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Ultrasonographic diagnosis of subclinical mastitis in buffalo (*Bubalus bubalis*)

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Abstract

A total number of 54 adult dairy buffalos were divided into 3 groups based on California Mastitis Test and Somatic Cell Count; group-1 (38 buffalos) with negative CMT and SCC; group-2 (12 buffalos) with positive CMT and SCC and group-3 (4 buffalos) with positive CMT and negative SCC. Ultrasonography of the mammary gland and supramammary lymph nodes was achieved via 7.5 MHz linear transducer. Subclinical mastitis was characterized ultrasonographically by homogenous hypoechoic parenchyma, hypoechogenic contents of gland cistern, the irregular contour lining of teat canal, slightly thickened teat wall, loss the characteristic three-layered appearance of the teat wall and overlapped ill distinct rosette of Furstenberg, papillary ducts and orifice. Superficial inguinal lymph node was enlarged, easily identified and completely hypoechogenic entire structure. Group 2 suffered from subclinical mastitis revealed significant ($P \leq 0.05$) increase in lymph node length, depth, elapse area, elapse volume and teat wall thickness, significant ($P \leq 0.05$) decrease in gray scale analysis of lymph node and significant ($P \leq 0.05$) increase in gray scale analysis of udder parenchyma and cisterns milk contents than that in group 1 and group 3. In conclusion, ultrasonography provides an accurate, non-invasive and rapid field technique for diagnosis of subclinical mastitis in buffalo.

Keywords: Ultrasonography; buffalo; subclinical mastitis

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Introduction

The buffalo's population represents the mainstream of milk production in Egypt (56%) with an annual 3.8% increase, the highest rate among other types of livestock producing milk (El-Naker et al., 2015).

Subclinical mastitis is considered to be one of the most prevalent production and economically important disease in dairy herds with varied incidence according to the stage of lactation (2.3%, 2.3%, and 12.9% in early, mid and late lactation stage, respectively) and season of lactation (40.90% and 25.71% during winter and summer months respectively) (Kotb et al., 2014).

Deleterious economic effects of subclinical mastitis included; adverse changes in the quantity and quality of milk, increased costs for treatment and early culling of the animals (Blowey and Edmondson, 2010). Serious hazard to public health as a result of some zoonotic diseases transmission through consumptions of infected low quality milk and milk products also must be considered (Fasulkov, 2012).

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Early and correct diagnosis of subclinical mastitis helps in accurate decision of treatment or culling and replacement program within the dairy system (Fasulkov, 2012). However, some authors (Karzis et al., 2007; Petzer et al., 2008) considered CMT as simple, inexpensive and rapid screening test for subclinical mastitis; others (Schaeren and Maurer, 2006; Radostits et al., 2007) proved that it is an unreliable method for diagnosing intramammary infection and therefore it must be confirmed by laboratory methods of SCC and bacteriological culture.

Kralickova et al. (2012) reported that nevertheless the elevated SCC is a consequence of infection; it may increase during the first few weeks of lactation and decrease at the maximum milk production. Hussein et al. (2015) reported that to avoid the limitations of CMT and SCC, udder and superficial inguinal lymph nodes ultrasonography are helpful in the diagnosis of subclinical mastitis in sheep.

Ultrasonography of the mammary gland has been used for describing the morphological appearance of normal udder and teats in ewes (Roval et al., 2008), diagnosis and monitoring of mammary pathological changes and differentiation of structures identified in udder parenchyma such as hematoma and abscess in cattle (Lazaridis et al., 2012). Ultrasonography of lymph nodes has also been used as a routine survey method in human (Bruneton et al., 1994), small animals (Wisner et al., 1991), cattle (Kofler et al., 1998) and sheep (Hussein et al., 2015). Moreover, Bradley et al. (2001) and Hussein et al. (2015) established a relationship between the degree of mastitis and alterations of supramammary lymph node size and architecture in cattle and sheep, respectively.

Many studies describe ultrasonographic examination of the ruminant mammary gland, but the available literatures appear to be free from the diagnostic role of mammary gland and supramammary lymph node ultrasonography in buffalo with subclinical mastitis. Therefore, the objective of the present study was to investigate the role of ultrasonography in the diagnosis of subclinical mastitis in buffalo.

