection (Djabri et al., 2002; Schukken et al., 2003).

Mastitis is the main reason for the use of antibiotics in cattle farming (De Briyne et al., 2014). The emergence of antimicrobial resistance leads to the need to reduce the use of antimicrobials, particularly in dairy farming. Plant-based products, probiotics, genetic selection and vaccination have been used for the treatment or prevention of mastitis (Francoz et al., 2017; Martin et al., 2018).

Numerous studies suggest that probiotics have great potential as a tool for improving health and well-being (Sanders et al., 2014). Trials of probiotics for treating mastitis in dairy cows had a successful result (Klostermann et al., 2008; Crispie et al., 2008). In Algeria, however, few studies have been conducted on the effect of probiotics on mastitis in dairy cows. In this paper, we investigated the therapeutic effect of a symbiotic (Symbioveba®) on subclinical mastitis in dairy cows and its effect on the physicochemical qualities of milk.

Material and methods

Animals

The study was carried out between February and May 2017 in a dairy cattle farm located in the Tipaza region (north-central Algeria). The dairy cows, total number 240, were of the Fleckvieh breed and in early lactation. No cows had clinical mastitis. Intensive type of farming was applied on the farm where the cows did not leave the barn.

Sampling

In a first step we performed a diagnosis of SCM in all cows (n=240) using the California Mastitis test (CMT) and cell count. Bacteriological analyses were then applied to cows diagnosed with SCM (n=58). In a second step, we divided all these cows with SCM into 2 lots, a control lot containing 21 cows (n=21) and an experimental lot containing 37 cows (n=37). Then, the symbiotic (Symbioveba®) was administered orally to the experimental lot once a month for three months at a rate of 50 ml of the symbiotic in 50 ml of water/cow, while the control lot was not treated. According the manufacturer's requirements it is sufficient to give the symbiotic once a month.

The symbiotic used is a purely biological product composed of: probiotics (*Lactobacillus* & *Saccharomycess cervicia*), medicinal plants (*Taraxacum officinalis*, *Zingiber officinalis*), enzymes, plant extracts and water, obtained with the exclusive MESEN® Patented process.

Milk sampling and laboratory analysis

The milk samples (n=58) were taken from the cows during morning milking before and after each symbiotic treatment (three times: first month - sample 1, second month - sample 2 and third month - sample 3), according to the aseptic procedures described by the National Mastitis Council (2004). The samples were immediately cultured or stored at 4°C for a maximum of 24h until cultured on standard bacteriological media.

Three laboratory analyses were performed: cell count, bacteriological analysis, and physico-chemical analysis (titratable acidity, butyric and protein content of the milk).

California mastitis test (CMT)

The CMT has been used to diagnose SCM. It was performed according to Quinn et al. (1999). The CMT results were interpreted using a score from 0 to 4 as follows: 0 - for no reaction, 1 - for trace, 2 - for weakly positive, 3 - for distinctly positive, and 4 - for strongly positive.

Somatic cell counts (SCC)

SCC were performed on individual samples of total milk from all four mammary quarters and on a Tank sample. These measurements were carried out using a Fossomatic® which stains cells with a fluorescent dye and then counts the number of fluorescing particles (Gonzalo et al., 2003).

Bacteriological control

Bacteriological diagnosis was carried out on all cows diagnosed with SCM (n=58) by the CMT, then after each administration of the symbiotic for the experimental lot and at the same time for the control lot. The bacteriological analyses were carried out according to the classical methods for isolating the most frequent bacteria in cow's milk (Ferney et al., 1966; Quinn et al., 1994).

Inoculation: Before sowing, a volume of 3 ml of each cow milk sample was taken sterile and introduced into a sterile tube containing Fontainebleau sand, which was itself sterile. This was vortexed for 5 s. This operation aims to break up the fat globules in the milk and thus release any trapped bacteria. The milk samples are inoculated at the rate of one drop of milk per Petri dish on Baird Parker agar for *Staphylococcus aureus* and TBX agar for *Escherichia coli* and Hektoen agar for Enterobacteriaceae. Incubation lasts 24-48 hours at 37°C. Colonies considered pathogenic are transplanted on nutrient agar to obtain a pure culture.

