

Milk acute phase proteins in the diagnosis of subclinical mastitis in dairy cattle

S. Bashir^{1*}, N. Hassan¹, M. Shaheen², A.A. Dar¹

¹Division of Veterinary Epidemiology and Preventive Medicine; ²Division of Veterinary Clinical, Faculty of Veterinary Sciences and Animal Husbandry, Sher e Kashmir University of Agricultural Sciences & Technology, Kashmir (SKUAST-K)- 190006, Jammu & Kashmir, India.

*Corresponding author e-mail: shahnazq44@gmail.com

Journal of Livestock Science (ISSN online 2277-6214) 17: 174-180
Received on 15/5/25; Accepted on 26/3/26; Published on 30/3/26
doi. 10.33259/JLivestSci.2026.174-180

Abstract

Subclinical mastitis (SCM) greatly influences the udder health, lowers milk quality and quantity. The gross clinical signs being absent, it is left undetected leading to high economic impacts on dairy farming. Cow-side indirect tests are employed for diagnosis besides the bacteriological culture (BC) as the gold standard. Growth of at least one colony of a major pathogen on culture media was taken as the criterion for a positive test. This study aims at assessing the acute phase proteins amyloid A (MAA) and haptoglobin (Hp) in milk as markers for the SCM diagnosis. 150 SCM positive and 150 SCM negative (based on the BC) milk samples were analysed for MAA and Hp using commercial ELISA kits. The results were compared by validity criteria for the Accuracy and Mann-Whitney's U test compared with BC. The median of MAA for SCM positive samples was 27.85 mg/L (19.20, 38.5) and that for SCM negative samples was 3.13 mg/L (2.00, 4.38), $p < 0.001$, with an accuracy of 83.33% and Cohen's kappa value as 0.82. The median of milk Hp for SCM positive samples was 32.93 mg/L (23.30, 47.45) and that for the SCM negative samples was 2.86 mg/L (1.71, 3.77), $p < 0.001$, with an accuracy of 69.64% and Cohen's kappa value as 0.61. The ROC analyses showed the AUC for MAA and milk Hp as 92.80% and 81.40% with $p < 0.0001$ validating their higher diagnostic validity for SCM detection. Both MAA and milk Hp had a good agreement with gold standard test and can be employed as markers for SCM detection. Besides, MAA has superiority over milk Hp in terms of diagnostic validity. Milk APP's should be used as combined screening procedure with other tests for accurate detection of SCM.

Keywords: Milk acute phase proteins; milk amyloid A; Milk haptoglobin; Subclinical mastitis

Introduction

Mastitis is the inflammation of the mammary gland characterized by a range of physical and chemical changes in milk and pathological changes in the udder tissues (Constable et al., 2017). The inflammatory response results in an increase in the blood proteins and white blood cells in the mammary tissue, which can then pass into milk. This response aims to destroy the irritant, repair the damaged mammary tissue and return the udder to its normal function. However, as a result of such inflammations, a loss in milk output is the consequence. Mastitis is regarded a disease of greatest global impact on dairy farming (Hogeveen et al., 2011; Ruegg, 2017; Aghamohammadi et al., 2018). It is the most frequent and economically devastating disease in dairy cattle leading to decreased milk production, poor quality milk, premature culling, cost of veterinary medical intervention (Danilov et al., 2019; Tommasoni et al., 2023). Over 80% of all the antibiotics used in dairy farming are for mastitis treatment alone (Ashraf and Imran, 2020).