Materials and Methods

Animals

This study was carried out on apparent healthy 54 adult dairy buffaloes (*Bubalus Bubalis*) of different ages (5-11 years), number of parities (3 to 7) and stage of the lactations (early, mild and late) belonging to the animal farm of the faculty of veterinary medicine, Benha University and 2 private buffalo farms in of El-Qalioupea and El-Monofia province.

Buffalos were classified into 3 groups based on the result of CMT and SCC; group 1 consisted of 38 buffalos with negative CMT and negative SCC ($167\pm25\times10^3$/ml); group 2 consisted of 12 buffalos with positive CMT and positive SCC ($361\pm87\times10^3$/ml) and group-3 consisted of 4 buffalos with positive CMT and negative SCC ($192\pm25\times10^3$/ml).

California Mastitis Test (CMT)

Milk samples were taken during milking time and CMT was performed for each quarter separately according to Moroni et al. (2006).

Somatic Cell Count (SCC)

Somatic Cell Count was performed by using (PortaSCC® Quick Test, PortaCheck, Inc., Moorestown, USA) for milk samples from each quarter separately. Animals were considered positive for subclinical mastitis when SCC was $250\text{-}500\times10^3$/ml milk with positive pathogen isolation Diahri et al (2002).

Ultrasonographic examination

Ultrasonographic examination was performed on each quarter and its corresponding supramammary lymph nodes by using 7.5 MHz linear transducer adjusted to portable ultrasound machine (Eickemeyer, Magic 1500, Co., Ltd, UK). All examinations were performed in the sagittal plane by direct contact method; the entire udder and teat were examined by placing the probe on the lateral surface of each quarter along its longitudinal axis moving upwards and downwards (Flock and Winter, 2006). The sonographic examination of the superficial inguinal lymph nodes was performed by placing the probe on the dorsal area and lateral to the caudal aspect of each hind quarter. The length, depth, area and volume of each node and the thickness of the teat wall were measured as reported by Hussein et al. (2015). All measurements were repeated three times and the mean was recorded for each animal.

Quantitative assessment of lymph node, udder parenchyma and content echogenicity was achieved by image brightness analysis (on gray scale units from 0 (black) to 255 (white) by using dedicated software Image J (Image J, NACL Co. Ltd., Tokyo, Japan) to obtain the mean gray value of the analyzed ultrasonographic image (Mostafa et al., 2016).

Data was statistically analyzed by one way ANOVA with post-hock Duncan multiple comparison test by using a statistical software program (SPSS for windows version 20, USA). Differences were considered significant at $P\leq0.05$ (Greiner et al., 2000).

Results

The supramammary lymph node was located caudal and dorsal to each hind quarter at depth of 1.5-2 cm beneath the udder skin. The normal lymph node could be identified as an oval-shaped structure with

thin echogenic capsule. The node's parenchyma appeared hypoechoic with a linear echogenic structure at the center representing the hilar area with its own vessels. In subclinical mastitis; the lymph node was enlarged, nearly lost its characteristic, highly echogenic hilus central area and the entire structure appeared completely hypoechogenic (Fig. 1).

Ultrasonographic examination of the normal quarters revealed homogenous hypoechogenic parenchyma with interspersed anechoic blood vessel, milk alveoli and lactiferous duct. The gland cisterns appeared as a large homogenous anechoic area with few hypoechogenic dotes corresponding to the milk. In subclinical mastitis; the mammary parenchyma appeared homogenous hypoechoic with a lack of clear visualization of milk alveoli and lactiferous duct. The gland cistern loss its anechogenicity and appeared with mixed hypoechogenic contents (Fig. 2).

The normal teat walls appeared as high reflective structure with three discrete layers; the outer hyperechoic layer, the middle thicker hypoechoic layer and the inner hyperechoic layer. The teat cistern appeared as a dilated anechoic area with few hypoechogenic dotes corresponding to the milk contents. In subclinical mastitis, the teat canal and cistern appeared with irregular contour lining, homogenous hypoechogenic contents, narrower lumen, slightly thickened wall and loss the characteristic three layered appearances (Fig. 3).