Identification of bacteria: Biochemical galleries API (Bio-Mérieux) have been used to identify staphylococci (Api Staph) and Enterobacteriaceae (Api 20E). Each type of colony isolated in pure culture is identified according to the usual bacteriological techniques.

Staphylococci: Identification of staphylococci was carried out first by the appearance of colonies on Baird Parker agar, by catalase and by coagulase test. The detection of related coagulase was performed using a rapid test (slide-test) in which the positive test is obtained when the bacteria agglutinate on a glass slide when mixed with plasma. Isolations suspected to be *Staphylococcus aureus* but which do not have related coagulase character may be tested for their production of free coagulase.

This test consists of inoculating in a tube 0.5 ml of plasma and 0.5 ml of Staphylococcus bouillon culture and incubating it

at 37°C. The production of enzymes results in a clot one to four hours after inoculation. Coagulase positive staphylococci were identified as *S. aureus* by transfer to DNA agar and then to the Api Staph gallery.

Coagulase-negative staphylococci were also identified using the Api Staph gallery.

The API®Staph BioMerieux® gallery is a standardised tool for the identification of Staphylococcus species through twenty biochemical tests. Near the benzene beak, the base of the API gallery is first moistened. Bacterial colonies are taken from the Petri dish and mixed with the Staph medium solution. The suspension is homogenized by vortexing. The microtubules of the gallery are filled with this suspension using a Pasteur pipette. For the ADH and URE tests, anaerobiosis is obtained by adding paraffin oil. The gallery is incubated at 37°C for 18 to 24 hours. Identification is done by converting the results obtained into a code that is entered into a numerical index (API web® Staph) (Murray et al., 2003).

Enterobacteriaceae: The search for Enterobacteriaceae was carried out using TBX agar colonies for *Escherichia Coli*. Enterobacteriaceae identification was performed by Gram staining, by the absence of oxidase. The identification of the different species was then carried out by examining the biochemical characters using the Api 20 E gallery.

Physico-chemical control: After each administration of the symbiotic we performed a control of certain physico-chemical parameters of the milk of each cow sample (n=58): titratable acidity - Dornic degrees (°D), protein and butyric rates - %. The titratable acidity of the milk was measured by the method of sodium hydroxide in the presence of phenolphthalein as an indicator. The Gerber method was applied for the butyric content (TB) and the Kjeldahl method for the protein content (TP).

Statistical analysis

For statistical analysis the statistical program R i386 3.0.2 for Windows GUI front-end, ANOVA, Chi square and multiple range tests were used. The threshold value of different tests was $p<0.05$.

Results

Cell count

The results of the cell counts for the three samples taken during the test period of the two lots (control, n=21 and experimental, n=37) are shown in Figure 1. The results show a similar tendency to reduce the number of somatic cells in the milk from the first to the third sample – 1.42 times in the control lot and much more - 3.47 times in the experimental lot. At the same time, the number of somatic cells in the milk of treated cows is significantly less than in the milk of control cows ($p<0.0001$). It is noteworthy that after each application of a symbiotic, the number of somatic cells decreases to a greater extent - in the milk of the first sample 1.19 times, in the second sample - 2.33 times and in the third sample - 2.92 times. The observed dependencies show that the number of somatic cells in the milk is influenced by both symbiotic treatment and possibly other factors.