Mastitis in dairy cattle mostly occurs as a low grade infection, a subclinical form in which systemic and local signs of inflammation are absent and with no visible abnormalities in milk, but an increase in milk leucocyte and bacterial count, and reduced milk production (Das et al., 2018). As such, SCM is left undiagnosed and untreated causing the infection to spread other quarters and cows. It is the most prevalent form with the highest economic burden worldwide (Iraguha et al., 2015). The pooled prevalence of subclinical mastitis (SCM) and clinical mastitis (CM) was 42% and 15%, respectively, worldwide. In India, the pooled prevalence of SCM and CM was 45% and 18%, respectively (Krishnamoorthy et al., 2021). Subclinical mastitis accounts for 60–70% of the total economic losses caused by all forms of mastitis and results in approximately three times greater production losses than clinical mastitis. (DeVliegher et al. 2012). In India, the monetary loss due to subclinical mastitis was estimated at INR 1390 per lactation, of which approximately 49% resulted from reduced milk value and 37% from veterinary expenses (Sinha et al., 2014). Clinical mastitis can be diagnosed by examining the udder and milk for visible abnormalities. However indirect tests are required for subclinical mastitis diagnosis based on increase in the concentration of immune cells and immune mediators during the inflammatory process and the indirect tests detect these changes (Jaeger et al., 2016; Hughes and Watson, 2018). SCM is routinely diagnosed by cow-side tests California mastitis test (CMT), somatic cell count (SCC) and electrical conductivity, later being the least efficient (Rasouli & Mosaferi, 2017). CMT is a handy and valuable test for subclinical mastitis and is considered a characteristic indicator of mammary gland infection (Souza et al., 2012). Iraguha et al. (2017) found CMT the preferred cow-side test with a Se (88.46%) and Sp (86.17%) and kappa agreement 'substantial' ($k=0.66$) after SCC for SCM detection. CMT has an accuracy of 70.97% for SCM detection and a good level of agreement to the gold standard test (Bashir et al., 2024). There are other inflammatory markers like some specific proteins which have been less explored for their diagnostic efficiency for SCM. For a profitable dairy farming and optimum health of dairy cattle, accurate detection of SCM is imperative.

Acute-phase proteins are a class of proteins whose serum concentrations increase in response to inflammation/Acute Phase Response which play a role in immune modulation by response by opsonising the microbes, complement activation, inert the radicals and trap the enzymes (Giagu et al., 2022). APP's are secreted by liver in serum and are employed as clinical biomarkers for diagnosis of inflammatory conditions including mastitis (Emily et al., 2024). However, elevated APP's in serum do not point out to any specific inflammatory condition or site. APP's secreted in tissues and fluids locally are more specific markers of inflammation in that organ even when there is no rise in their serum levels (Thomas et al., 2015) and can prove more reliable indicators for inflammatory status of the local tissue than the systemic concentration. Thus, APP's secreted by the udder tissue under inflammation may serve as a diagnostic marker for subclinical mastitis by their detection in milk. In cattle, haptoglobin (Hp) and amyloid A (SAA) are the most sensitive and major APPs which show a substantial rise in serum in response to inflammation (Kalmus et al., 2013). Serum amyloid A is also synthesized in the udder called mammary associated amyloid A (MAA) and is excreted in milk during mastitis (Hussein et al., 2018). MAA and Hp in milk could be more specific and sensitive indicators of subclinical mastitis (Wollowski et al., 2021). MAA levels increase faster in milk than the serum amyloid A and are several times higher in subclinical or chronic mastitis than normal. The mammary gland is a well-known source of an MAA variant (Stanek et al., 2024). In udder, the lipoteichoic acid of Gram-positive bacteria and lipopolysaccharides in cell wall of Gram-negative bacteria stimulate the secretion of MAA (Wu et al., 2020).

This study was undertaken to ascertain the diagnostic value of MAA and milk Hp for subclinical mastitis detection with bacteriological culture as the reference test for SCM. In India, studies have been carried out on APP's in serum in mastitic animals, however, their detection in milk, in subclinical mastitis cases is lacking.

Materials and methods

Sample collection

Sampling was carried out at Mountain Livestock Research Institute (MLRI) and Veterinary Clinical Complex (VCC), Faculty of Veterinary Sciences, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir. All the procedures and tests were done in the Division of Veterinary Epidemiology and

Preventive Medicine, FVSc and AH, SKUAST-K over a period of three months. For comparative study, 200 quarter milk samples collected from 64 cross bred jersey cows suspected to suffer from SCM based on the case definition framed for SCM. About 10 mL of quarter milk samples were collected aseptically after disinfection of teat surface with 70% ethanol soaked cotton in separate sterile wide mouth vials after discarding 2-3 streams. The samples were kept in an ice box and carried immediately to the laboratory and kept at 4°C in a refrigerator for further investigations. These were subjected to cultural examination/bacteriological culture (BC) as the 'Gold Standard' test (Crosson et al., 2015).

Bacteriological culture of milk

Bacteriological cultures were carried out according to the standard microbiological procedures of inoculation and incubation (Jaeger *et al.*, 2016). 10 µL of milk were cultured on nutrient agar, blood agar and MacConkey agar. Plates were incubated at 37°C and examined 24-48 h later. Colonies were identified by morphology and gram staining. Only major pathogens of mastitis were considered for positive test. The sample was considered positive for SCM if at least one colony (1 cfu/ml) of a major pathogen was detected (Gonçalves et al., 2023).

