

Ultrasonography of the supramammary lymph nodes for diagnosis of bovine chronic subclinical mastitis

Khoramian, B.¹; Vajhi, A.^{2*}; Ghasemzadeh-Nava, H.³; Ahrari-Khafi, M. S.⁴
and Bahonar, A.⁵

¹Department of Clinical Sciences, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran; ²Department of Surgery & Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ³Department of Theriogenology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran; ⁴Department of Clinical Sciences, School of Veterinary Medicine, Shiraz University, Shiraz, Iran; ⁵Department of Food Hygiene and Public Health, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

*Correspondence: A. Vajhi, Department of Surgery & Radiology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. E-mail: avajhi@ut.ac.ir

(Received 13 Apr 2013; revised version 9 Jun 2014; accepted 23 Aug 2014)

Summary

Currently, somatic cell count (SCC) and bacterial culture are considered as the gold standard of detecting subclinical Mastitis. Mastitis leads to proliferation of lymphocytes in the supramammary lymph nodes and subsequent enlargement of ipsilateral lymph node. Ultrasonography can be used to survey these changes. A portable ultrasound machine with a 2-5 MHz convex transducer was used to identify the supramammary lymph node size in 35 cows in a herd with chronic *Staphylococcus aureus* mastitis. After pre-milking udder preparation, a California mastitis test (CMT) was performed and individual milk samples were taken from each quarter for bacterial culture and somatic cell count (SCC) in accordance with NMC recommendations. The mean length (range 5.77-12.90 cm) and width (range 2.07-7.41 cm) of the lymph node were 9.2 and 4.03 cm, respectively. There was a positive correlation between lymph node size (length and depth) and culture of milk samples on ipsilateral quarters. Also, there was a significant difference correlation between CMT or mean log SCC of each side and size of supramammary lymph node in the same side. This study showed significant changes in supramammary lymph node dimensions in mastitis cases, so ultrasonography of this lymph node is probably a useful method for mastitis detection, especially in situations that test on milk is impossible.

Key words: Supramammary lymph node, Ultrasonography, Mastitis, *Staphylococcus aureus*, Dairy cow

Introduction

Mastitis is considered to be the most prevalent production disease in dairy herds worldwide and the most economically important disease of the dairy industry. Diagnosis of mastitis pathogens is generally performed by traditional culture followed by biochemical tests on bacterial isolates (Oliver *et al.*, 2004). Conventional microbiological methods have been the gold standard for identification of bacteria from milk (Gillespie and Oliver, 2005). However, in some groups of cows such as heifers and dry cows monitoring of mastitis by these tests that are performed on milk is impossible.

In cattle, the four quarters of the mammary gland are completely separated anatomically and the two quarters on each side (right or left) are connected to the ipsilateral supramammary lymph nodes by lymphatic ducts (Kimura *et al.*, 2005). In the most cows there are two lymph nodes on each side (Bradley *et al.*, 2001). When cows suffer from mastitis, lymphocytes in the ipsilateral supramammary lymph nodes are activated and proliferated and then migrated into the mammary gland to fight bacterial infection (Soltys and Quinn, 1999; Kehrlí and Harp, 2001). They migrate through blood circulation, lymph nodes, lymphatic system, and then

back to blood circulation (Von Andrian and Mempel, 2003).

Ultrasonography is a routine method to survey lymph nodes in human (Praye *et al.*, 1990; Bruneton *et al.*, 1994), small animals (Wisner *et al.*, 1991) and cattle (Braun *et al.*, 1994; Kofler *et al.*, 1998; Bradley *et al.*, 2001). Supramammary lymph nodes that are located superficially are relatively well demarcated ultrasonographically from the surrounding fat and udder tissue with an echogenic capsule, a hypoechoic cortical area and a hyperechoic inner medulla/hilar area. In dairy cows it is expected that supramammary lymph nodes have different size and architecture according to the infectious status (Bradley *et al.*, 2001).

Bradley *et al.* (2001) has done the only study on relationship between mastitis and supramammary lymph node size and showed that the lymph node on sides which were positive in a California mastitis test (CMT) were significantly larger than those on sides which were negative but it was not correlated with somatic cell count (SCC).