The normal rosette of Furstenberg appeared as 2-3 parallel, short hyperechoic lines extending from the teat cistern into the papillary duct which could be defined as a thin, bright white line at the end of the teat. The papillary orifice appeared as a small anechoic structure at the tip of the teat. In subclinical mastitis, the rosette of Furstenberg, papillary ducts and papillary orifice were overlapped, ill distinct and could not be differentiated (Fig. 4).

The mean lymph node length, depth, elapse area, elapse volume and teat wall thickness of group 2 (4.94±0.29, 3.88±0.74, 10.62±1.59, 33.69±2.67 and 1.05±0.03, respectively) showed significant ($P \leq 0.05$) increase than group 1 (2.71±0.36, 1.58±0.28, 4.16±0.66, 16.57±1.27 and 0.63±0.01, respectively) and group 3 (2.63±0.34, 1.56±0.24, 3.96±1.01, 16.07±1.57 and 0.63±0.01, respectively). The mean lymph node gray value of group 2 (70.92±5.34) showed significant ($P \leq 0.05$) decrease than group 1 (108.86±5.17) and group 2 (107.41±3.63). The mean gray value of udder parenchyma and cisterns milk (66.51±3.51 and 17.92±3.76, respectively) showed significant ($P \leq 0.05$) increase than group 1 (49.00±3.05 and 3.21±1.31, respectively) and group 3 (48.22±3.82 and 4.28±0.77, respectively). There was a non-significant ($P \leq 0.05$) difference between the measurements and values recorded in group 1 and group 3 (Table 1).

Discussion

Conserving a healthy mammary gland in ruminants is urgent in modern animal husbandry in order to achieve high amount and quality milk production (Fasulkov, 2012). Subclinical mastitis is a common problem of concern in buffaloes worldwide; therefore, quick and accurate diagnosis of such type of mastitis is extremely important to avoid the subsequent serious economic losses (Kotb et al., 2014).

In the present investigation, somatic cell counts of 4 cases with positive CMT were less than the standard range. This observation is in agreement with the finding reported by Sharma et al. (2010) who recorded 227 CMT positive samples, subjected to SCC, in which 201 samples were positive only and the other 26 samples were false positive.

Even SCC of milk samples is the most standard and reliable test for identifying subclinical mastitis closest to the bacteriological results (Fragkou et al., 2014). The method is very expensive, requires a skilled personnel, adequate laboratory facilities and time.

Table 1: Mean ± Standard Deviation, Minimum value (Min) and Maximum value (Max) of SCC, lymph node length (Ln. length), depth (Ln. Depth), elapse area (Ln. E.A), elapse volume (Ln. E.V), teat wall thickness (Teat W.T.) and gray scale value of lymph node (Ln. Gray s.), gland parenchyma (Paren. Gray s.) and milk contents (Milk Gray s.)

Items	Group-1 (n=38)			Group-2 (n=12)			Group-3 (n=4)		
	Mean± SD	Min	Max	Mean± SD	Min	Max	Mean± SD	Min	Max
CMT	Negative			Positive			Positive		
SCC (×10³/ml)	167±25[a]	120	215	359±86[b]	240	495	192±25[a]	165	225
Ln. Length (cm)	2.71±0.36[a]	2.08	3.20	4.94±0.29[b]	4.53	5.53	2.63±0.34[a]	2.13	2.87
Ln. Depth (cm)	1.58±0.28[a]	1.22	2.09	3.88±0.74[b]	3.05	5.05	1.56±0.24[a]	1.35	1.89
Ln. E.A (cm²)	4.16±0.66[a]	3.17	5.34	10.62±1.59[b]	7.93	13.19	3.96±1.01[a]	2.69	5.13
Ln. E.V (cm³)	16.57±1.27[a]	14.03	18.20	33.69±2.67[b]	29.09	36.69	16.07±1.57[a]	13.89	17.32
Teat W.T.	0.63±0.01[a]	0.49	0.71	1.05±0.03[b]	0.90	1.23	0.63±0.01[a]	0.62	0.65
Ln. Gray s.	108.86±5.17[a]	101.11	118.09	70.92±5.34[b]	60.09	77.77	107.41±3.63[a]	104.22	111.05
Paren. Gray s.	49.00±3.05[a]	43.12	54.12	66.51±3.51[b]	60.40	71.18	48.22±3.82[a]	43.03	52.01
Milk Gray s.	3.21±1.31[a]	1.25	5.36	17.92±3.76[b]	12.14	3.33	4.28±0.77[a]	24.71	5.02