MAA and Hp ELISA

MAA and milk Hp were estimated as using ELISA Kit (Bioassay Technology Laboratory, China). The procedure was carried out as per the kit's protocol. All reagents were prepared as instructed. Milk samples were diluted with sample diluent as instructed. Samples, standards and biotinylated antigen were added to the plate wells and the plates incubated for 60 min at 37°C. Washings were carried out followed by adding avidin-horse radish peroxidase. After incubation and washings, substrate solutions were added leading to the development of a blue colour. Stop solution was added to terminate the reaction leading to the change in colour from blue to yellow. Optical density (OD) was read within 10 minutes at 450 nm in an ELISA reader (Cytation, BioTek, USA). Cut-off/lowest detection limit (LOD) of the assay was calculated from mean of three controls (negatives) + (3 x SD of negatives).

Statistical analyses

Comparison of MAA and milk Hp was carried out with Mann-Whitney's U test. Receiver operating characteristic (ROC) curves were used to determine the comparison with BC by assessing the area under curve (AUC) and calculating the diagnostic validity criteria. Significance level was set at $p < 0.05$.

Results

Box plot (Fig. 1) shows the concentration of MAA with BC negative and positive categories. Lowest detection limit (LOD) for the assay was 0.7 µg/mL. The median value for positive samples (SCM) of MAA was 27.85 mg/L (19.20, 38.5) whereas median value for the control samples was 3.13 mg/L (2.00, 4.38). The interquartile range (IQR) for BC positive and control samples was 19.30 and 2.38, respectively. Mann-Whitney U test of the data gave p-value of < 0.001 (statistically significant). Fig. 1.

Box plot (Fig. 2) shows the concentration of milk Hp with BC negative and positive categories. LOD for the assay was 0.4 µg/mL. The median value for positive samples (SCM) of milk Hp was 32.93 mg/L (23.30, 47.45) whereas median value for the control samples was 2.86 mg/L (1.71, 3.77). The interquartile range (IQR) for BC positive and control samples was 24.15 and 2.06, respectively. Mann-Whitney U test of the data gave p-value of < 0.001 (statistically significant). Fig. 1.

Table 1 presents the Diagnostic Accuracy of MAA and milk Hp with BC. The Cohen's kappa value for MAA was 0.82 and that for milk Hp as 0.61.

Table 1: ROC analysis of milk acute phase proteins

Test Variable	AUC (%)	P-Value
MAA (µg/ml)	92.8±2.3	0.0001*
Hp (µg/ml)	81.4±4.0	0.0001*

*Significant, ROC: receiver operating curve, AUC: area under curve, MAA: milk amyloid A, Hp: haptoglobin

Table 2: Diagnostic accuracy of milk acute phase proteins

Test	Se %	Sp %	PPV %	NPV %	Accuracy %	Cohen's Kappa
MAA	94.00	90.00	96.57	83.33	93.00	0.82
Hp	88.67	78.00	92.36	69.64	86.00	0.61

Se: sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, MAA: milk amyloid A, Hp: haptoglobin

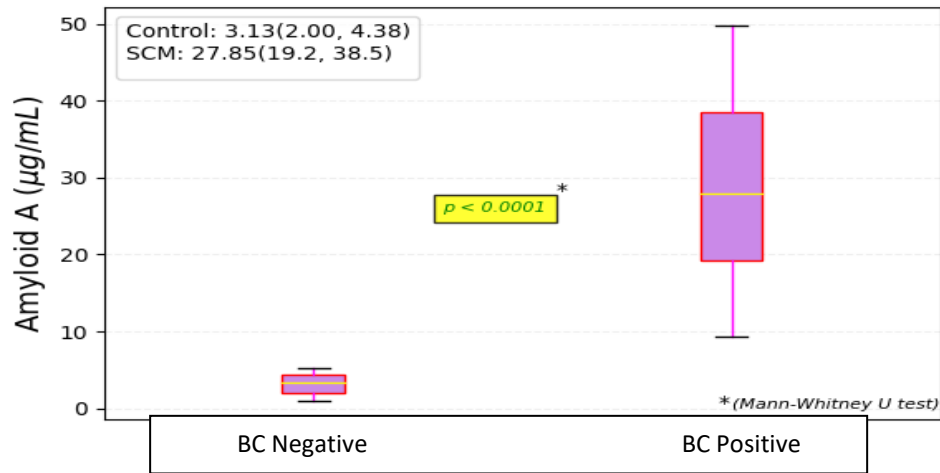


Fig 1. Box and Whisker plot for milk MAA; ($p < 0.001$); SCM, subclinical mastitis