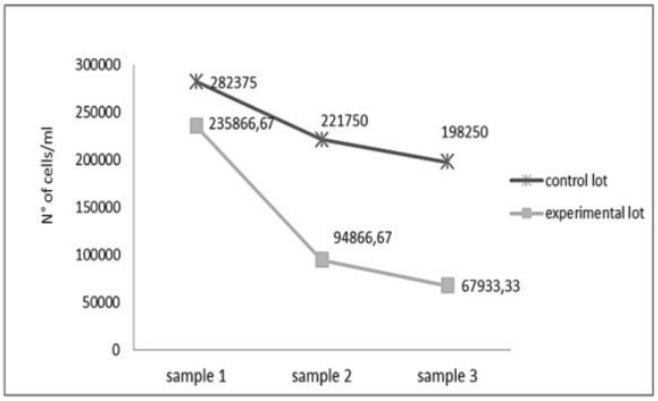

Figure 1. Average values of the somatic cell counts (No of cells/ml) in the milk of the cows from the two lots

Bacteriological analysis

Prevalence of different species of bacteria prior to symbiotic administration

A number of 58/240 (24.16%) cows were found to have subclinical mastitis by the CMT. Bacteriological analysis revealed that 34% of samples from SCM contained a single bacterial species. Staphylococci were isolated from 51/58 (87.93%) of the mastitis cows, of which 21 were *S. aureus* strains (36.20%) and 30 were coagulase-negative *Staphylococcus* strains (51.72%). Enterobacteriaceae were isolated from 36/58 (62.07%) of the mastitis cows, of which 21 (36.20%) were *E. coli* strain and 15 (25.86%) were other strains of Enterobacteriaceae.

Prevalences of S. aureus and E. coli isolated after symbiotic administration

The prevalences of the different bacterial species after administration of the symbiotic to the two lots are shown in Figure 2. The prevalence of *S. aureus* and *E. coli* decreased significantly in the experimental lot compared to the control lot ($p<0.001$).

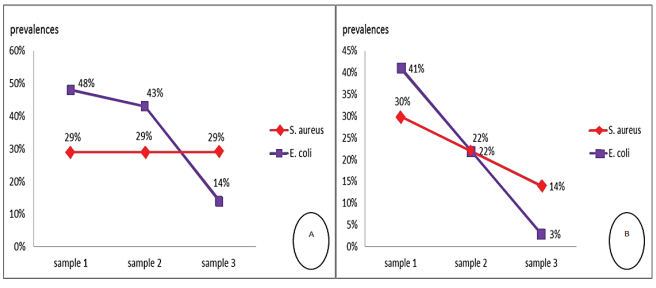

Figure 2. Prevalence of *S. aureus* and *E. coli* in the control (A) and experimental (B) lots during the three samples taken after each symbiotic administration

Physicochemical analysis of milk

The average monthly values of titratable acidity in the milk of the cows in the control lot were higher when compared to the experimental lot ($p<0.001$) (Table 1, Figure 3). In the first month the mean values obtained were 20.35°D and 21.40°D

for the experimental and control lot, in the second month 16.75°D and 19.80°D, and in the third month 17.85°D and 18.30°D, respectively. Titratable acidity decreased significantly in the first month in all cows treated with the symbiotic when compared to the control lot ($p<0.001$). This trend continues in the remaining two months.

Table 1. Average monthly values of titratable acidity, butyric and protein rates in the milk of the cows of the treated and control lot

Sample	Treated lot, n=37			Control lot, n=21		
	Titratable acidity, °D	Butyric rate, %	Protein rate, %	Titratable acidity, °D	Butyric rate, %	Protein rate, %
Sample 1	20.35±1.03	2.93±0.76	3.17±0.81	21.4±1.08	3.37±0.52	3.18±0.71
Sample 2	16.75±0.85	2.63±0.72	2.84±0.63	19.8±0.91	3.35±0.63	2.77±0.89
Sample 3	17.85±0.92	2.22±0.67	2.89±0.87	18.3±1.02	2.64±0.47	2.88±0.83

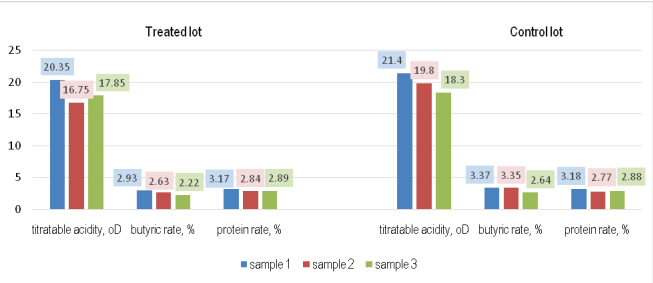

Figure 3. Evolution of the values of the different physicochemical parameters of the milk of the control and treated lots (titratable acidity, °D; butyric and protein rates, %).

