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The Correlation Between Ultrasonographic and Laboratory Findings of Mastitis in Buffaloes (Bubalus Bubalis)

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Abstract: The aims of this study are to through light on the importance of ultrasonography as a useful, accurate and relatively quick tool for diagnosis of mastitis in buffaloes and to correlate the laboratory results with ultrasonographic findings. In the present study, mastitis was diagnosed in 55 lactating buffaloes by clinical findings, California Mastitis Test (CMT), Somatic Cell Count (SCC), microbiological tests and ultrasonography. Out of 55 examined buffaloes, 25 and 30 animals had clinical and subclinical mastitis. Udder and teats ultrasonography of buffaloes with subclinical mastitis showed irregular contour of teat canal and sinus, loss of the three layered appearance of the affected teat wall, overlapped papillary duct and rosette of Furstenberg and clarity image of udder parenchyma and gland sinus. In clinical mastitis, ultrasonographic examination revealed thickened teat wall, loss of the three layered appearance of the teat wall, complete obstruction of teat canal and disappearance of rosette of Furstenberg. The teat cistern had irregular lining and filled with homogenous hypoechoic milk. Milk alveoli appeared as anechoic cavities filled with hypoechoic fluid. Buffaloes with parenchymatous abscesses showed complete obstruction of the teat canal and cistern with hyperechoic materials and multiple abscesses filled with hyperechoic caseated pus and surrounded by hyperechoic thick capsules. Udder fibrosis revealed disappearance of teat canal and cistern, diffuse hyperechoic small cordial echoes and complete replacement of milk alveoli with hyperechoic fibrous tissues. Somatic cell count ranged between 320 000 and 476000 cell/ ml of milk in subclinical mastitis. Both mixed and single infections were recorded in in 90.9% and 9.1% of the collected milk samples. The most common isolated pathogens were *S. aureus*, environmental bacteria and *Candida spp*. Marbocil and Norfloxacin were the most sensitive antibiotics. In conclusion, ultrasonography and laboratory diagnosis are complementary to each other in correct diagnosis of mastitis in buffaloes.

Key words: Ultrasonography · Buffaloes · Mastitis · Somatic Cell Count · California Mastitis Test

INTRODUCTION

Mastitis is a combination of physical, chemical and microbiological changes in the milk with pathological changes in the glandular tissue of the udder that affects both the quality and quantity of milk causing high economic losses for the dairy industry and serious hazard for public health [1]. The magnitude of these changes in an individual animal varies with the severity and duration of the infection and the causative microorganisms [2]. This infection may be with one or more of different micro-organisms such as bacterial contamination, mycotic infection and sometimes viral. These microorganisms produce toxins that can directly damage milk-producing tissue of the udder [1, 2].

Infection of the udder usually takes place directly through teat canal however; organisms may get settled in the mammary tissues via blood as in case of tuberculous mastitis [3].

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Mastitis contributes to decrease milk yield, compositional changes in milk, high costs for treatment and early culling of the animals. Early diagnosis and treatment of mastitis decreases these economic losses [4].

Nowadays, ultrasonography is widely used in bovine practice [5]. Udder and teat ultrasonography was performed in cattle for diagnosis of milk flow disturbances, different inner anatomical structures of the teat, cisternitis, tissue proliferation, foreign bodies, milk stones, congenital changes, hematoma and abscess [2].

The ultrasound examination of the udder parenchyma is mainly performed using the direct contact method with lower frequency linear probes (3.5 – 5 MHz) while examination of the teat is most commonly conducted by the water bath technique with a help of a 7.5 MHz linear probes [2, 6].

Final diagnosis of bovine mastitis depends mainly upon laboratory diagnosis. Several laboratory tests are useful predictors of mastitis such as California Mastitis Test (CMT), Somatic Cell Count (SCC) and microbial diagnosis [7].

The majority of published studies about mastitis are related to etiology, control and prevention [8]. Therefore, the present study was focused on the correlation between ultrasonographic and laboratory findings of mastitis in buffaloes.

MATERIALS AND METHODS

Animals: The present study was carried out on fifty five dairy buffaloes suffering mastitis in a research farm at Giza governorate, Egypt during the period between January 2012 and January 2013. The affected animals were examined in standing position under sedation with Xlyazin HCL given intramuscularly at a dose of 0.1mg/Kg body weight. The affected buffaloes were subjected to clinical examination and all data were recorded.

