

# Concentration of Acute Phase Proteins in Milk: A New Tool for Mastitis Diagnosis

**Felipe Morales Dalanezi, Sâmea Fernandes Joaquim, Rodrigo Costa da Silva, Elizabeth Moreira dos Santos Schmidt and Helio Langoni\***

*Department of Veterinary Hygiene and Public Health, School of Veterinary Medicine and Animal Sciences, Brazil*

**Submission:** February 28, 2017; **Published:** March 22, 2017

\***Corresponding author:** HelioLangoni, Department of Veterinary Hygiene and Public Health, School of Veterinary Medicine and Animal Sciences, Universidade Estadual Paulista, Botucatu, São Paulo State, Brazil, Email: hlangoni@fmvz.unesp.br

## Abstract

Mastitis is a disease of the mammary gland, caused by pathogens like bacteria, fungi, virus, and algae. Bacteria are the principal agents, and the infection caused by them can be classified in contagious and environmental. Each of them present's different and specific characteristics and control measures. The disease is responsible for high economic losses in the dairy production. In this way, it is necessary to minimize the losses. To attenuate the losses of the dairy producers, a precise and quickly diagnosis of mastitis is necessary. Currently, the tests used in dairy science are not so efficient, present's low sensitivity, or provide low help to the veterinarian and/or producer to control mastitis on time to avoid new cases and solve those in course. Acute phase proteins maybe are produced in mammary gland and have a change on its concentration. The method to measure of these proteins can be implanted in a dipping machine like SCC, and help the professionals to determine which cow needs the treated. The aim of this paper is to explain how the APPs can be used as a new and better tool for the mastitis diagnosis.

**Keywords:** Bovine; Dairy science; M-SAA3; CRP

## Introduction

Mastitis presents a significant worldwide impact in milk production, showing an annual cost at least US\$ 2,000,000,000 to dairy producers of United States. The major part of amount is related to the reduction of milk production (US\$115/cow/year), treatment cost (US\$50/cow/year), and animal mortality (US\$14/cow/year), totalizing US \$179/cow/year with clinical mastitis [1]. Mastitis is characterized by a multiple etiology mammary gland infection, being more frequently caused by bacteria [2], that triggers an inflammatory response [3]. Mastitis can be classified as subclinical or clinical mastitis. The clinical form of mastitis is characterized by abnormal milk production, and can presents mammary gland alterations or systemic signs [4,5]. The subclinical mastitis present an inflammatory cell mobilization to the mammary gland, causing an increase on somatic cell count (SCC) [6,7].

Nowadays, new diagnostics tools are available, and aim to facilitate the mastitis diagnostic as well as to analyses the disease prognosis [8]. The acute phase proteins (APP) are potential biomarkers of inflammation produced in the liver and others extra-hepatics tissues like mammary gland, presenting a promising future to aid the diseases diagnosis in veterinary medicine [9]. During the inflammatory, APPs can improve the

concentration to 100-fold [10]. These proteins can be detected in various body fluids, and blood [11], APPs is produced in specifics organs and tissues which provide local inflammation [12]. However, APPs produced and released by the mammary gland can be more specific and sensitive for mastitis than the blood ones [13]. In bovines, the principal APPs are the Haptoglobin (Hp), Lipo polysaccharides binding protein (LBP), Serum A Amyloid (SAA) and  $\alpha$ 1-acid glycoprotein (AGP) [9]. The APPs can be classified as major, moderate and minor, according to the inflammatory response. The four APPs aforementioned are major APPs. Mammary associated Serum Amiloid A isoform 3 (M-SAA3) and C-Reative Proteins (CRP) are important APPs found in milk. They have been measured in milk as biomarkers of mastitis [14,15].

## Discussion

The SAA belong to a big family of proteins known as one of the most reactive APPs [16]. The major part of the isoforms of SAA is produced in the liver, and unleashed at the bloodstream [17]. The isoform 3 of the SAA (M-SAA3) identified in the bovine milk is produced by mammary cells and acts combating the local inflammation in the udder [18]. Health cows presents an increase of the M-SAA3 production for the epitelial cells of the teat, when stimulated with prolactin, however this increase is not followed

by the production of SAA. It demonstrates that the M-SAA3 is the most important isoform to the mammary gland [19].