Butyric rate in the milk produced by the cows of both lots decreased constantly and to similar extent during the study period – 1.31 times in the experimental lot and 1.28 times in the control lot. Butyric rate of the milk from symbiotic treated cows were lower – from 1.15 times in the first sample to 1.27 times in the second sample compared to the control group, but the differences were not statistically significant.

The protein rate decreased significantly ($p<0.01$) in both lots during the first two samples (from 3.17% to 2.89% and from 3.18% to 2.77% for the experimental and control lots, respectively). However, no significant differences were observed in the butyric and protein rates between the two lots throughout the study period.

Discussion

In this study, we tested the efficacy of a symbiotic on subclinical bovine mastitis and studied its effect on the physicochemical composition of milk. It was found that 58/240 (24.16%) cows had SCM and no cows were detected with clinical mastitis. The predominance of SCM compared to clinical mastitis has been reported in other studies (Dego and Tareke, 2003; Sori et al., 2011; Zeryehun et al., 2013). This is because SCM is undiagnosed and breeders pay little attention, unlike clinical mastitis, for which the breeder is required to treat it (Karimuribo et al., 2006; Girma, 2010). The results obtained are lower in comparison with the results of Abebe et al. (2016) and Amer et al. (2018) who found prevalence close to 60%.

It was found that more than 34% of milk samples from SCM contained a single bacterial species. This is lower in comparison with the results of other studies where these parameter values ranged between 58.6% and 76.7% (Ramisse et al., 1982; David et al., 1988; Schukken et al., 1989; Fabre et al., 1991; Sargeant et al., 1998).

The prevalence of subclinical poly-bacterial mastitis in our study was 66%. This result is higher than those obtained in many other reports (from 1.3% to 11.10%) (Ramisse et al., 1982; Fabre et al., 1991, 1997; Bradley, 2002).

Milk SCC is widely used as an indicator of intramammary infection, as the number of these cells increases markedly during clinical or subclinical mastitis (Schukken et al. 2003). This study revealed that the average SCC in cows from the control lot was higher than that of cows from the experimental lot in all three samples ($p<0.0001$). Those results clearly indicate that the symbiotic has a positive effect on the microbiological quality of the milk.

S. aureus is a major pathogen causing great problems in dairy cattle farming due to its contagious nature (transmission to milking machines and animals, intra-mammary persistence, antibiotic resistance) and causing heavy economic losses mainly due to the reduction in quality and quantity of milk produced (Wallemacq et al., 2010). *S. aureus* is responsible for a significant proportion of SCM in dairy cows (Kasozi et al., 2014). The frequency of *S. aureus* in clinical mastitis ranges from 7 to 40% (Fox and Gay, 1993).

The established prevalence of *S. aureus* (36.20%) in the present investigation is close to the results reported by Nagahata et al. (2007) and Busato et al. (2000) and higher compared to the proportions found by other authors (Messadi et al., 1990; Martel, 1991). The discrepancies in these studies could be attributed to the difference in the breed, management system, and the epidemiological status (Radostits et al., 2007).

Coagulase-negative staphylococci are responsible for the moderate increase in somatic cell concentration in milk (Fabre et al., 1997). The prevalence of coagulase-negative staphylococci in subclinical mammary infections in dairy cows varies from country to another, depending on the period and the authors, ranging from 23 to 78% (Poutrel, 2005). They were isolated in 51.72% of the mastitis samples in our survey. This finding is comparable to the results of Bussato et al. (2000) and higher than the data reported by Fabre et al. (1991, 1997). However, it is inferior to the results of Hamiroune et al. (2016). The high prevalence of coagulase-negative staphylococci isolated in our study is due to poor hygienic milking conditions. Several studies have shown that the application of disinfection of teats after milking contributes to the decrease of the

prevalence of coagulase-negative staphylococci (Fox and Gay, 1993; Poutrel, 2005).

