Preliminary *in vivo* Evaluation of Hydroalcoholic *Eucalyptus camaldulensis* Extract as an Alternative Intramammary Therapy for Bovine Mastitis

Nathalia Rocha[1], Natalia Hernández[1], Judyanna Aguirre[1], Jhosep Quiroz[1], Luis M Salinas[1], Byron Flores[1]

[1]*Universidad Internacional Antonio de Valdivieso, 47000, Rivas, Nicaragua*

Keywords: *antimicrobial activity, bovine mastitis, dairy cattle, Eucalyptus, intramammary treatment.*

Abstract. *Bovine mastitis is an inflammatory condition of the mammary gland that induces physical, chemical, and bacteriological alterations in milk, as well as structural changes in mammary tissue. These impacts highlight the need for practical, viable, and cost-effective therapeutic alternatives. The objective of this exploratory, preliminary study was to assess the potential efficacy of a hydroalcoholic extract of Eucalyptus camaldulensis as an alternative antimicrobial treatment for bovine mastitis. The sample consisted of 40 quarters with mastitis corresponding to 10 lactating cows, divided into two groups of 20 quarters in the control group (5 cows) and 20 (5 cows) in the group treated with the application of three intramammary doses of the E. camaldulensis extract. CMT scores showed no statistically significant differences between the control and treatment groups, either before or 48 hours after the third administration (OR = 0.63; 95% CI: 0.11–3.70; P = 0.610). Evaluation of the antimicrobial activity of E. camaldulensis extract demonstrated a significant reduction (P < 0.001) in bacterial load 24 hours after the third intramammary application. This effect was not observed in the control group (P ⩾ 0.05), as determined by the marginal means test of the mixed-effects model. Based on the findings of this pilot study, the hydroalcoholic extract of E. camaldulensis demonstrated a reduction in bacterial load, suggesting possible in vivo antimicrobial activity. However, because somatic cell count (SCC) was not measured and inflammatory response was assessed only through the California Mastitis Test (CMT), the study cannot determine whether the treatment influenced mammary inflammation or clinical resolution of mastitis.*

Introduction

Bovine mastitis is an inflammation of the mammary gland that can lead to physical, chemical, and bacteriological alterations in milk, as well as structural changes in glandular tissue (Goulart, 2022). From a clinical perspective, clinical mastitis is characterized by visible signs of inflammation of the mammary gland, such as redness and swelling, as well as the presence of clots or other macroscopic changes in the milk, which may be accompanied by more severe systemic signs in acute cases. In contrast, subclinical mastitis does not present overt clinical signs but is associated with an increased somatic cell count and a reduction in milk yield, making it difficult to detect without specific diagnostic tests such as somatic cell counting or the CMT (Morales-Ubaldo, 2023). Intramammary inflammation is associated with an increase in somatic cell count (SCC) in milk, and the magnitude of this increase varies depending on the bacterial species involved. Because SCC is a key marker of inflammatory status and recovery, the absence of post-treatment SCC measurements in the present study precludes an objective assessment of inflammatory resolution (Ramuada, 2024).

Epidemiologically, the subclinical form is far more prevalent than the clinical form, making it a major challenge for diagnosis and treatment. The absence of visible clinical signs necessitates the use of diagnostic methods for early pathogen identification and the selection of targeted therapies, which in turn may help address the increasing emergence of antimicrobial-resistant bacterial strains. In addition, continuous monitoring and the exploration of alternative therapeutic approaches are required to improve udder health and treatment efficiency (Tommasoni, 2023).

Bovine mastitis is one of the most common diseases in dairy herds and represents one of the greatest economic burdens for the dairy industry. It may be present in clinical or subclinical forms. Factors such as general animal management practices and improper milking procedures, whether performed manually or mechanically, facilitate the harmful activity of common pathogenic microorganisms, including *Streptococcus agalactiae, Staphylococcus aureus, Streptococcus uberis*, *Klebsiella* spp., *Escherichia coli, and Enterococcus* spp. (Morales-Ubaldo, 2023).

