

OBSERVATIONS ON ACUTE PHASE PROTEINS AND VARIOUS DIAGNOSTIC TESTS FOR UDDER HEALTH

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ABSTRACT

In the present study, different parameters, with special emphasis on acute phase proteins, were evaluated in healthy and mastitic udders of dairy animals. Concentration of acute phase proteins was determined in milk and serum samples (ten each) collected from mastitic cows and buffaloes. Milk samples were also subjected to NAG-ase, spot trypsin inhibition assay, California mastitis test, somatic cell count and electrical conductivity test. In mastitic cows and buffaloes a significant increase in concentrations of acute phase proteins and other parameters was observed as compared to healthy ones. The advantage of haptoglobin and serum amyloid A over other parameters is attributable to the fact that both are not present in the milk of healthy dairy cows and buffaloes and are not influenced by factors other than mastitis. Therefore, estimation of acute phase proteins in milk was found to be a useful diagnostic tool to detect clinical and sub clinical mastitis.

Key words: Mastitis, acute phase proteins, diagnostic tests

Mastitis is a costly and frequently occurring disease of dairy animals. In India Dua (2001) has estimated the annual losses to the tune of Rs. 6053.21 crores due to mastitis in cows and buffaloes. Several strategies have been tried to control mastitis in dairy animals. Monitoring of udder health is an essential component of mastitis control program. Monitoring the inflammatory process in the mammary gland can be done using several parameters. Recently, research has been focused on acute phase proteins which undergo substantial changes in concentration following infection, inflammation and trauma (Haghkhah *et al.*, 2010). In cattle, the most sensitive acute phase proteins are haptoglobin (Hp) and serum amyloid A (SAA).

Few reports on acute phase proteins as indicator of mastitis are available from abroad (Eckersall *et al.*, 2001; 2006), however, there seems to be no report from India. Therefore, the present study was planned to compare acute phase proteins with other inflammatory indicators such as trypsin inhibition assay (TIA), electrical conductivity test (EC), California mastitis test (CMT) and somatic cell counts

(SCC) in healthy and mastitic quarters of dairy cows and buffaloes.

MATERIALS AND METHODS

Milk Samples: Quarter milk samples 10 each from clinical mastitic cows and buffaloes and equal number from healthy animals were collected and subjected to estimation of acute phase proteins and other indirect tests viz. NAG-ase test (Kitchen *et al.*, 1978), TIA (Samad and Awaz, 1997), SCC (Schalm *et al.*, 1971), CMT (Doxy, 1985) and EC with the help of a portable mastitis detector.

Estimation of Acute Phase Proteins: The concentrations of acute phase proteins viz. SAA, serum haptoglobin (SHp), milk amyloid A (MAA), and milk haptoglobin (MHP) were determined with available kits (PhaseTM Range, Tridelta, Development Ltd., Ireland) as per the instructions of the manufacturers. The assay of amyloid A was based on the solid phase sandwich ELISA. The milk samples were initially diluted 1:50, and all the samples including the standards, were tested in duplicate.

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RESULTS AND DISCUSSION

Both in cows and buffaloes suffering from clinical mastitis, a significant increase in concentrations of amyloid A and haptoglobin was observed as compared to healthy animals indicating that milk Hp and MAA accurately reflect the degree of inflammation (Table 1). There seems to be no report available in the literature on the concentrations of acute phase proteins in healthy and mastitic buffaloes. However, a few reports in cows are available from other countries. Studies conducted by different workers (Eckersall *et al.*, 2001; Eckersall *et al.*, 2006; O'Mahony *et al.*, 2006; Kovac *et al.*, 2007) have also demonstrated higher concentrations of SAA and haptoglobin in serum of cows suffering from clinical mastitis.

Also there was a significant increase in values of other parameters namely NAG-ase activity, EC and SCC. Both SCC and CMT are indices of the same biological parameter. Earlier studies (O'Mahony *et al.*, 2006; Kovac *et al.*, 2007; Haghkhah *et al.*, 2010) reported a positive relationship between SCC and acute phase proteins. SCC has been accepted as the dairy industry's standard indicator for mastitis. But practically SCC alone cannot be used as a marker to identify infected quarters. Raised SCC in the absence of intramammary inflammation may be encountered in early lactation (Dohoo, 1993) and late lactation (de Haas *et al.*, 2002). NAG-ase test was found less practical for detecting mastitis because of lengthy assay time, tedious sample preparation and unsatisfactory procedures. Amongst animal side tests, mean values of CMT and TIA were not obtained as the tests were non

parametric but both the tests gave comparable results with other parameters. Our findings are in close agreement with those reported by Saluja *et al.* (2004) who found CMT as a simple reliable quick and inexpensive test for the diagnosis of mastitis having good correlation with SCC. These tests including NAG-ase activity, SCC, EC, CMT, acute phase proteins and TIA can be used to screen the herd at quarter level but the limitation of CMT and TIA is that both the tests are based on visible reaction which may misclassify the positive and negative test results. In conclusion, our study in confirmation with previous reports from abroad provide strong evidence for production of significant amount of acute phase proteins in milk during mastitis. These can be rapid and sensitive markers of inflammation as compared to SCC. The advantage of haptoglobin and serum amyloid A over other markers of mastitis is attributable to the fact that they are not present in the milk of healthy dairy cows and buffaloes and are not influenced by factors other than mastitis. Therefore, estimation of acute phase proteins in milk can be used as a diagnostic tool to detect mastitis and to monitor herd health

ACKNOWLEDGEMENTS

Thanks are due to Sh. Randhir Singh and Sh. Bhupendra Singh of College Central Lab for their help and co-operation.