Ultrasonographic Examination: It was conducted with ultrasonographic device (ECM–Novico, Exagyne, France) connecting with a 5-8 MHz linear transducer. The method of examination was carried out as mentioned before [2, 6]. Before scanning, teats and udder were cleaned thoroughly with warm water then ultrasound coupling gel (Ultrasound Gel, Jaay Vee Meditech International, Pondicherry, India) was applied. Both sagital and transverse planes were performed and the images were printed on Polaroid paper.

Water bath method was applied to examine the teats by ultrasound. The examined teat was dipped in a polyethylene cup filled with water then the transducer was applied in both vertical and horizontal planes of the outer wall of the polyethylene cup [9].

Laboratory Diagnosis

California Mastitis Test (CMT): It was applied to milk samples after discarding first three strips of fore milk to detect clinical and subclinical mastitis according to Schalm and Noor Lander [10].

Somatic Cell Count (SCC): Milk samples were collected from all udder quarters according to Andrews *et al*. [11]. About 150 infrared Milk Analyzer (Somacount, France) was used in this count. Samples contained flakes; clots or other unusual aspects were discarded. Animals were considered positive for subclinical mastitis when SCC was 250000-500000 cells/ ml milk with positive pathogen isolation and considered positive for clinical mastitis when SCC was above 500000 cells/ ml milk according to Diabri *et al*. [12].

Microbiological Examination: A total of 220 quarter milk samples were aseptically collected during the morning milking for cultivation according to the National Mastitis Council [13]. From each sample, 0.1 ml of milk was plated on blood agar, Mannetol salt agar, Edwer's media and Macconky agar and incubated for 24- 48 hours at 37°C. In addition, Dextrose Sabaurd agar was used for isolation of mycotic infections at 25°C for one week. A quarter was considered culture-positive when a growth of at least one colony was detected.

Bacteria were identified according to colony morphology and Gram-staining. For Gram-positive cocci, catalase test with hydrogen peroxide (3%) was used to differentiate between catalase-positive staphylococci and catalase-negative cocci. Coagulase test was carried out using sterile rabbit plasma to distinguish *Staphylococcus aureus* (coagulase-positive) from non-*aureus staphylococci* (coagulase-negative). *Streptococci* were subdivided into aesculin-positive cocci and aesculin-negative cocci (*Streptococcus agalactiae* and other *Streptococcus*). CAMP-test was used to differentiate *S. agalactiae* from *S. dysgalactiae. Enterobacteriace* were identified as Gram negative bacilli.

Sensitivity Test: It was performed by using Muller-Hinton agar media and antibiotic sensitivity discs (Oxoid). The types of antibiotic sensitivity discs were selected according to Carter and Cole [14].

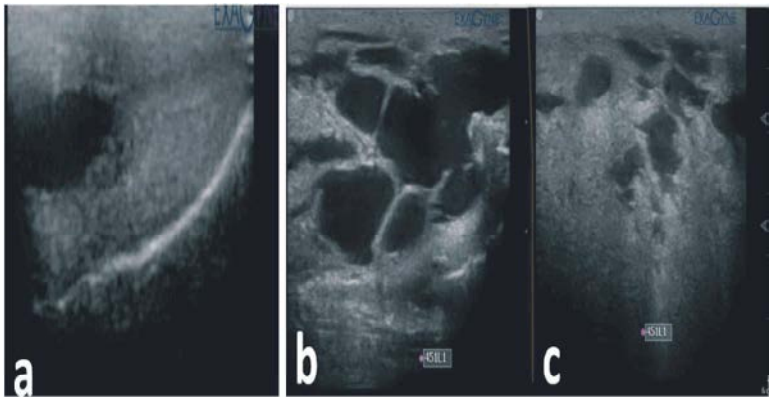

Fig. 1: (a): Teat ultrasonogram in a buffalo with subclinical mastitis showing irregular contour of teat canal and sinus, absence of demarcation between the three layers of the affected teat wall and overlapped papillary duct and rosette of Furstenberg. (b): Ultrasonogram of the milk alveoli in a buffalo with subclinical mastitis showing anechoic fluid with suspended hypoechoic dots before milking. (c): Ultrasonogram of the same milk alveoli in figure (b) after milking showing increased echogenicity of the fluid.

RESULTS

Out of 55 examined buffaloes, 25 animals representing 45.5% had clinical mastitis with severe clinical symptoms including: fever and hotness, tenderness, redness and swelling of the udder. While the other 30 buffaloes (54.5%) had subclinical mastitis.