The inappropriate use of antibiotics in veterinary medicine poses risks to both human and animal health due to the development of antimicrobial resistance, as observed in *Staphylococcus aureus* and its resistance to penicillin. One of the major concerns of the World Health Organization (WHO) and the World

Corresponding author: byronfloressomarriba@gmail.com

Organization for Animal Health (WOAH) is the growing bacterial resistance to antibiotics, as well as the increasing costs this imposes on livestock production and the residual effects on consumers. This issue has stimulated research aimed at identifying viable, practical, and cost-effective therapeutic alternatives (Ul-Hamid, 2023). Among these alternatives, natural compounds have gained attention for their ability to stimulate innate defense mechanisms and reduce mammary tissue inflammation, thereby promoting healing and enhancing the resilience of the affected tissue (Askari, 2025).

Eucalyptus was introduced to the Americas by Europeans in the late nineteenth century and has since been used for its medicinal properties to treat numerous diseases, including those of bacterial origin. This plant contains polyphenolic compounds, flavanones, and hydrolysable tannins, which confer strong antioxidants and antimicrobial activity. Recent studies have demonstrated its antimicrobial efficacy against *S. aureus*, *Klebsiella* spp. and *E. coli* (Uriol Plasencia, 2019), and depending on the concentration of *E. camaldulensis* extract, bacteriostatic or bactericidal effects can be obtained against different strains of *S. aureus* (Salari, 2006).

Currently, the use of medicinal plants as alternative treatments for mastitis has not been widely implemented, despite the limited reports of bacterial resistance to plant-derived products. In Nicaragua, there is previous evidence demonstrating the *in vitro* antimicrobial capacity of the hydroalcoholic extract of *Eucalyptus globulus* (Flores-Somarriba, 2021). However, its potential antimicrobial effect has not yet been preliminarily explored in vivo in cows with mastitis, and its cytotoxicity remains unknown. The objective of this exploratory, preliminary study was to assess the potential efficacy of a hydroalcoholic extract of *E. camaldulensis* as an alternative antimicrobial treatment for bovine mastitis. Given the absence of somatic cell count measurements and the short follow-up period, the study was not designed to fully evaluate the inflammatory component of mastitis and should therefore be interpreted as generating preliminary hypotheses rather than confirmatory evidence.

Material and Methods

Preparation of the *Eucalyptus camaldulensis* Extract

Two kilograms of *E. camaldulensis* leaves were collected and washed with 5 L of distilled water to remove extraneous substances. The leaves were then air-dried on metal trays at room temperature and under shade for 7 days. Subsequently, 400 g of the dried leaves were weighed using a digital balance and ground with a conventional blender to obtain a fine powder.

A volume of 1800 mL of 70% (v/v) ethyl alcohol was measured and poured into an Erlenmeyer flask containing the powdered plant material. The mixture was covered with aluminum foil to prevent evaporation and allowed to macerate for 24 h. After this period, the extract was filtered through Whatman No. 1 filter paper into another Erlenmeyer flask. Simultaneously, 100 mL of 70% (v/v) ethyl alcohol was placed in a separate Erlenmeyer flask as a control. Both flasks were left uncovered at room temperature for 7 days. As a final step to remove residual particles, the extract was transferred to a particle separation funnel and left for 48 h until a clear, impurity free liquid was obtained (Flores-Somarriba, 2021). To ensure bacterial sterility prior to *in vivo* application, the extract was inoculated onto tryptic soy agar and 5% blood agar and incubated aerobically for 48 h at 35°C.