The means within the same row having different superscript are significantly different at level ($P \leq 0.05$)

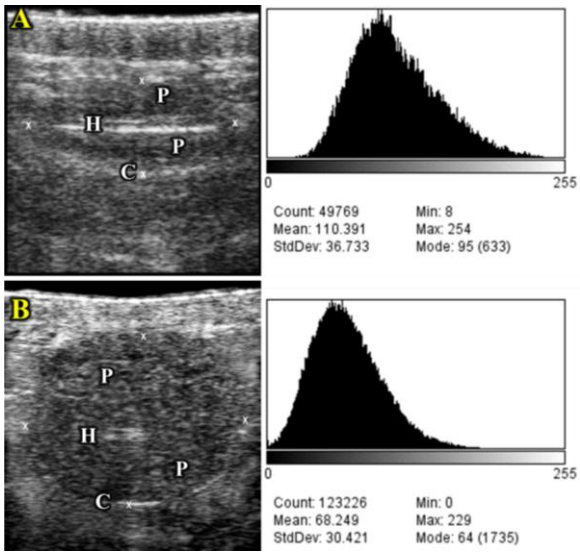

Fig. 1: Ultrasonographic examination of supramammary lymph and its crossponding gray scale histogram analysis; in normal (A) and in subclinical mastitis (B). Showing the lymph node capsule (C), parenchyma (P) and hilar area (H)

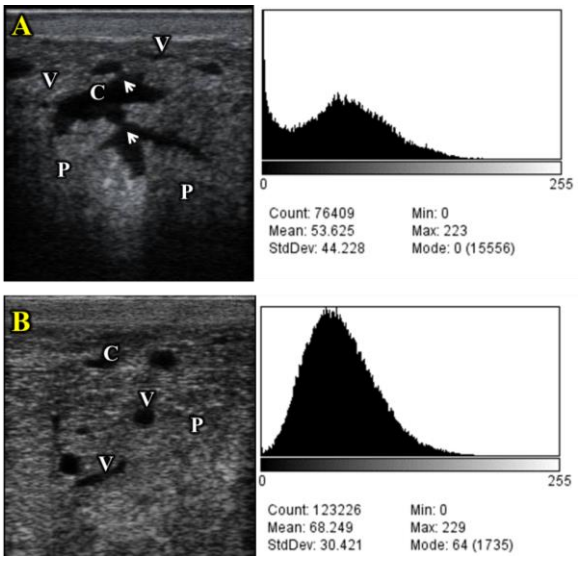

Fig. 2: Ultrasonographic examination of buffalo mammary gland and its crossponding gray scale histogram analysis; in normal (A) and in subclinical mastitis (B). Showing the gland parenchyma (P), gland cistern (C) lactiferous duct (arrow) and blood vessels (V)

To avoid the limitations of CMT and SCC, ultrasonography particularly superficial inguinal lymph nodes are useful in diagnosis of subclinical mastitis in sheep (Hussein et al., 2015).

The results of ultrasonographic examination were consistent with the result of SCC in the three studied groups. In the same accordance Bradley et al. (2001) reported that ultrasonographic measurements of superficial inguinal lymph node varied among animals according to the somatic cell count and are useful for diagnosis of subclinical mastitis in cattle.

The mean lymph node dimensions (length, depth, elapse volume and elapse area), the mean teat wall thickness, the mean gray value of udder parenchyma and cisterns milk in group 2 showed significant ($P \leq 0.05$) increase in comparison with group 1 and group 3. In the same agreement, Dingwell et al. (2004) and Hussein et al. (2015) reported that the ultrasonographic length, depth and area of the superficial inguinal lymph nodes were significantly ($P<0.05$) increased in the udder infected groups. The sensitivity of lymph node length, depth and elapse area to sub-clinical mastitis was 96, 92 and 94% respectively, in contrast the sensitivity of SCC was 94% and CMT was 68.8%.