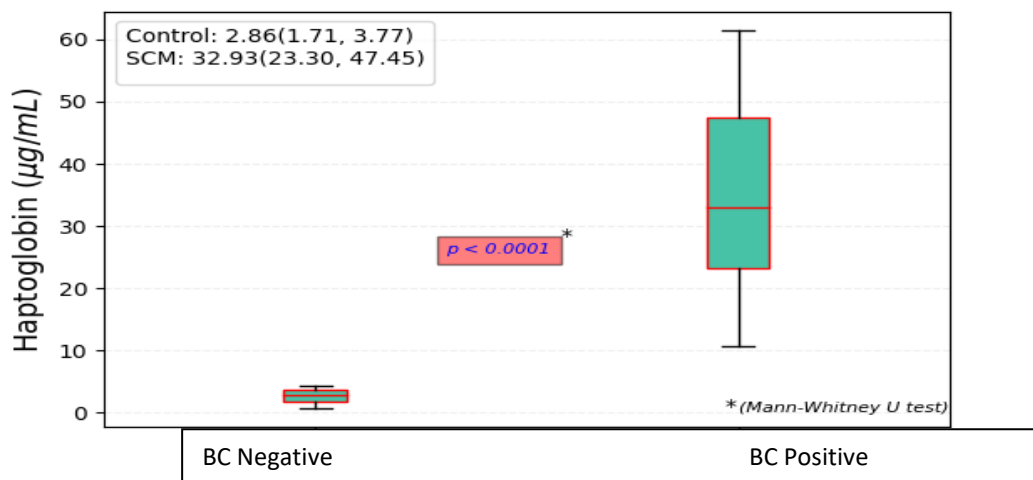


Fig 2. Box and Whisker plot for milk Hp A; ($p < 0.001$); SCM, subclinical mastitis

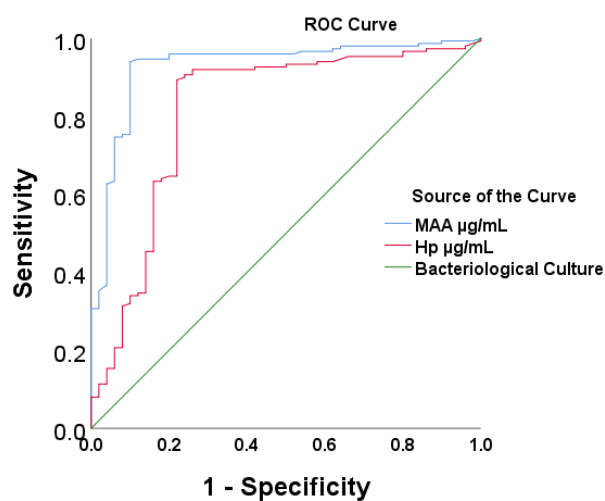


Fig 3. Receiver operating characteristic curve of MAA and Hp; ($p < 0.001$)

Discussion

Inflammation triggers the release of cytokines, particularly interleukins and tumour necrosis factor-alpha (TNF- α), which stimulate the production of acute phase proteins (APPs) as part of the innate immune response

(Dalanezi et al., 2020). These non-enzymatic proteins have emerged as alternative biomarkers for detecting intramammary infection (IMI) in addition to conventional indicators (Giagu et al., 2022). Elevated APP concentrations are observed not only during acute inflammation but also in subclinical or chronic mastitis (Ghasemian and Asri-Rezaei, 2024). In cattle, serum amyloid A (SAA) and haptoglobin (Hp) are considered the major APPs associated with inflammatory responses (Hussein et al., 2018; Iliev and Georgieva, 2019).