The results obtained show that 36/58 (62.07%) SCM samples were caused by enterobacteria, of which 21/58 were caused by *E. coli* (36.20%). This is higher in comparison with the results of Fabre et al. (1991, 1997) and Sargeant et al. (1998), and comparable to the findings of Martel (1991) and Bradley (2002). Poor bedding maintenance, poor hygiene and poor housing of animals in general could explain the high frequency of *E. coli* isolated in this study.

After administering the symbiotic to the experimental lot, we observed a significant decrease in the level of *S. aureus* and *E. coli* in the milk from the first introduction of the symbiotic. This significant decrease is probably due to the antimicrobial action of the symbiotic. This hypothesis is consistent with the report of Beecher et al. (2009). Symbiotics induce a decrease in the digestive pathogen charge with an increase in the beneficial digestive flora and consequently a decrease in the environmental contamination of cows (Chaucheyras-Durand et al., 2008). Certain probiotic bacteria have shown an ability to inhibit the invasion of bovine mammary epithelial cells by *E. coli* and *S. aureus* (Assis et al., 2015).

The value of the titratable acidity of normal fresh milk from non-mastitic cows varies from 16 to 18°D (Veisseyre, 1979). In our study, the titratable acidity of the milk of the two lots is higher and this is due to the SCM. Exceptions are the second and third samples from the treated with symbiotic cows where the titratable acidity is within the above-mentioned borders (Table 1, Figure 3). In mastitis milk the titratable acidity increases due to the degradation of lactose into other acids in addition to lactic acid and lipids (Veisseyre, 1979). The activity of lactic acid bacteria was significantly lower in milk from SCM due to the activity of pathogens that inhibit the activity of lactic acid bacteria. The acidity of milk can be an indicator of the quality of raw milk because it allows an assessment of the amount of acid produced by the bacteria (Veisseyre, 1979). We found that titratable acidity in symbiotic treated cows was lower (average 18.3°D) compared to the control cows (average 19.8°D), indicating the antimicrobial role of the symbiotic. However, in the control lot, the average acidity was still high (18.3-21.4°D).

In our study, no significant difference was detected between the values obtained for the protein and butter content of the milk of the two lots (control and experimental) throughout the study period. This clearly indicates that symbiotics do not have a negative influence on the fat and protein composition of the milk. This result corroborates the observations of Oetzel et al. (2007).

Conclusion

The symbiotic appears to be effective in the control of subclinical mastitis (SCM) in dairy cows. In fact, it was found that administration of the symbiotic to cows with SCM resulted in a significant decrease in milk somatic cell counts (SCC) and the prevalence of *S. aureus* and *E. coli* responsible of SCM. Physiochemical quality of the milk (titratable acidity, butyric and protein content) was not altered by the administration of the symbiotic. It would be interesting to continue this study in cows with clinical mastitis in order to gain further knowledge on the effect of symbiotics on clinical mastitis.

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References

Abebe R, Hatiya H, Abera M, Megersa B and Asmare K, 2016. Bovine mastitis: prevalence, risk factors and isolation of *Staphylococcus aureus* in dairy herds at Hawassa milk shed, South Ethiopia. BMC Veterinary Research, 12(1), 270. https://doi.org/10.1186/s12917-016-0905-3

Amer S, Gálvez FLA, Fukuda Y, Tada C, Jimenez IL, Valle WFM and Nakai Y, 2018. Prevalence and etiology of mastitis in dairy cattle in El Oro Province, Ecuador. Journal of Veterinary Medical Science, 80(6), 861-868. https://doi.org/10.1292/jvms.17-0504

Assis BS, Germon P, Silva AM, Even S, Nicoli JR and Loir YL, 2015. *Lactococcus lactis* V7 inhibits the cell invasion of bovine mammary epithelial cells by *Escherichia coli* and *Staphylococcus aureus*. Beneficial Microbes. 6(6), 879-886. https://doi.org/10.3920/bm2015.0019

Bauer AW, Kirby WMM, Sherris JC and Turck M, 1966. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology, 36, 493-496.