Ultrasonographic examination of the normal buffalo mammary gland revealed homogenous hypoechogenic parenchyma with interspersed anechoic blood vessel, milk alveoli, lactiferous duct, gland cisterns. The teat cistern defined only when filled with milk as a dilated anechoic structure with few hypoechogenic dotes. The papillary duct visualized as a single hypoechoic zone and its boundary with the teat cistern appeared as round anechoic structures corresponding to the venous ring of Furstenberg. These results were in accordance to Nak et al. (2005), Rambabu et al. (2008), Fasulkov (2012) and Kotb et al. (2014).

The rosette of Furstenberg appeared as 2-3 parallel, short hyperechoic lines extending from the teat cistern into the papillary duct. These results were in agreement with Khol et al. (2006). On the other hand Nak et al. (2005) described the rosette of Furstenberg as a homogenous hyperechoic structure located directly above the teat canal.

The normal teat wall appeared as high reflective structure with three discrete layers; outer hyperechoic layer, middle thicker hypoechoic layer and inner hyperechoic layer. In support of this claim, Szencziová and Strapák (2012) proved that the histologic image of the teat wall appeared as a threefold layered structure, the outer teat skin layer, the middle fibro-muscular vascularized layer and the inner boundary mucous membrane layer. Because of this density difference, the wall layer could be distinguished ultrasonographically.

Subclinical mastitis mammary parenchyma appeared homogenous hypoechoic with ill distinct milk alveoli and lactiferous duct. This observation is in good agreement with the finding reported by Hussein et al. (2015) and Kotb et al. (2014) and they added that the milk alveoli with subclinical mastitis showed anechoic fluid with suspended hypoechoic dots before milking and increased echogenicity after milking as a consequence of the concentration of the somatic cells in the residue of milk after milking.