In the present study, APPs were measured in milk rather than serum to evaluate their diagnostic potential for subclinical mastitis (SCM). Serum acute phase responses are often non-specific because elevated APP levels may occur in various inflammatory conditions unrelated to mastitis. Therefore, measuring APPs locally in the mammary gland during inflammation provides greater diagnostic specificity (Thomas et al., 2015). While increased serum Hp and SAA levels may reflect systemic inflammation, their elevation in milk is more specifically associated with IMI (Pyorala et al., 2011). Consequently, milk amyloid A (MAA) and Hp have been proposed as sensitive biomarkers for SCM detection in cattle (Kovac et al., 2011; Kalmus et al., 2013; Miglio et al., 2013). These proteins increase primarily in infected mammary quarters under both experimental and natural IMI conditions (Wollowski et al., 2021). Bochniarz et al. (2017) also reported that cows with SCM did not differ significantly from healthy controls in serum amyloid A levels, whereas milk concentrations were significantly elevated, indicating local synthesis within the mammary gland during infection. This supports the diagnostic relevance of milk APPs, particularly MAA, which has been shown to correlate strongly with bacteriological culture results.

For comparative evaluation, bacteriological culture (BC) of milk was considered the gold standard for SCM diagnosis (Dohoo et al., 2011) BC remains the most reliable method for identifying IMI pathogens (Crosson et al., 2015). In this study, a milk sample was classified as culture positive when at least one colony of a major pathogen was detected, consistent with previously recommended criteria (Nyman et al., 2014; Cunha et al., 2020). Jashari et al. (2015) further demonstrated that the detection of ≥ 1 colony (approximately 100 CFU/mL) of a major pathogen correlates strongly with somatic cell count (SCC) and indicates SCM. Accordingly, isolation of any major pathogen on milk culture was considered evidence of subclinical infection in the present study.

Milk MAA concentrations in control samples (SCM negative) ranged from 0.9 to 5.2 $\mu\text{g/mL}$, whereas culture-positive samples showed values between 8.3 and 49.7 $\mu\text{g/mL}$. Statistical analysis using the Mann–Whitney U test revealed a significant difference between groups ($p < 0.001$). The median MAA concentration in SCM samples was 27.85 mg/L (19.20–38.5) compared with 3.13 mg/L (2.00–4.38) in controls. ROC analysis yielded an area under the curve (AUC) of 92.8% ($p < 0.001$), indicating excellent diagnostic performance. The sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy were 94.00%, 90.00%, 96.57%, 83.33%, and 93.00%, respectively. Cohen’s kappa value of 0.82 indicated almost perfect agreement between MAA detection and bacteriological culture.

Similarly, milk Hp concentrations ranged from 10.4 to 61.2 $\mu\text{g/mL}$ in SCM samples and from 0.6 to 4.4 $\mu\text{g/mL}$ in control samples. The Mann–Whitney U test showed a statistically significant difference between groups ($p < 0.001$). The median Hp concentration was 32.93 mg/L (23.30–47.45) in SCM samples compared with 2.86 mg/L (1.71–3.77) in controls. ROC analysis demonstrated an AUC of 81.4% ($p < 0.001$). The calculated sensitivity, specificity, PPV, NPV, and accuracy were 88.67%, 78.00%, 92.36%, 69.64%, and 86.00%, respectively. Cohen’s kappa value of 0.61 indicated substantial agreement with bacteriological culture.

The findings clearly demonstrate that SCM-negative samples exhibited significantly lower APP concentrations than infected samples. Both MAA and Hp showed strong diagnostic validity and good agreement with the gold standard bacteriological culture. However, MAA showed superior diagnostic performance compared with Hp, as indicated by higher AUC, sensitivity, specificity, and kappa agreement values. The higher specificity of MAA is particularly advantageous for accurately identifying true negative samples and reducing false-positive diagnoses. Therefore, MAA may be considered a more reliable biomarker for SCM detection.

The results of this study are consistent with earlier investigations evaluating milk APPs in mastitis diagnostics. Thomas et al. (2015) reported a threshold of 7.9 $\mu\text{g/mL}$ for Hp in normal milk. Jaeger et al. (2016) suggested a cut-off value of 3.9 $\mu\text{g/mL}$ for MAA and reported sensitivity and specificity of 81.4% and 96.6%, respectively. Szczubial et al. (2012) reported MAA concentrations ranging from 5.24 to 19.04 $\mu\text{g/mL}$ in pathogen-free milk and from 8.76 to 74.75 $\mu\text{g/mL}$ in *Staphylococcus aureus*-associated SCM. Similarly, Wollowski et al. (2021) reported threshold values of 1.81 $\mu\text{g/mL}$ for MAA (Se = 0.77, Sp = 0.83) and 5.40 $\mu\text{g/mL}$ for Hp (Se = 0.96, Sp = 0.99). These reported ranges broadly align with the findings of the present study.