It was also observed that M-SAA3 is a bio indicator of mastitis more trustworthy than the Hp, with a better correlation to the inflammation of the mammary gland [20]. M-SAA3 stimulates the innate response of the immunity system of the udder, and also has antibacterial action against *Escherichia coli*, *Streptococcus uberis* and *Pseudomonas aeruginosa* [21]. Cows experimentally infected with *Staphylococcus aureus* presented an increase to the M-SAA3 concentration earlier in milk than blood [20]. Extra mammary infections can elevate the serum SAA concentration, but does not interfere to the M-SAA3 concentration in milk. Thus, this isoform is not influenced by new inflammations [12].

As SAA, CRP is also produced in the liver and unleashed in the bloodstream [22-24]. CRP decreases tissue damage, destroys pathogens, and assists tissue regeneration [24,25]. Despite being considerate a minor APP due the small variation in blood concentration during inflammatory process, in mastitis cases in dairy cows the serum concentration of CRP observed is, approximately, 1083ng/mL, while in health cows it is 82ng/mL [15]. In milk of cows with clinical mastitis (SCC greater than 200,000), the mean concentration is 32.64ng/mL, and ranges from 1.8 to 172.47ng/mL [10]. Moreover, it was demonstrated that the CRP does not follow the variation in the SCC of the milk. In this way, the CRP concentration is more sensitive to the mammary infections than the SCC [10,15,26]. It is believed that the high range observed in the concentration of this APP should be caused by the different types of causative agents of clinical mastitis, and highlights the importance of this protein to the diagnosis of mastitis caused by different pathogens.

The determination of these proteins (M-SAA3 and CRP) can be performed by the immunoassay technique, which measures during the dipping? It was demonstrated that the clinical mastitis caused by different pathogens can cause a greater or a smaller increase in the concentration of the APP in the milk [8]. In the same work, the authors also observed that several mastitis generates a large liberation of these proteins once compared with mild or moderate level mastitis. Therefore, it is possible that the pathogens causing severe mastitis stimulate a higher liberation of these proteins.

## Conclusion

More studies are necessary to determine the concentration of these APPs and the correlation of these concentrations with the cause of mastitis. In this way, new methods based in the APPs in milk would improve the mastitis diagnosis and it will be the future of the diagnosis making the time-consuming techniques like microbiologic culture less needful.

## References

1. Bar D, Tauer LW, Bennett G, González RN, Hertl JA, et al. (2008) The cost of generic clinical mastitis in dairy cows as estimated by using dynamic programming. *J Dairy Sci* 91(6): 2205-2214.