Evaluation of the Cytotoxicity of the Extract

A 1% suspension of human red blood cells was prepared. From this suspension, 100 µL was mixed with 100 µL of the hydroalcoholic extract of *E. camaldulensis* at concentrations of 200, 100, 50, 25, 12.5, and 6.75 mg/mL. Simultaneously, a control was prepared by mixing 100 µL of the erythrocyte suspension with 100 µL of saline solution (0.85% NaCl). All mixtures were incubated at 37°C for 30 min and subsequently examined under a microscope to assess the presence of hemolysis in comparison with the control. A cytopathic effect was considered present at concentrations showing hemolysis (Meza et al., 2016).

Determination of Bovine Mastitis

Somatic cell count (SCC) was not measured in this study due to logistical constraints; therefore, inflammatory status was evaluated only using the CMT, which served as a qualitative field screening tool. Subclinical mastitis screening was performed using the CMT. Prior to sampling, the mammary quarters were cleaned with a disinfectant, dried with disposable paper towels, and disinfected with 70% alcohol. The first streams of milk were discarded, and one to two streams per quarter were collected onto the CMT paddle. Subsequently, an equal volume of CMT reagent was added, mixed, and the presence of gel formation was evaluated as an indicator of subclinical mastitis. The paddle was rinsed between animals to prevent cross-contamination (Ranasinghe, 2021).

To confirm mastitis and identify the bacterial agent, the samples were cultured aerobically on 5% blood agar. Identification was carried out based on colony characteristics, Gram staining, catalase test, growth on mannitol salt agar, and coagulase tests on slide and in tube (Mahamed, 2023).

Experimental Design

A controlled and randomized experimental study was conducted. Forty quarters from 10 cows were included; the cows were selected based on

a previous study to ensure that all four quarters presented mastitis according to the CMT and had intramammary infection confirmed by *Staphylococcus aureus*. In the experimental group, each of the four mammary quarters (20 quarters in total) received an intramammary inoculation of 5 mL of a hydroalcoholic extract of *E. camaldulensis* at a concentration of 50 mg/mL. Treatments were administered three times at 7-day intervals (days 0, 7, and 14).

In the control group, each mammary quarter received 5 mL of sterile saline according to the same administration schedule as the treatment group. To evaluate changes in bacterial load, *Staphylococcus* counts were quantified using Petrifilm® Staph Express Count Plates both before the first treatment and 24 h after the third treatment (Nagasawa, 2020). Additionally, the CMT was performed prior to treatment initiation and again 48 h after the third treatment.

Because this study was designed as a preliminary pilot investigation, no formal sample size calculation was performed. Consequently, the number of animals included was limited and the study should be considered statistically underpowered for confirmatory inference.

Statistical Analysis

To evaluate treatment efficacy, CMT scores, treated as an ordinal repeated outcome variable, were analyzed using a cumulative logit ordinal mixed-effects regression model. The model included fixed effects for group (treatment vs. control), time (baseline vs. post-treatment), and their interaction. To account for the clustered and repeated-measures structure of the data, cow was included as a random effect.

Odds ratios (ORs) and their 95% confidence intervals (95% CIs) were estimated from the model. Predicted probabilities for each CMT score category were calculated based on the fitted model. Statistical significance was set at $P < 0.05$. In contrast, bacterial load was analyzed using a linear mixed-effects model, including the experimental group, sampling time, and their interaction as fixed effects, and cow as a random effect, with mammary quarters nested within each cow. The model was fitted by restricted maximum likelihood (REML). Adjusted marginal means were estimated, and post hoc comparisons were performed when appropriate. Analyses were conducted in excel and R (R Foundation for Statistical Computing, Vienna, Austria).

Ethics Statement

The research protocol was approved by the Department of Veterinary Medicine and Animal Science. All procedures were performed by highly qualified veterinarians affiliated with the Universidad Internacional Antonio de Valdivieso (UNIAV). The study was conducted in accordance with Law No. 747, which regulates the protection and welfare of domestic and domesticated wild animals.

Results

Hemolytic Activity of the Extract

Hemolytic activity was detected at extract concentrations of 200 and 100 mg/mL. No hemolytic activity was observed at lower concentrations.