Beecher C, Daly M, Berry DP, Klostermann K, Flynn J, Meaney W, Hill C and McCarthy TV, 2009. Administration of a live culture of *Lactococcus lactis* DPC3147 into the bovine mammary gland stimulates the localhost immune response, particularly IL-1beta and IL-8 gene expression. Journal of Dairy Research, 76, 340-348.

Blum SE, Heller ED and Leitner G, 2014. Long term effects of *Escherichia coli* mastitis. Veterinary Journal, 201, 72-77. https://doi.org/10.1016/j.tvjl.2014.04.008

Bradley AJ, 2002. Bovine mastitis: an evolving disease. Veterinary Journal, 164, 2, 116-128.

Busato A, Trachsel P, Schallibaum M and Blum JW, 2000. Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland. Preventive Veterinary Medicine, 44, 205-220.

Chaucheyras-Durand F, Walker ND and Bach A, 2008. Effects of active dry yeasts on the rumen microbial ecosystem: Past, present and future. Animal Feed Science and Technology, 145(1-4), 5-26.

Chebel RC, 2007. Mastitis effects on reproduction. NMC Regional Meeting Proceedings, 43-55.

Crispie F, Alonso-Gomez M, O'Loughlin C, Klostermann K,

Flynn J, Arkins S, Meaney W, Paul Ross R and Hill C, 2008. Intramammary infusion of a live culture for treatment of bovine mastitis: effect of live lactococci on the mammary immune response. Journal of Dairy Research, 75(3), 374-84.

David RC, Legrand M, Nicolas JA and Thomasson C, 1988. Bactériologie des mammites bovines. Résultats d'enquête de terrain. Bulletin de la Société Vétérinaire Pratique de France, 72, 529-539 (F).

De Briyne N, Atkinson J, Pokludova L and Borriello SP, 2014. Antibiotics used most commonly to treat animals in Europe. Veterinary Record, 175(13), 325. https://doi.org/10.1136/vr.102462

Dego OK and Tareke F, 2003. Bovine mastitis in selected areas of southern Ethiopia. Tropical Animal Health and Production, 35, 3, 197-205.

Dhakal IP and Thapa BB, 2002. Economic impact of clinical mastitis in the buffaloes in Nepal. Buffalo Journal, 2, 225-34.

Djabri B, Bareille N, Beaudeau F and Seegers H, 2002. Quarter milk somatic cell count in infected dairy cows: a meta-analysis. Veterinary Research, 33, 335-57.

Fabre JM, Berthelot X, Morvan H, Lebret P, Blanc MF and Blanc MC, 1991. Estimation de la fréquence des différents germes responsables d'infections mammaires dans le Sud Ouest de la France. Revue de Médecine Vétérinaire, 142, 823-829 (F).

Fabre JM, Morvan H, Lebreux B, Houffschmitt P and Berthelot X, 1997. Estimation de la fréquence des différents germes responsables de mammites en France. Partie 1: mammites cliniques. Bulletin du Groupe Technique Vétérinaire, 552, 17-23 (F).

Ferney J, Oudar J and De Saint Aubert G, 1966. Diagnostic bactériologique des mammites. Revue de Médecine Vétérinaire, 17, 845-858 (F).

Fox LK and Gay JM, 1993. Contagious mastitis. Veterinary Clinics of North America - Food Animal Practice, 9, 475-487.

Francoz D, Wellemans V, Dupre JP, Roy JP, Labelle F, Lacasse P and Dufour S, 2017. Invited review: a systematic review and qualitative analysis of treatments other than conventional antimicrobials for clinical mastitis in dairy cows. Journal of Dairy Science, 100, 7751-70. https://doi.org/10.3168/jds.2016-12512

Girma D, 2010. Study on prevalence of dairy cows around Holeta Areas, West Shewa Zone of Oromia Region, Ethiopia. Global Veterinaria, 5, 6, 318-323.