The purpose of the present study was identification of the relationship between microbiological test, CMT and SCC with size of supramammary lymph nodes using ultrasonography in a herd with *Staphylococcus aureus* mastitis problem.

Materials and Methods

Thirty-five cows in a herd with a problem of *Staphylococcus aureus* mastitis were selected for this study. All of the selected cows had a history of 4 to 7 lactation number and were not in late lactation period. Cows were housed in the loose-housing dairy barns and were milked three times a day. Body condition was scored (BCS) on scale from 1 to 5 (where, 1= emaciated to 5= extremely fat) once a month (Edmonson *et al.*, 1989).

A portable ultrasound machine (Sonosite[®], MicroMaxxTM) with a 2-5 MHz convex transducer (contact area 6 × 1.5 cm) was used to identify the supramammary lymph node size. The length (dorsoventral dimension) and depth (caudocranial dimension) were measured three times by in-built machine software, it was repeated at least three times for each lymph node for more accuracy.

The anatomical position of superficial supramammary lymph nodes was found according to the descriptions of Bradley *et al.* (2001).

Individual milk samples were taken from each quarter for culture and somatic cell count. After pre-milking udder preparation, teat ends were scrubbed with alcohol and allowed to dry, foremilk was stripped from each quarter and milk sample was immediately taken. A CMT (scored as normal, trace, 1, 2 and 3) was performed on each quarter of cows. For bacteriological culture, all laboratory procedures were performed in accordance with NMC recommendations (National Mastitis Council, 1999). Direct microscopic method was used for somatic cell count in milk samples.

For statistical analysis, the sum of the mastitis scores of two quarters in each side was calculated (0= N, 1= trace of CMT test, 2= first degree of CMT, 3= second degree of CMT, 4= third degree of CMT, and 5= clinical mastitis) and the mean dimensions of the lymph nodes in the same side were analyzed using nonparametric Spearman rank correlation. Utilization of Pearson correlation coefficients were computed between the average SCC and dimensions of the same lymph nodes and differences between size of lymph nodes and culture of milk samples were analyzed by ANOVA test. Data were analyzed using SPSS package version 16 and $P \leq 0.05$ were considered significant.

Results

The mean (\pm SD) length of the lymph nodes was 9.2 (\pm 0.27) cm (range 5.77-12.90) and their mean (\pm SD) depth was 4.03 (\pm 0.23) cm (range 2.07-7.41) (Figs. 1A-B). The only microorganism isolated in this study was *Staphylococcus aureus*. Tukey tests indicated that in the cows in which only one quarter of each side was positive in bacterial culture, the mean (\pm SD) of length was significantly larger than that of two culture negative quarter sides (10.68 ± 1.38 vs. 8.04 ± 1.72 ; $P < 0.01$). In the cows with only one quarter of each side positive in culture, the depth was significantly larger than the cows

with negative culture of both quarters on each sides (6.17 ± 1.37 vs. 3.90 ± 1.31 ; $P = 0.01$). However, there was not a significant difference in the lymph node size between one and two quarters positive on each side ($P = 0.945$).

The Spearman's correlation test indicated a positive relation between cumulative scores of CMT in both quarters of each side and ipsilateral supramammary lymph node size (length, $r = 0.39$, $P = 0.008$ and depth, $r = 0.44$, $P = 0.001$).

The mean (\pm SD) individual cell count was 2039×10^3 ($\pm 4284 \times 10^3$) (log SCC=7, range 5 to 8). Our results revealed a positive correlation between right lymph node length and mean log SCC on right quarters ($r = 0.446$, $P = 0.043$) and a positive correlation between left node length and mean log SCC on left quarters ($r = 0.532$, $P = 0.007$). Also, a significant positive correlation is observed between depth of nodes and mean log SCC in right ($r = 0.471$, $P = 0.031$) and left ($r = 0.561$, $P = 0.004$) sides.