CMT Scores: Ordinal Regression Analysis

The cumulative logit ordinal regression model showed a non-significant tendency toward higher CMT scores in the treatment group compared with the control group (OR = 3.46; 95% CI: 0.94–12.70; $P = 0.061$). The main effect of sampling time (baseline vs. follow-up) was not statistically significant (OR = 1.59; 95% CI: 0.28–8.82; $P = 0.599$). Likewise, the Group × Time interaction was not statistically significant (OR = 0.63; 95% CI: 0.11–3.70; $P = 0.610$).

Predicted Probabilities of CMT Scores

In the control group at baseline, the highest predicted probability corresponded to CMT score 2 (0.59; 95% CI: 0.46–0.72), followed by CMT score 3 (0.25; 95% CI: 0.08–0.42) and CMT score 1 (0.16; 95% CI: 0.03–0.29). After follow-up, the predicted probability of CMT score 2 remained the highest (0.59; 95% CI: 0.47–0.72), followed by CMT score 1 (0.23; 95% CI: 0.06–0.41) and CMT score 3 (0.18; 95% CI: 0.03–0.32).

In the treatment group at baseline, the predicted probability was highest for CMT score 2 (0.50; 95% CI: 0.33–0.67), followed by CMT score 3 (0.42; 95% CI: 0.21–0.63) and CMT score 1 (0.08; 95% CI: 0.001–0.16). After treatment, predicted probabilities were 0.49 (95% CI: 0.31–0.66) for CMT score 2, 0.43 (95% CI: 0.21–0.66) for CMT score 3, and 0.08 (95% CI: 0.001–0.15) for CMT score 1 (Fig. 1).

Bacterial Load Analysis

The linear mixed-effects model revealed a significant effect of treatment group on bacterial load ($\beta = -0.590$; SE = 0.164; $z = -3.61$; $P < 0.001$). The main effect of sampling time was not statistically significant ($\beta = 0.064$; SE = 0.114; $z = 0.56$; $P = 0.576$). However, a significant Group × Time interaction effect was observed ($\beta = 0.440$; SE = 0.161; $z = 2.74$; $P = 0.006$), (Table 1).

Estimated marginal means indicated that, prior to treatment, bacterial load was 4.12 $\log_{10}$ in the treatment group and 4.27 $\log_{10}$ in the control group, with no statistically significant difference between the groups (difference = –0.15 $\log_{10}$; 95% CI: –0.42 to 0.12; $P = 0.28$). After treatment, bacterial load was 3.62 $\log_{10}$ in the treatment group and 4.21 $\log_{10}$ in the control group. At this time point, the difference between the groups was –0.59 $\log_{10}$ (95% CI: –0.91 to –0.27; $P < 0.001$), (Fig. 2).

For the mixed-effects model, variance attributable

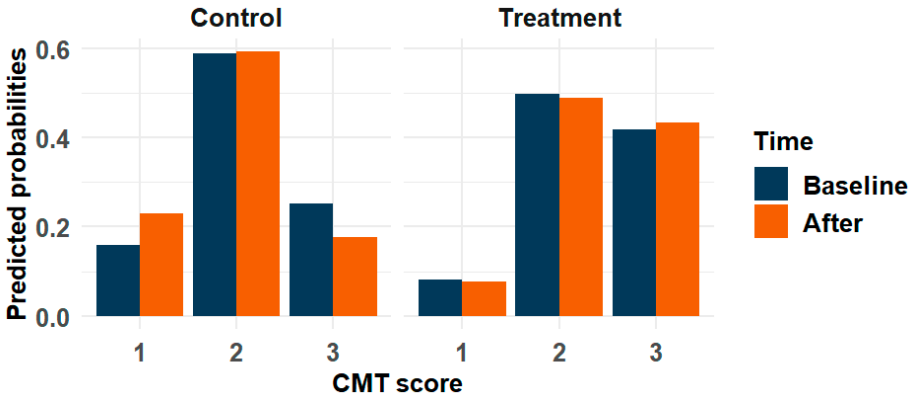

Fig. 1. Predicted probabilities of CMT scores in cows with subclinical mastitis at baseline and 48 hours after treatment with saline solution (control) or *Eucalyptus camaldulensis* (treatment)