Variations in APP concentrations reported across studies may result from differences in etiological agents, infection severity, host response, and individual animal variability (Kovačević-Filipović et al., 2012). Previous studies have also demonstrated a positive association between elevated MAA levels and increased SCC in milk (Bochniarz et al., 2017). In the present study, elevated milk Hp and MAA concentrations were significantly associated with bacteriological culture positivity, further supporting their diagnostic relevance in detecting SCM.

Conclusions

Overall, the findings of the present study demonstrate that milk acute phase proteins, particularly milk amyloid A and haptoglobin, are reliable biomarkers for the detection of subclinical mastitis in dairy cattle. Both

markers showed strong diagnostic validity and good agreement with bacteriological culture, the current gold standard. However, milk amyloid A exhibited superior diagnostic performance, with higher sensitivity, specificity, and agreement values compared with haptoglobin. These results suggest that measurement of milk APPs, especially MAA, could serve as a rapid, sensitive, and non-invasive diagnostic approach for early detection of subclinical mastitis, thereby facilitating timely intervention and improved udder health management in dairy herds.

Acknowledgements

Authors are deeply obliged to SKUAST-Kashmir for providing the funds for the study and Mountain Livestock Research Institute, SKUAST-Kashmir for the facilitation of sampling.

Conflict of interest

The authors declare no conflict of interest.

References

- 1) Aghamohammadi, M., Haine, D., Kelton, D.F., Barkema, H.W., Hogeveen, H., Keefe, G. P., Dufour, S. (2018). Herd-level mastitis associated costs on Canadian dairy farms. *Frontiers in Veterinary Science*, 5,100.
- 2) Ashraf, A. and Imran, M. (2020) Causes, types, etiological agents, prevalence, diagnosis, treatment, prevention, effects on human health and future aspects of bovine mastitis. *Animal Health Research Reviews*, 21(1), 36-49.
- 3) Bashir, S., Shaheen, M., Hassan, N., Muhee, A., Hussain, I., Khan, H.M., Bhat, M.A. and Dar, A.A. (2024). Comparative Accuracy of Cow-Side Tests for Detection of Subclinical Mastitis. *Journal of Animal Research*, 14(05), 309-315.
- 4) Bochniarz, M., Zdzisinska, B., Wawron, W., Szczubial, M., Dabrowski, R. (2017). Milk and serum IL-4, IL-6, IL-10, and amyloid A concentrations in cows with subclinical mastitis caused by coagulase-negative staphylococci. *Journal of Dairy Science*, 100, 9674-9680.
- 5) Constable, P. D., Hinchcliff, K. W., Done, S.H., Grunberg, W. (2017). *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*. 11th ed., Vol.(1), Elsevier, pp 1904.
- 6) Crosson, C., Meriaux, L., Decers, T., Belvalette, M. (2015). A screening method using milk amyloid A measurement in cow milk to significantly reduce the use of intramammary antibiotics at drying off. *ICAR Handbook of Technical Services*, 9, 35-43.
- 7) Cunha, A.F., Andrade, H.M., Souza, F.N., Fialho Júnior, L.C., Rosa, D.L.S.O., Ramos Sanchez, E.M., Gidlund, M., Goto, H., Brito, M.A. V.P., Guimarães, A.S., Lage, A.P. (2020). Comparison of antibody repertoires against *Staphylococcus aureus* in healthy and infected dairy cows with a distinct mastitis history and vaccinated with a polyvalent mastitis vaccine. *Journal of Dairy Science*, 103(5), 4588-4605.
- 8) Dalanezi, F.M., Schmidt, E.M.S., Joaquim, S.F., Guimarães, F.F., Guerra, S.T., Lopes, B.C., Cerri, R.L.A., Chadwick, C., Langoni, H. (2020). Concentrations of acute-phase proteins in milk from cows with clinical mastitis caused by different pathogens. *Pathogens*, 9(9), 706.
- 9) Danilov, M.S., Valitova, N.V., Vorobyov, A.L., Asangaliev, E.A., Kalachev, A.A. 2019. Needles of *Abies Sibirica* in the Treatment of Subclinical Mastitis in Cows. *Journal of Livestock Science* 10:9-18. doi: 10.33259/JLivestSci.2019.9-18
- 10) Das, D., Panda, S. K., Jena, B. and Sahoo, A. K. 2018. Economic impact of subclinical and clinical mastitis in Odisha, India. *International Journal of Current Microbiology and Applied Sciences* 7(3):3651-3654.
- 11) De Vlieghe, S., Fox, L.K., Piepers, S., McDougall, S., Barkema, H.W. (2012) Invited review: Mastitis in dairy heifers: nature of the disease, potential impact, prevention, and control. *Journal of Dairy Science*, 95(3), 1025-40.
- 12) Dohoo, I.R., Smith, J., Andersen, S., Kelton, D.F., Godden, S. (2011). Diagnosing intramammary infections: Evaluation of definitions based on a single milk sample. *Journal of Dairy Science*, 94(1), 250-261.
- 13) Emily, L., O'Reilly, V., Lorenzo, V., Francisco, M. P., Theo, P., Ruth, Z., Nicola, B., Han, Q. H., Mark, McL., Anita, H., Andrea, G., Josipa, K., Vladimir, M.P., David, E. (2024). Biomarker and proteome analysis of milk from dairy cows with clinical mastitis: Determining the effect of different bacterial pathogens on the response to infection, *Research in Veterinary Science*, 172, 2024, 105240.
- 14) Ghasemian, S.O., Asri-Rezaei S. (2024). Inflammatory Markers in Cows Affected by subclinical mastitis due to *Escherichia coli* and *Staphylococcus* infections. *Archives of Razi Institute*.79(6), 1319-1328.
- 15) Giagu, A., Penati, M., Traini, S., Dore, S., Addis, M.F. (2022). Milk proteins as mastitis markers in dairy ruminants - a systematic Review. *Veterinary Research Communications*, 46, 329–351.
- 16) Gonçalves, J.L., Lee, S.H.I., Camargo, C.H., Zanella, R.C., Silva, N.C.C., Rall, V.L.M., Cue, R.I., Dos Santos, M.V. (2023). Molecular characterization of persistent subclinical mastitis-causing *Staphylococcus aureus* from dairy farms. *Brazilian Journal of Microbiology*, 54(2):1181-1189.
- 17) Hogeveen, H., Huijps, K., Lam, T.J. (2011). Economic aspects of mastitis: New developments. *New Zealand Veterinary Journal*, 59, 16-23.