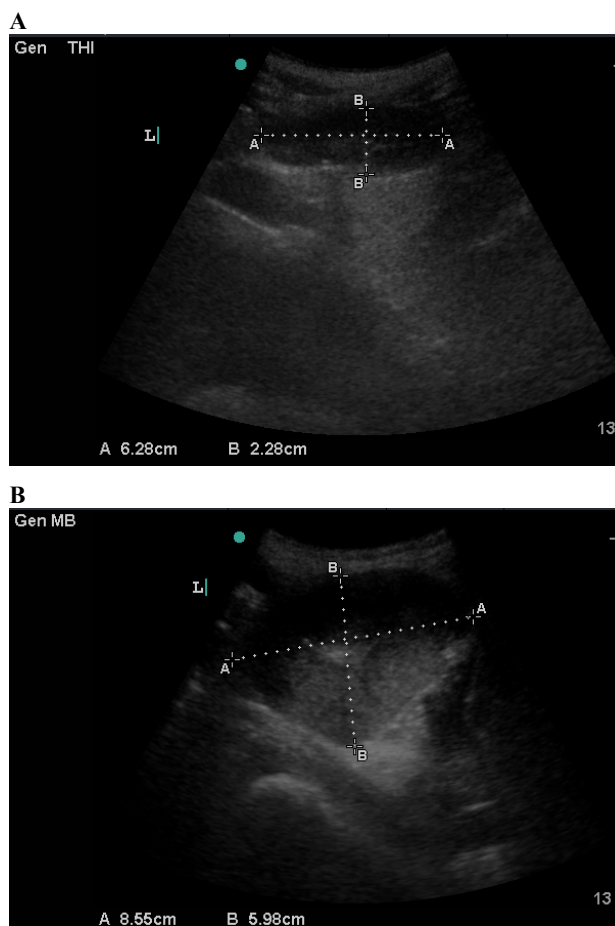


Fig. 1: Ultrasonographic image of a supramammary lymph node taken by a portable ultrasound machine and a 5 MHz convex transducer. Fig. A: An uninfected case, and Fig. B: An infected case. A: Length (dorsoventral dimension) of supramammary lymph node, and B: Depth (caudocranial dimension) of supramammary lymph node

Discussion

In a previous study Bradley *et al.* (2001) used

ultrasound machine with a 7.5 MHz linear transducer with a depth of field 8 cm, which was not a limit for measuring the depth of the nodes, but the width of the field was only 5 cm, that was less than the length of nodes. In order to overcome this limitation a 2-5 MHz convex transducer with a maximum of 30 cm depth of field was employed that could easily measure the entire length of the nodes.

The range in size of the supramammary lymph node reported in previous anatomical studies was 3-9 cm for length and 1-2 cm for depth (Turner, 1952) and with ultrasonographic measurement was 3.5-15 cm (mean 7.4 cm) for length and 1.2-5.7 cm (mean 2.5 cm) for depth (Bradley *et al.*, 2001). The mean length (range 5.77-12.9) and depth (range 2.07-6.41) of the lymph nodes in this study (infected and uninfected cows) were 9.2 and 3.9 cm, respectively, which were slightly larger than those previously reported in uninfected cows. This may be due to these cows being selected from a herd with chronic and severe *Staphylococcus* mastitis problems with very high somatic cell count (2039×10^3) and more than four lactation periods. Milk culture is the gold standard for identification of bacteria from milk and will identify the presence of mastitis pathogens in milk but SCC and CMT will provide a determination of the level of infection.

Our results indicated that in the cows in which one or two quarters of each side were positive in bacteriological culture, the length and depth of ipsilateral nodes were significantly larger than sides which had two quarters with negative culture. However, there was not a significant difference between one and two quarters positive in culture. Although quarters of the mammary gland are separated but the quarters in each side are connected to the same supramammary lymph node, so mastitis in one quarter of each side has a greater effect on increasing supramammary lymph node size rather than both quarters. The findings of this study showed a positive correlation between cumulative scores of CMT in both quarters of each side and ipsilateral supramammary lymph node size. These findings are in accordance with Bradley *et al.* (2001) who reported the lymph nodes size on sides which were positive in a CMT were significantly larger than those on sides which were negative. Furthermore, our results revealed a positive correlation between lymph node length and mean log SCC in both sides, also a significant positive correlation was seen between depth of nodes and mean log SCC in the same sides. This result contradicts a previous study that found the mean dimensions of the nodes in the cows with the higher cell counts though were slightly longer than those of the cows with the lower cell counts, but this difference was not significant, however in that study there was a significant relationship between CMT test result and mean dimensions of the nodes (Bradley *et al.*, 2001). This difference between results of two studies can be due to level of infection or type of microorganisms isolated from quarters.