Table 1. Estimates of fixed effects on bacterial load

Effects	Estimation (β)	Standard error	z value	*P*-value
Intercept (Control – After)	4.206	0.116	36.33	<0.001
Group (Treatment vs. Control)	–0.590	0.164	–3.61	<0.001
Time (Before vs. After)	0.064	0.114	0.56	0.576
Group x Time	0.440	0.161	2.74	0.006

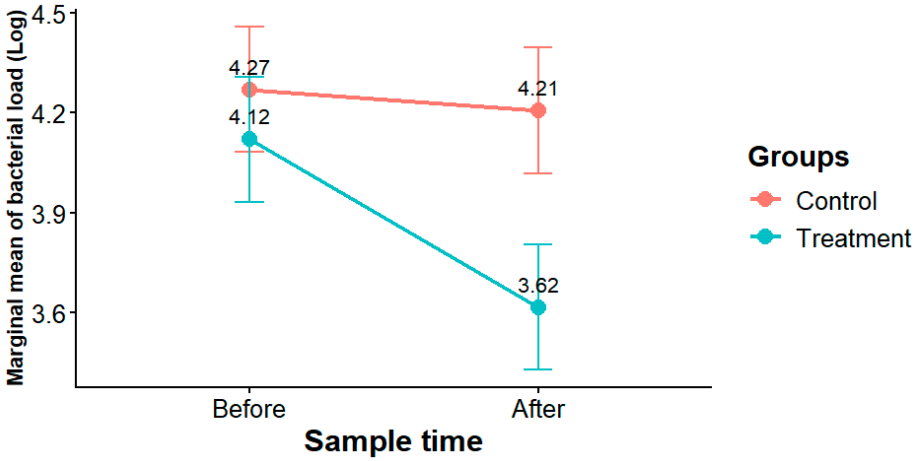

Fig. 2. Estimated marginal means of bacterial load before and after treatment (24 h after the third application) in the control and treatment groups. Points represent the estimated marginal means, and error bars indicate 95% confidence intervals.

to differences between cows was estimated at 0.035, whereas residual variance was 0.129.

Discussion

The present pilot study explored the potential antimicrobial effect of a hydroalcoholic extract of *E. camaldulensis* in cows with mastitis. The main finding was a reduction in bacterial growth after treatment, suggesting that the extract may exert antimicrobial activity under field conditions.

The cytotoxicity findings of the present study indicate that the hydroalcoholic extract of *E. camaldulensis* does not induce a cytopathic effect at concentrations below 50 mg/mL. Notably, this study represents one of the few investigations to assess *in vitro* cytotoxicity of a plant-derived product intended for the treatment of subclinical mastitis. The evaluation of *in vitro* cytotoxicity constitutes a critical prerequisite prior to intramammary application, as it ensures the absence of deleterious effects on mammary gland cells. This approach is consistent with the study by Kaseke (2023), who conducted preliminary toxicity assessments before performing *in vivo* trials, thereby adhering to fundamental principles of animal welfare and ethical research.

The lack of observable changes in CMT scores may be partially explained by the temporal dynamics of the inflammatory response within the mammary gland.

In mastitis, inflammatory indicators often require longer periods to return to baseline levels following treatment, particularly when tissue repair and immune regulation are still ongoing. In the present study, the assessment of inflammation was limited to CMT and did not include somatic cell count (SCC), which is considered a more sensitive indicator of leukocyte infiltration associated with mammary inflammation. Consequently, inflammatory markers regulated by immune mediators may remain elevated during the early post-treatment period, as the mammary immune response may not have fully resolved within 48 h after treatment (Peckler, 2025).