Gonzalo C, Martinez JR, Carriedo JA and San Primitivo F, 2003. Fossomatic cell-counting on ewe milk: comparison with direct microscopy and study of variation factors. Journal of Dairy Science, 86, 138-145.

Hamiroune M, Berber A and Boubekeur S, 2016. Evaluation of the bacteriological quality of raw cow's milk at various stages of the milk production chain on farms in Algeria. Revue Scientifique et Technique-Office International des Epizooties, 35(3), 925-946.

Karimuribo ED, Fitzpatrick JL, Bell CE, Swai ES, Kambarage DM, Ogden NH, Bryant MJ and Rench NP, 2006. Clinical and subclinical mastitis in smallholder dairy farms in Tanzania: risk, intervention and knowledge transfer. Preventive Veterinary Medicine, 74, 1, 84-98.

Kasozi KI, Tingiira JB and Vudriko P, 2014. High prevalence of subclinical mastitis and multidrug resistant *Staphylococcus aureus* are a threat to dairy cattle production in Kiboga District (Uganda). Open Journal of Veterinary Medicine, 4(04), 35-43. https://dx.doi.org/10.4236/ojvm.2014.44005

Kehrli ME and Shuster DE, 1994. Factors affecting milk somatic cells and their role in health of the bovine mammary gland. Journal of Dairy Science, 77, 619-27.

Klostermann K, Crispie F, Flynn J, Ross RP, Hill C and Meaney W, 2008. Intramammary infusion of a live culture of *Lactococcus lactis* for treatment of bovine mastitis: comparison with antibiotic treatment in field trials. Journal of Dairy Research, 75(3), 365-73.

Lukas JM, Hawkins DM, Kinsel ML and Reneau JK, 2005. Bulk tank somatic cell counts analyzed by statistical process control tools to identify and monitor subclinical mastitis incidence. Journal of Dairy Science, 88, 3944-52. https://doi.org/10.3168/jds.s0022-0302(05)73080-0

Martel JL, 1991. Le diagnostic bactériologique des mammites. In: Les mammites de la vache laitière. Société Française de Buiatrie Edit., Paris, 18-19 décembre 1991, pp. 75-80 (F).

Martin P, Barkema HW, Brito LF, Narayana SG and Miglior F, 2018. Symposium review: novel strategies to genetically improve mastitis resistance in dairy cattle. Journal of Dairy Science, 101, 2724-36. https://doi.org/10.3168/jds.2017-13554

Messadi L, Benmiled L and Haddad N, 1990. Mammites bovines en Tunisie: Bactéries responsables et antibiorésistance. Revue de Médecine Vétérinaire, 142, 313-319 (F).

Murray PR, Baron EJ, Jorgensen JH, Pfaller MA and Yolken RH, 2003. Manual of Clinical Microbiology. 8th Edition. American Society for Microbiology, Washington, D.C., p. 2310

Nagahata H, Ito H, Maruta H, Nishikawa Y, Suskino H, Matski S, Higuchi H, Okuhira A and Anri A, 2007. Controlling highly prevalent *Staphylococcus aureus* mastitis from dairy farm. Journal of Veterinary Medicine Science, 69, 893-898.

National Mastitis Council (NMC), 2004. Microbiological procedures for the diagnosis of udder infection. 3. Arlington: National Mastitis Council Inc.

Oetzel GR, Emary KM, Kautz WP and Nocek JE, 2007. Direct-fed microbial supplementation and health and performance of pre- and postpartum dairy cattle: a field trial. Journal of Dairy Science, 90, 2058-2068.

Poutrel B, 2005. Les staphylocoques coagulase-négative impliqués dans les infections mammaires: des athogènes émergents. Journées Européennes organisées par la Société Française de Buiatrie, 151-156 (F).

Quinn PJ, Carter ME, Markey B and Carter GR, 1994. Clinical veterinary microbiology. Edit. Wolfe, p. 648.

Quinn PJ, Carter ME, Markey B and Carter GR, 1999. Clinical Veterinary Microbiology. 1. London: Elsevier Ltd. No. SF 780.2. C54.