On the basis of the results of this study that showed dimensions of supramammary lymph node change in

mastitis cases, ultrasonography of supramammary lymph node is probably a quite useful method for confirmation of mastitis cases, future research should be done to apply this method for mastitis detection in some groups of cows such as heifers and dry cows where test on milk is impossible and can be used for selective dry cow therapy or additional treatment in the late stage of dry period, it can also be useful for selective treatment of heifer's mastitis before parturition.

References

- Bradley, KJ; Bradley, AJ and Barr, FJ** (2001). Ultrasonographic appearance of the superficial supramammary lymph nodes in lactating dairy cattle. *Vet. Rec.*, 148: 497-501.
- Braun, U; Fohn, J and Pusterla, N** (1994). Ultrasonographic examination of the ventral neck region in cows. *Am. J. Vet. Res.*, 55: 14-21.
- Bruneton, JN; Balu-Maestro, C; Marcy, PY; Melia, P and Mourou, MY** (1994). Very high frequency (13 MHz) ultrasonographic examination of the normal neck: detection of normal lymph nodes and thyroid nodules. *J. Ultras. Med.*, 13: 87-90.
- Edmonson, AJ; Lean, IJ; Weaver, LD; Farver, T and Webster, G** (1989). A body condition scoring chart for Holstein dairy cows. *J. Dairy Sci.*, 72: 68-78.
- Gillespie, BE and Oliver, SP** (2005). Simultaneous detection of mastitis pathogens, *Staphylococcus aureus*, *Streptococcus uberis*, and *Streptococcus agalactiae* by multiplex real-time polymerase chain reaction. *J. Dairy Sci.*, 88: 3510-3518.
- Kehrli, ME and Harp, JA** (2001). Immunity in the mammary gland. *Vet. Clin. N. Am. Food A.*, 17: 495-516.
- Kimura, K; Harp, JA; Goff, JP and Olsen, SC** (2005). Lymphocytes from one side of the bovine mammary gland migrate to the contra lateral gland and lymph node tissue. *Vet. Immunol. Immunop.*, 108: 409-415.
- Kofler, J; Schilcher, F and Buchner, A** (1998). Ultrasonographic appearance of normal superficial cervical and subiliac lymph nodes in cattle. *Vet. Rec.*, 142: 425-428.
- Oliver, SP; Gonzalez, RN; Hogan, JS; Jayarao, BM and Owens, WE** (2004). *Microbiological procedures for the diagnosis of bovine udder infection and determination of milk quality*. 4th Edn., National Mastitis Council Inc., Verona, WI. PP: 1-8.
- Praye, L; Winkelbauer, H; Gritzmann, N; Winkelbauer, F; Helmer, M and Pehamberger, H** (1990). Sonographic versus palpation in the detection of regional lymph-nodes, metastases in patients with malignant melanoma. *Eur. J. Cancer*. 26: 827-830.
- Soltys, J and Quinn, MT** (1999). Selective recruitment of T-cell subsets to the udder during staphylococcal and streptococcal mastitis: analysis of lymphocyte subsets and adhesion molecule expression. *Infect. Immun.*, 67: 6293-6302.
- Turner, CW** (1952). *The mammary gland 1: anatomy of the udder of cattle and domestic animals*. 2nd Edn., Columbia, Lucas Bros, MO. PP: 1-40.
- Von Andrian, UH and Mempel, TR** (2003). Homing and cellular traffic in lymph nodes. *Nat. Rev. Immunol.*, 3: 867-878.
- Wisner, ER; Mattoon, JS; Nyland, TG and Baker, TW** (1991). Normal ultrasonographic anatomy of the canine neck. *Vet. Radiol.*, 32: 185-190.