This interpretation is consistent with previous studies reporting reductions in inflammatory indicators only after longer observation periods. For example, Zelaya Mendoza (2017) reported a decrease in somatic cell counts in cows with mastitis following intramammary administration of a 20% *Hamelia patens* extract after a longer treatment period. Although anti-inflammatory properties have been described for essential oils of *E. camaldulensis* in experimental models (Mondal, 2021), the present study did not directly evaluate the inflammatory response. Therefore, no conclusions can be drawn regarding potential anti-inflammatory effects of the hydroalcoholic extract evaluated here.

The reduction in bacterial presence observed after intramammary administration of the *E. camaldulensis* extract suggests that the formulation may exert antimicrobial activity within the mammary gland environment. The antimicrobial activity observed is consistent with previous studies reporting inhibitory effects of *E. camaldulensis* extracts against bacterial pathogens associated with bovine mastitis, particularly *Staphylococcus* species. Experimental studies have demonstrated that extracts and essential oils of this species can inhibit the growth of *S. aureus* and other pathogenic bacteria through the action of bioactive phytochemicals such as phenolic compounds and terpenoids (Al-Hadid, 2022; Aouadhi, 2024). Furthermore, previous *in vitro* research conducted in Nicaragua reported promising antimicrobial activity of *Eucalyptus* against *S. aureus* isolates from bovine mastitis (Flores-Somarriba, 2021), supporting the findings of the present study.

The antimicrobial properties of *Eucalyptus* are attributed to its complex chemical composition, which includes cineole, terpene hydrocarbons, aldehydes, ketones, terpenes, terpenoids (e.g., 1,8-cineole, carvacrol), and aromatic compounds such as cinnamaldehyde and eugenol. The bactericidal activity of these compounds has been associated mainly with phenols and monoterpenes, which can interact directly with the bacterial cytoplasm or, due to their hydrophobic nature, integrate into the lipid components of the bacterial cell membrane. This interaction results in increased membrane permeability, leading to ion leakage and subsequent cell death (Montero-Recalde, 2019).

Several methodological limitations should be considered when interpreting these findings. First, the study included a relatively small sample size (five cows per group), and because it was designed as a pilot trial, it is statistically underpowered to detect modest treatment effects or to support confirmatory conclusions regarding clinical efficacy. Second, the inflammatory response of the mammary gland was not directly evaluated because somatic cell count (SCC), the standard quantitative indicator of mammary inflammation, was not measured (Huang, 2022). Instead, inflammation was assessed using the CMT, a practical screening tool that provides only a semi-quantitative estimate of milk cellularity and cannot replace direct SCC measurements. Finally, the follow-up period was limited to 48 hours after the final intramammary administration, which is likely insufficient to evaluate the resolution of mammary inflammation or the longer-term recovery of the mammary gland. Moreover, because bacterial load was assessed only 24 hours after the third treatment, the persistence of the antimicrobial effect could not be determined. Consequently, the present findings should be interpreted cautiously and considered hypothesis-generating rather than definitive evidence of treatment effectiveness.

Conclusion

Within the scope of this pilot study, the hydroalcoholic extract of *E. camaldulensis* demonstrated an acceptable *in vitro* safety profile at concentrations below 50 mg/mL, suggesting potential suitability for intramammary application. Although no significant changes in CMT scores were observed within the 48-hour post-treatment period, a reduction in bacterial load was detected in cows with mastitis, indicating possible *in vivo* antimicrobial activity. However, because somatic cell count (SCC) was not measured and inflammatory response was assessed only through CMT, the present study cannot determine whether the treatment promoted resolution of mammary inflammation or clinical recovery from mastitis. Because of the small number of animals included, the study is underpowered for confirmatory inference, and the results should therefore be interpreted as hypothesis-generating observations that require validation in larger, adequately powered trials. These findings provide an initial basis for further investigation of *E. camaldulensis* as a potential natural antimicrobial candidate for the management of bovine mastitis.