- 18) Hughes, K., Watson, C.J. (2018). The mammary microenvironment in mastitis in humans, dairy ruminants, rabbits and rodents: a one health focus. *Journal of Mammary Gland Biology and Neoplasia*, 23, 27-41.
- 19) Hussein, H.A., Abd El-Razik, K.E., Gomma, A.M., Elbayoumy, M.K., Abdelrahman, K.A., Hosein, H.I. (2018). Milk amyloid A as a biomarker for diagnosis of subclinical mastitis in cattle. *Veterinary World*, 11, 34-41.
- 20) Iliev, P.T., Georgieva, T.M. (2019). Acute phase biomarkers of diseases in small ruminants: an overview. *Bulgarian Journal of Veterinary Medicine*, 22, 1-12.
- 21) Iraguha, B., Hamudikuwanda, H., Mushonga, B. (2015). Bovine mastitis prevalence and associated risk factors in dairy cows in Nyagatare District, Rwanda. *Journal of the South African Veterinary Association*, 86(1), 1-6.
- 22) Iraguha, B., Hamudikuwanda, H., Mushonga, B., Kandiwa, E., Mpatswenumugabo, J.P. (2017). Comparison of cow-side diagnostic tests for subclinical mastitis of dairy cows in Musanze district, Rwanda. *Journal of the South African Veterinary Association*, 88:1464.
- 23) Jaeger, S., Virchow, F., Torgerson, P.R., Bischoff, M., Biner, B., Hartnack, S., Ruegg, S.R. (2016). Test characteristics of milk amyloid A ELISA, somatic cell count, and bacteriological culture for detection of intramammary pathogens that cause subclinical mastitis. *Journal of Dairy Science*, 100, 7419-7426.
- 24) Jashari, R; Piepers, S., Vlieghe, S. (2015). Evaluation of the composite milk somatic cell count as a predictor of intramammary infection in dairy cattle. *Journal of Dairy Science*, 99, 9271-9286.
- 25) Kalmus, P., Simojoki, H., Pyorala, S., Taponen, S., Holopainen, J., Orro, T. (2013). Milk haptoglobin, milk amyloid A, and N-acetyl-beta-D-glucosaminidase activity in bovines with naturally occurring clinical mastitis diagnosed with a quantitative PCR test. *Journal of Dairy Science*, 96, 3662-70.
- 26) Kovač, G., C. Tothova, O. Nagy, H. Seidel. (2011). Milk amyloid A and selected serum proteins in cows suffering from mastitis. *Acta Veterinaria Brno*, 80, 3-9.
- 27) Kovačević-Filipović, M., Ilić, V., Vujčić, Z., Dojnov, B., Stevanov-Pavlović, M., Mijačević, Z., Božić, T. (2012). Serum amyloid A isoforms in serum and milk from cows with *Staphylococcus aureus* subclinical mastitis. *Veterinary Immunology and Immunopathology*, 145(1), 120-128.
- 28) Krishnamoorthy, P., Goudar, A. L., Suresh, K. P., Roy, P. (2021). Global and countrywide prevalence of subclinical and clinical mastitis in dairy cattle and buffaloes by systematic review and meta-analysis. *Research in Veterinary Science*, 136, 561-586,