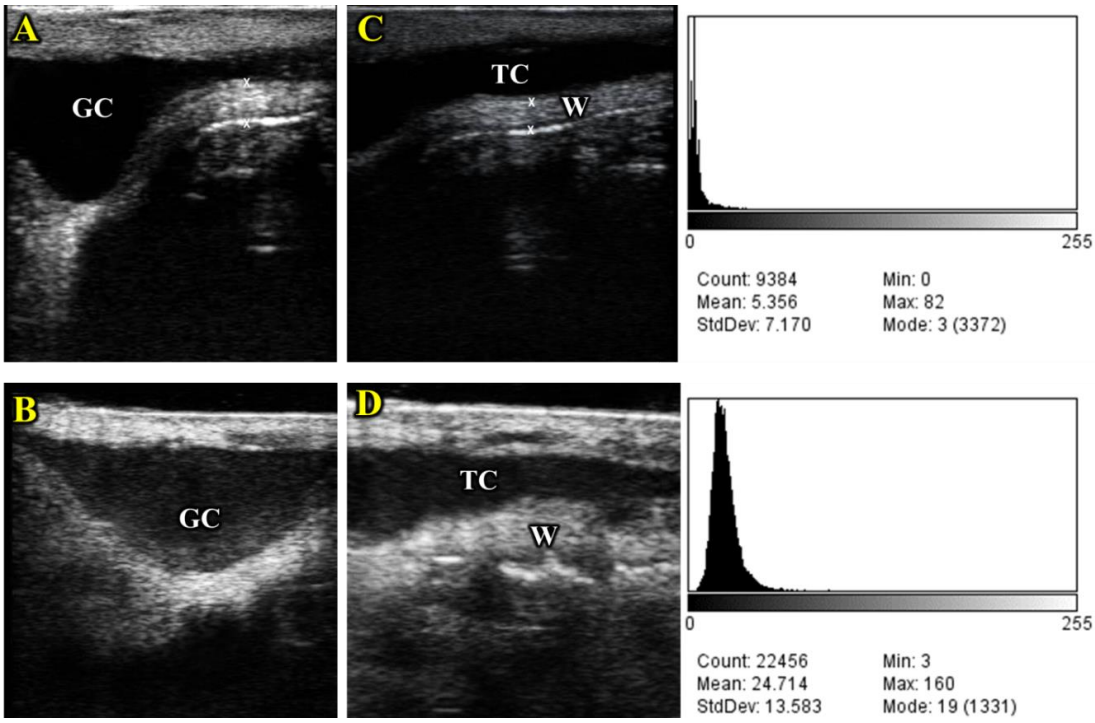

Fig. 3: Ultrasonographic examination of mammary gland cistern and teat cistern and the corresponding gray scale histogram analysis of their milk contents in normal (A & C) and in subclinical mastitis (B & D). Showing gland cistern (GC), teat cistern (TC) and teat wall (T).

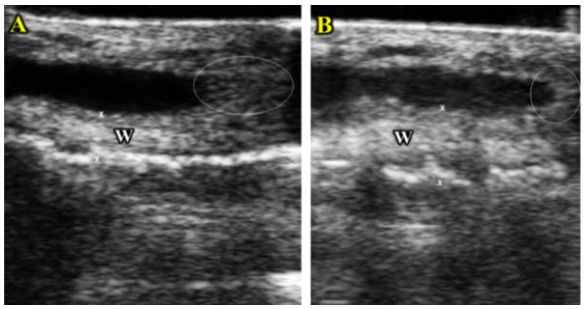

Fig. 4: Ultrasonographic examination of buffalo teat; in normal (A) and in subclinical mastitis (B). Showing the teat wall (W) and rosette of Furstenberg (circle).

Gland cistern in subclinical mastitis loss its anechogenicity and appeared with mixed hypoechogenic contents. The teat cistern appeared with homogenous hypoechogenic, irregular contour lining, narrower lumen, slightly thickened wall and loss the characteristic three layered appearances. The rosette of Furstenberg, papillary ducts and papillary orifice were overlapped and ill distinct. Szencziova and Strapak (2012) and Kotb et al. (2014) attributed those clear ultrasonographic changes to the slight inflammatory reaction and directly damage of milk-producing tissue of the udder as a result of bacterial, mycotic and sometimes viral infections.

Ultrasonographically, the supramammary lymph node was located caudodorsal to each hind quarter at depth 1.5-2 cm beneath skin. The normal lymph node appeared as oval-shaped structure with thin echogenic capsule, hypoechoic parenchyma and linear echogenic central area representing the node hilus. This observation is in agreement with the finding in previous studies on sheep (Hussein et al., 2015) and cattle (Bruneton et al., 1994).

Subclinical mastitis lymph node was easily identified, enlarged, nearly lost their highly echogenic hilus central area and the entire structure appeared completely hypoechogenic. In the same accordance Bradley et al. (2001) and Hussein et al. (2015) found significant changes in the dimensions, internal architecture and ultrasonographic pattern of supramammary lymph node of cattle with subclinical mastitis.

Soltys and Quinn (1999) and Bradley et al. (2001) attributed the aforementioned result to the lymphocytes in the ipsilateral supramammary lymph nodes of the affected quarter are rapidly activated, proliferate and then migrate to the mammary gland to fight bacterial infection. This phenomenon was incriminated in the relationship between subclinical mastitis and alterations happen to the supramammary lymph node.

Quantitative ultrasonographic assessment of the lymph node, udder parenchyma and content echogenicity was achieved through measurements and

image brightness analysis using Image J. Pillen et al. (2009), Grani et al. (2015) and Mostafa et al. (2016) have previously used the same technique for accurate interpretation of soft tissue ultrasonographic image based on quantitative assessment rather than qualitative evaluation which is more descriptive and less accurate.

In conclusion, ultrasonography provides non-invasive and rapid field technique for accurate diagnosis of subclinical mastitis in buffalo. Moreover, ultrasonographic examination of the udder and the superficial inguinal lymph node was recommended to minimize the adverse changes in the quantity and quality of milk, decrease costs of treatment, improve the culling program of animals and prevent the serious hazard to the public health.

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