References

1. Al-Hadid, K.J., Al-Karablieh, N., Abu-Irmaileh, B., Sharab, A., Jaafreh, A.M.A. Antibacterial, Antifungal, Antioxidant, and Anti-Proliferative Effects of Eucalyptus camaldulensis and Pistacia atlantica Ethanol Extracts: doi.org/10.26538/tjnpr/v6i2.7. Tropical Journal of Natural Product Research. 2022. Vol. 6. P. 207–212.
2. Aouadhi, C., Jouini, A., Maaroufi, K., Maaroufi, A. Antibacterial Effect of Eight Essential Oils against Bacteria Implicated in Bovine Mastitis and Characterization of Primary Action Mode of Thymus capitatus Essential Oil. Antibiotics. 2024. Vol. 13. P. 237. https://doi.org/10.3390/antibiotics13030237.
3. Askari, S., Zomorodi, A.R., Aflakian, F. Alternative treatment candidates to antibiotic therapy for bovine mastitis in the post-antibiotic era: a comprehensive review. Microbial Pathogenesis. 2025. Vol. 205. P. 107684. https://doi.org/10.1016/j.micpath.2025.107684.
4. Flores-Somarriba, B., Mejía-Solorzano, J.L., Morales, M., Mora-Sánchez, B., Torres, D., Sheleby-Elias, J. Efecto inhibitorio del extracto hidroalcohólico de "Eucalipto" Eucalyptus globulus en bacterias aisladas de vacas con mastitis. Kasmera. 2021. Vol. 49. P. e49235288–e49235288. https://doi.org/10.5281/zenodo.5338773.
5. Goulart, D.B., Mellata, M. Escherichia coli Mastitis in Dairy Cattle: Etiology, Diagnosis, and Treatment. Frontiers in Microbiology. 2022. Vol. 13. P. 928346. https://doi.org/10.3389/fmicb.2022.928346.
6. Huang, C.-H., Kusaba, N. Association between differential somatic cell count and California Mastitis Test results in Holstein cattle. JDS Communications. 2022. Vol. 3. P. 441–445. https://doi.org/10.3168/jdsc.2022-0249.
7. Kaseke, T. Actividad antibacteriana de plantas medicinales en el tratamiento de la mastitis en vacas lecheras: una revisión sistemática - Kaseke - 2023 - Medicina y ciencia veterinaria - Biblioteca en línea de Wiley. 2023.
8. Mahamed, S.A., Omer, A.I., Osman, N.Y., Ahmed, M.A. Isolation and identification of Staphylococcus aureus from bovine milk and community awareness on public health significance of mastitis in and around Jigjiga, Somali region, Ethiopia. Heliyon. 2023. Vol. 9. https://doi.org/10.1016/j.heliyon.2023.e20981.
9. Meza, J.B., Álvarez, J.C., Forero, J.F., Wilches, M.C.G. Evaluación de la cito-genotoxicidad in vitro en eritrocitos y linfocitos humanos de los extractos de Croton niveus, Piper marginatum e Hyptis suaveolens, especies vegetales utilizadas en medicina tradicional del Departamento del Atlántico en el año 2016. 2016.
10. Mondal, M., Quispe, C., Sarkar, C., Bepari, T.C., Alam, Md.J., Saha, S., Ray, P., Rahim, M.A., Islam, M.T., Setzer, W.N., Salehi, B., Ahmadi, M., Abdalla, M., Sharifi-Rad, J., Kundu, S.K. Analgesic and Anti-Inflammatory Potential of Essential Oil of Eucalyptus camaldulensis Leaf: In Vivo and in Silico Studies. Natural Product Communications. 2021. Vol. 16. P. 1934578X211007634. https://doi.org/10.1177/1934578X211007634.
11. Montero-Recalde, M., Morocho-Núñez, M.J., Avilés-Esquivel, D., Carrasco-Cando, Á., Erazo-Gutierrez, R. Eficacia antimicrobiana del aceite esencial de eucalipto (Eucalyptus spp) sobre cepas de Escherichia coli y Staphylococcus aureus subsp. aureus. Revista de Investigaciones Veterinarias del Perú. 2019. Vol. 30. P. 932–938. https://doi.org/10.15381/rivep.v30i2.16099.