- 29) Miglio, A., Moscati, L., Fruganti, G., Pela, M., E., Scoccia, E., Valiani, A., Maresca, C. (2013). Use of milk amyloid A in the diagnosis of subclinical mastitis in dairy ewes. *Journal of Dairy Research*, 80, 496-502.
- 30) Nyman, A.K., Waller, K.P., Bennedsgaard, T.W., Larsen, T., Emanuelson, U. (2014). Associations of udder-health indicators with cow factors and with intramammary infection in dairy cows. *Journal of Dairy Science*, 97, 5459-5473.
- 31) Pyorala, S., Hovinen, M., Simojoki, H., et. al. (2011). Acute phase proteins in milk in naturally acquired bovine mastitis caused by different pathogens. *Veterinary record*, 168(20), 535.
- 32) Rasouli, A., Mosafieri, S. 2017. Accuracy rate of mastitis on-farm test (udder check) in comparison to somatic cell count (S.C.C) in dairy herd. *Journal of Livestock Science* 8: 191-195
- 33) Ruegg, P. L. (2017). A 100-year review: Mastitis detection, management, and prevention. *Journal of Dairy Science*, 100,10381-10397.
- 34) Sinha, M.K., Thombare, N.N., Mondal, B. (2014). Subclinical mastitis in dairy animals: incidence, economics, and predisposing factors. *Scientific World Journal*, 2014, 523984.
- 35) Souza, F.N., Blagitz, M.G., Penna, C.F.A.M., Della Libera, A.M.M.P., Heinemann, M.B., Cerqueira, M.M.O.P. (2012). Somatic cell count in small ruminants: friend or foe? *Small Ruminant Research*, 107, 65-75.
- 36) Stanek, P., Żółkiewski, P., Januś, E. (2024). A Review on Mastitis in Dairy Cows Research: Current Status and Future Perspectives. *Agriculture*. 14(8), 1292.
- 37) Szczubiał, M., Dabrowski, R., Kankofer, M., Bochniarz, M., Komar, M. (2012). Concentration of serum amyloid A and ceruloplasmin activity in milk from cows with subclinical mastitis caused by different pathogens. *Polish Journal of Veterinary Science*, 2, 291-296.
- 38) Thomas, F.C., Waterston, M., Hastie, P., Parkin, T., Haining, H., Eckersall, P.D. (2015). The major acute phase proteins of bovine milk in a commercial dairy herd. *BMC Veterinary Research*, 11, 207.
- 39) Tommasoni, C., Fiore, E., Lisuzzo, A., Giancesella, M. (2023). Mastitis in Dairy Cattle: On-Farm Diagnostics and Future Perspectives. *Animals (Basel)*, 13(15), 2538.
- 40) Wollowski, L., Heuwieser, W., Kossatz, A., Addis, M.F., Puggioni, G. M. G., Laurent Meriaux, L., Bertulat, S. (2021). The value of the biomarkers cathelicidin, milk amyloid A and haptoglobin to diagnose and classify clinical and subclinical mastitis. *Journal of Dairy Science*, 104, 2106-2122.
- 41) Wu Y, Sun Y, Zhang Z, Chen J, Dong G. (2020). Effects of peptidoglycan, lipoteichoic acid and lipopolysaccharide on inflammation, proliferation and milk fat synthesis in bovine mammary epithelial cells. *Toxins (Basel)*, 12(8), 497.