2. Langoni H, Silva AV, Cabral KG, Domingues PF (1998) Aspectos etiológicos na mastite bovina: Flora bacteriana aerobica. *Rev Bras Med Veterinária* 20(5): 204-210.
3. Fuenzalida MJ, Fricke PM, Ruegg PL (2015) The association between occurrence and severity of subclinical and clinical mastitis on pregnancies per artificial insemination at first service of Holstein cows. *J Dairy Sci* 98(6): 3791-3805.
4. Pinzón-Sánchez C, Ruegg PL, (2011) Risk factors associated with short-term post-treatment outcomes of clinical mastitis. *J Dairy Sci* 94(7): 3397-3410.
5. Smith B (2014) *Large Animal Internal Medicine*. Mosby, St Louis, USA, pp. 1702.
6. Guimarães FF, Nóbrega DB, Richini-Pereira VB, Marson PM, Pantoja JCF, et al. (2013) Enterotoxin genes in coagulase-negative and coagulase-positive staphylococci isolated from bovine milk. *J Dairy Sci* 96(5): 2866-2872.
7. Langoni H, da S Penachio D, CC Citadella J, Laurino F, Faccioli-Martins PY, et al. (2011) Aspectos microbiológicos e de qualidade do leite bovino. *Pesqui Vet Bras* 31(12): 1059-1065.
8. Pyörälä S, Hovinen M, Simojoki H, Fitzpatrick J, Eckersall PD, et al. (2011) Acute phase proteins in milk in naturally acquired bovine mastitis caused by different pathogens. *Vet Rec* vol 168(20): 535.
9. Ceciliani F, Ceron JJ, Eckersall PD, Sauerwein H (2012) Acute phase proteins in ruminants. *J Proteomics* 75(14): 4207-4231.
10. Thomas FC, Waterston M, Hastie P, Parkin T, Haining H, et al. (2015) The major acute phase proteins of bovine milk in a commercial dairy herd. *BMC Vet Res* 11(1): 207.
11. Lecchi C, Dilda F, Sartorelli P, Ceciliani F (2012) Widespread expression of SAA and Hp RNA in bovine tissues after evaluation of suitable reference genes. *Vet Immunol Immunopathol* 145(1-2): 556-562.
12. Nielsen BH, Jacobsen S, Andersen PH, Niewold TA, Heegaard PMH (2004) Acute phase protein concentrations in serum and milk from healthy cows, cows with clinical mastitis and cows with extramammary inflammatory conditions. *Vet Res* 154(12): 361-365.
13. Gronlund U, Sandgren C, Waller K (2005) Haptoglobin and serum amyloid A in milk from dairy cows with chronic sub-clinical mastitis. *Vet Res* 36(2): 191-198.
14. Eckersall PD, Young FJ, McComb C, Hogarth CJ, Safi S (2001) Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet Rec* 148(2): 35-41.
15. Schrodll W, Kruger M, Hien TT, Fuldner M, Kunze R (1995) C-reactive protein as a new parameter of mastitis. *Tierarztl Prax* 23(4): 337-341.
16. Bing Z, Reddy SAG, Ren Y, Qin J, Liao WSL (1999) Purification and characterization of the serum amyloid A3 enhancer factor. *The Journal of Biological Chemistry* 274(35): 24649-24656.
17. Uhlar CM, Whitehead AS (1999) Serum amyloid A, the major vertebrate acute-phase reactant. *Eur J Biochem* 265(2): 501-523.
18. Weber A, Weber AT, McDonald TL, Larson MA (2006) *Staphylococcus aureus* lipoteichoic acid induces differential expression of bovine serum amyloid A3 (SAA3) by mammary epithelial cells: Implications for early diagnosis of mastitis. *Vet Immunol Immunopathol* 109(1-2): 79-83.
19. Larson MA, Weber A, Weber AT, McDonald TL (2005) Differential expression and secretion of bovine serum amyloid A3 (SAA3) by mammary epithelial cells stimulated with prolactin or lipopolysaccharide. *Vet Immunol Immunopathol* 107(3-4): 255-264.
20. Eckersall PD, Young FJ, Nolan AM, Knight CH, McComb C, et al. (2006) Acute Phase Proteins in Bovine Milk in an Experimental Model of *Staphylococcus aureus* Subclinical Mastitis. *J Dairy Sci* 89(5): 1488-1501.

21. Molenaar AJ, Harris DP, Rajan GH, Pearson ML, Callaghan MR, et al. (2009) The acute-phase protein serum amyloid A3 is expressed in the bovine mammary gland and plays a role in host defence. *Biomarkers* 14(1): 26-37.
22. Clyne B, Olshaker JS (1999) The C-reactive protein. *J Emerg Med* 17(6): 1019-1025.
23. Baumann H, Gaudie J (1994) The acute phase response. *Immunol Today* 15(2): 74-80.
24. Sarikaputi M, Morimatsu M, Syuto B, Saito M, Naiki M (1991) A new purification procedure for bovine C-reactive protein and serum amyloid P component. *Int J Biochem* 23(10): 1137-1142.
25. Horadagoda NU, Knox KM, Gibbs HA, Reid SW, Horadagoda A, et al. (1999) Acute phase proteins in cattle: discrimination between acute and chronic inflammation. *Vet Rec* 144(16): 437-441.
26. Lee WC, Hsiao HC, Wu YL, Lin JH, Lee YP, et al. (2003) Serum C-reactive protein in dairy herds. *Can J Vet Res* 67(2): 102-107.



This work is licensed under Creative Commons Attribution 4.0 License  
DOI: [10.19080/JDVS.2017.01.555565](https://doi.org/10.19080/JDVS.2017.01.555565)

**Your next submission with Juniper Publishers  
will reach you the below assets**

- Quality Editorial service
- Swift Peer Review
- Reprints availability
- E-prints Service
- Manuscript Podcast for convenient understanding
- Global attainment for your research
- Manuscript accessibility in different formats  
( Pdf, E-pub, Full Text, Audio)
- Unceasing customer service

Track the below URL for one-step submission  
<https://juniperpublishers.com/online-submission.php>