12. Morales-Ubaldo, A.L., Rivero-Perez, N., Valladares-Carranza, B., Velázquez-Ordoñez, V., Delgadillo-Ruiz, L., Zaragoza-Bastida, A. Bovine mastitis, a worldwide impact disease: Prevalence, antimicrobial resistance, and viable alternative approaches. Veterinary and Animal Science. 2023. Vol. 21. P. 100306. https://doi.org/10.1016/j.vas.2023.100306.
13. Nagasawa, Y., Kiku, Y., Sugawara, K., Yabusaki, N., Oono, K., Fujii, K., Suzuki, T., Maehana, K., Hayashi, T. Rapid Staphylococcus aureus Detection from Clinical Mastitis Milk by Colloidal Gold Nanoparticle-Based Immunochromatographic Strips. Frontiers in Veterinary Science. 2020. Vol. 6. https://doi.org/10.3389/fvets.2019.00504.
14. Peckler, G.L., Adcock, S.J.J. Evaluating somatic cell count, the California mastitis test, and infrared thermography for subclinical mastitis detection in meat ewes. Research in Veterinary Science. 2025. Vol. 193. P. 105791. https://doi.org/10.1016/j.rvsc.2025.105791.
15. Ramuada, M., Tyasi, T.L., Gumede, L., Chitura, T. A practical guide to diagnosing bovine mastitis: a review. Frontiers in Animal Science. 2024. Vol. 5. https://doi.org/10.3389/fanim.2024.1504873.
16. Ranasinghe, R.M.S.B.K., Deshapriya, R.M.C., Abeygunawardana, D.I., Rahularaj, R., Dematawewa, C.M.B. Subclinical mastitis in dairy cows in major milk-producing areas of Sri Lanka: Prevalence, associated risk factors, and effects on reproduction. Journal of Dairy Science. 2021. Vol. 104. P. 12900–12911. https://doi.org/10.3168/jds.2021-20223.
17. Salari, M.H., Amine, G., Shirazi, M.H., Hafezi, R., Mohammadypour, M.. Antibacterial effects of Eucalyptus globulus leaf extract on pathogenic bacteria isolated from specimens of patients with respiratory tract disorders. Clinical Microbiology and Infection: The Official Publication of the European Society of Clinical Microbiology and Infectious Diseases. 2006. Vol. 12. P. 194–196. https://doi.org/10.1111/j.1469-0691.2005.01284.x.
18. Tommasoni, C., Fiore, E., Lisuzzo, A., Gianesella, M. Mastitis in Dairy Cattle: On-Farm Diagnostics and Future Perspectives. Animals: an Open Access Journal from MDPI. 2023. Vol. 13. P. 2538. https://doi.org/10.3390/ani13152538.
19. Ul-Hamid, A., Baig, N., Haider, A., Hakeem, A.S., Ikram, M. Using biologically synthesized TiO2 nanoparticles as potential remedy against multiple drug resistant Staphylococcus aureus of bovine mastitis. Scientific Reports. 2023. Vol. 13. P. 18785. https://doi.org/10.1038/s41598-023-45762-4.
20. Uriol Plasencia, D.E. Actividad antimicrobiana in vitro de los extractos hidroalcohólicos de frutos de aguaymanto (physalis peruviana l.) y de hojas de eucalipto (eucalyptus globulus labill) frente a staphylococcus aureus. Universidad Privada Antenor Orrego. 2019.
21. Zelaya Mendoza, M.U. Evaluación del extracto de Hamelia patens como alternativa de tratamiento para el control de la mastitis subclínica en bovinos, municipio de El Sauce en período de julio a septiembre del 2016. 2017.