Radostits OM, Gay KW, Hinchcliff CC and Constable PD,

2007. Mastitis. In: Veterinary Medicine: A Text Book of Disease of Cattle, Sheep, Pigs, Goats, and Horses, 10th edition, pp. 674-762, Bailliere Tindall, London, UK.

Ramisse J, Brement AM, Lamarre C, Viaud MA and Breard A, 1982. Résultats d'une enquête sur les mammites en Vendée. Le Point Vétérinaire, 13, 63-73 (F).

Roy S, Vishwakarma P, Roy M and Sharma M, 2009. Prevalence and control of bubaline mastitis in Chhattisgarh state in India. Pakistan Journal of Zoology, 9, 281-7.

Salvador RT, Beltran JMC, Abes NS, Gutierrez CA and Mingala CN, 2012. Short communication: prevalence and risk factors of subclinical mastitis as determined by the California Mastitis Test in water buffaloes (*Bubalis bubalis*) in Nueva Ecija. Philippines. Journal of Dairy Science, 95, 1363-6.

Sanders ME, Lenoir-Wijnkoop I, Salminen S, Merenstein DJ, Gibson GR, Petschow BW, Nieuwdorp M, Tancredi DJ, Cifelli CJ, Jacques P and Pot B, 2014. Probiotics and prebiotics: prospects for public health and nutritional recommendations. Annals of the New York Academy of Sciences, 1309, 19-29. https://doi.org/10.1111/nyas.12377

Santos MV and Fonseca LFL, 2007. Estratégias para controle de mastite e melhoria da qualidade do leite. 1a. Pirassununga (Sp).

Sargeant JM, Morgan A, Scott H, Leslie KE, Ireland MJ and Bashiri A, 1998. Clinical mastitis in dairy cattle in Ontario: frequency of occurrence and bacteriological isolates. Canadian Veterinary Journal, 39, 33-38.

Schukken YH, Smit JAH, Grommers FJ, Vandegeer D and Brand A, 1989. Effect of freezing on bacteriology culturing of mastitis milk samples. Journal of Dairy Science, 72, 1900-1906.

Schukken YH, Wilson DJ, Welcome F, Garrison-Tikofsky L and Gonzalez RN, 2003. Monitoring udder health and milk quality using somatic cell counts. Veterinary Research, 34, 579-96.

Sharma N, Gupta SK, Sharma U and Hussai K, 2007. Treatment of clinical mastitis in buffalo case report. Buffalo Bulletin, 26, 56-8.

Sharma N, Singh NK and Bhadwal MS, 2011. Relationship of somatic cell count and mastitis: an overview. Asian-Aust. Journal Animal Science, 24, 429-38.

Shuster DE, Harmon RJ, Jackson JA and Hekman RW, 1991. Endotoxin mastitis in cows milked four times daily. Journal of Dairy Science, 74, 1527-38.

Singh RS and Bansal BK, 2004. Variation in selected components of milk among different milk fractions and its relevance to diagnosis of mastitis in buffaloes. Buffalo Journal, 3, 213-24.

Sori T, Hussien J and Bitew M, 2011. Prevalence and susceptibility assay of *Staphylococcus aureus* isolated from bovine mastitis in dairy farms of Jimma town, South West Ethiopia. Journal of Animal and Veterinary Advances, 10, 6, 745-749.

Veisseyre R, 1979. Technologie du lait constitution, récolte, traitement et transformation du lait. 3éme édition. Edition la maison rustique, Paris, p. 714 (F).

Wallemacq H, Girard B, Lekeux P and Bureau F, 2010. La vaccination contre les mammites à *Staphylococcus aureus* chez la vache laitière. Annales de Médecine Vétérinaire, 154, 16-29 (F).

Zeryehun T, Aya T and Bayecha R, 2013. Study on prevalence, bacterial pathogens and associated risk factors of bovine mastitis in small holder dairy farms in and around Addis Ababa, Ethiopia. Journal of Animal Plant Science, 23, 1, 50-55.