REFERENCES

de Hass, Y., Barkema, H.W. and Veerkamp, R.F. (2002). The effect of pathogen specific clinical mastitis on the lactation curve for

Table 1
Acute phase proteins and other inflammatory indicators in healthy and clinical mastitic cows and buffaloes

| Parameters | Buffalo | | Cow | |
|---------------------------------|--------------------------------|-----------------------------------|--------------------------------|----------------------------------|
| | Healthy (n=10) | Mastitic (n=10) | Healthy (n=10) | Mastitic (n=10) |
| Milk amyloid A(μ g/ml) | 0.087 \pm 0.029 ^b | 5.50 \pm 2.20 ^a | 0.022 \pm 0.01 ^b | 10.10 \pm 1.40 ^a |
| Milk haptoglobin(μ g/ml) | 28.96 \pm 5.27 ^b | 243.00 \pm 76.91 ^a | 16.20 \pm 3.10 ^b | 216.00 \pm 42.01 ^a |
| Serum amyloid A (μ g/ml) | 1.096 \pm 0.21 ^b | 194.55 \pm 28.66 ^a | 0.69 \pm 0.23 ^b | 118.49 \pm 31.91 ^a |
| Serum haptoglobin (μ g/ml) | 18.60 \pm 3.26 ^b | 2915.30 \pm 306.25 ^a | 62.60 \pm 24.33 ^b | 724.30 \pm 307.32 ^a |
| NAGase (OD) | 0.17 \pm 0.01 ^b | 1.01 \pm 0.16 ^a | 0.21 \pm 0.03 ^b | 0.78 \pm 0.13 ^a |
| Electrical conductivity (mS) | 4.94 \pm 0.14 ^b | 5.40 \pm 0.71 ^a | 2.58 \pm 0.08 ^b | 8.50 \pm 0.35 ^a |
| Somatic cell count(105/ml) | 0.57 \pm 0.05 ^b | 7.78 \pm 0.30 ^a | 0.97 \pm 0.97 ^b | 8.02 \pm 0.36 ^a |

*P<0.05 in comparison to healthy control.

- somatic cell count. *J. Dairy Sci.* **85**:1314-1323.
- Dohoo, I.R. (1993). An evaluation of the validity of individual cow somatic cell count from cows in early lactation. *Prev. Vet. Med.* **16**:103-110.
- Doxy, C.L. (1985). Clinical Pathology and Diagnostic Procedure. (2nd edn.), Bailliere Tindal, London.
- Dua, K. (2001). Studies on incidence, etiology and estimated economic losses due to mastitis in Punjab and India - An update. *Indian Dairyman* **53**: 41-48.
- Eckersall, P.D., Young, F.J., McComb, C., Hogarth, C.J., Safi, S., Weber, A., McDonald, T., Nolan, A.M. and Fitzpatrick, J.L. (2001). Acute phase proteins in serum and milk from dairy cows with clinical mastitis. *Vet. Rec.* **148**: 35-41.
- Eckersall, P.D., Young, F.J., Nolan, A.M., Knight, C.H., McComb, C., Waterston, M.M., Hogarth, C.J., Scott, E.M. and Fitzpatrick, J.L. (2006). Acute phase proteins in bovine milk in an experimental model of *Staphylococcus aureus* subclinical mastitis. *J. Dairy Sci.* **89**:1488-1501.
- Haghighi, M., Nazifi, S. and Jahromi, A. (2010). Evaluation of milk haptoglobin and amyloid A in high producing dairy cattle with clinical and subclinical mastitis in Shiraz. *Comp. Clin. Pathol.* **19**: 547-552.
- Kitchen, B. J., Middleton, G. and Salmon, M. (1978). Bovine milk N-acetyl-b-D-glucosaminidase and its significance in the detection of abnormal udder secretions. *J. Dairy Res.* **45**: 15-20.
- Kovac, G., Popelkova, M., Tkacikova, L., Burdova, O. and Ihnat, O. (2007). Interrelationship between somatic cell count and acute phase proteins in serum and milk of dairy cows. *Acta Vet. Brno.* **76**: 51-57.
- O'Mahony, M.C., Healy, A.M., Harte, D., Walshe, K.G., Torgerson, P.R. and Doherty, M.L. (2006). Milk amyloid A: correlation with cellular indices of mammary inflammation in cows with normal and raised serum amyloid A. *Res. Vet. Sci.* **80**:155-161.
- Saluja, P.S., Gupta, S.L. Kapur, M.P. and Sharma, A. (2004). Prevalence of bovine mastitis in an organized dairy herd. *Indian Vet. J.* **84**: 1404-1405.
- Samad, A. and Awaz, K.B. (1997). A rapid spot test based on detection of trypsin inhibitors in milk to diagnose bovine sub-clinical mastitis. *Int. J. Anim. Sci.* **12**: 97-101.
- Schalm, O.W., Carol, E.J. and Jain, N.C. (1971). Bovine Mastitis. Lea and Febiger, Philadelphia.
- Sharma, A. and Kapur, M.P. (2000). Prevalence of sub clinical mastitis in cows at an organized farm. *Haryana Vet.* **39**: 13-16.

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