Ultrasonographic Findings: Ultrasonographic examination provided additional information on the status of the udder and teats. Udder and teats ultrasonography of buffaloes with subclinical mastitis showed; irregular contour of teat canal and sinus, loss of the three layered appearance of the affected teat wall, overlapped papillary duct and rosette of Furstenberg and clarity image of udder parenchyma and gland sinus (Figure 1a). The milk alveoli showed anechoic fluid with suspended hypoechoic dots before milking (Figure 1b) and increased echogenicity after milking (Figure 1c).

In clinical mastitis, ultrasonographic examination revealed; thickened teat wall, loss of the three layered appearance of the teat wall, complete obstruction of teat canal and disappearance of rosette of Furstenberg (Figure 2a). The teat cistern had irregular lining and filled with homogenous hypoechoic milk (Figure 2b). Milk alveoli in buffaloes with clinical mastitis caused by pyogenic bacteria, appeared as anechoic cavities filled with homogenous hypoechoic fluid (Figure 2c). Udder with clinical mastitis caused by *Staph. aureus* mixed with *Candida spp.,* showed milk alveoli with hypoechoic fluid and suspended hyperechoic flakes (Figure 2d).

Seven buffaloes with clinical mastitis developed multiple parenchymatous abscesses (Figure 3a). All of these buffaloes were infected with either *C. pyogen* and / or *Staph. aureus.* Ultrasound examination revealed complete obstruction of the teat canal and cistern with hyperechoic mass (Figure 3b). The udder parenchyma showed multiple abscesses filled with hyperechoic caseated pus and surrounded by hyperechoic thick capsules (Figure 3c). The parenchymatous abscesses caused by *Staph. aureus* were less echogenic than that caused by *C. pyogen* (Figure 3d).

Udder fibrosis was recorded in four quarters of three mastitic buffaloes (Figure 4a). Ultrasonographic findings included; disappearance of teat canal and cistern, diffuse hyperechoic small cordial echoes (Figure 4b) and complete replacement of milk alveoli with hyperechoic fibrous tissue (Figure 4c). Ultrasonography of the other normal quarters showed homogenous and hyperechoic glandular tissue with anechoic milk alveoli.

Laboratory Findings

California Mastitis Test (CMT): Out of 55 examined buffaloes, CMT revealed 30 buffaloes suffering subclinical mastitis with different degrees.

Somatic Cell Count: Buffaloes with subclinical mastitis had somatic cell count ranged between 320 000 and 476000 cell/ ml of milk.

Microbiological Findings: Cultures with mixed species of bacterial isolates were found in 200 samples representing 90.9% of the total examined milk samples. While single

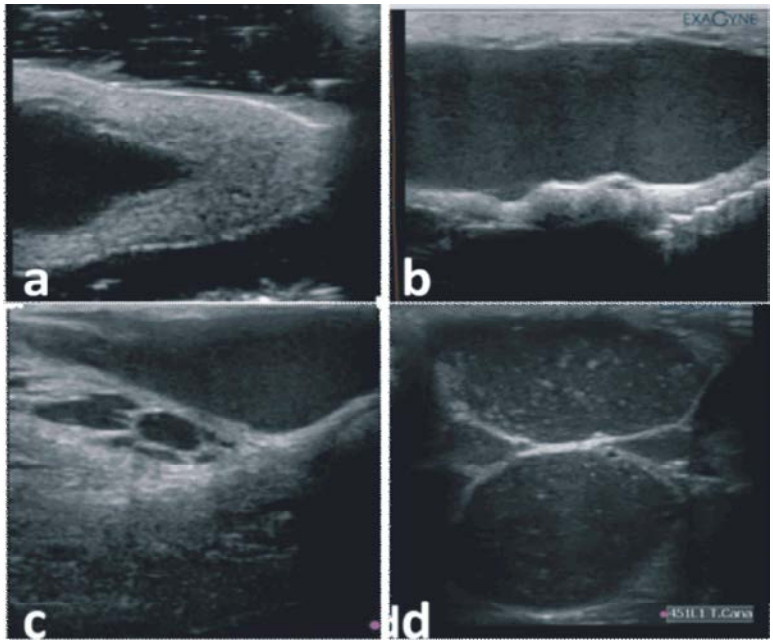

Fig. 2: (a): Teat ultrasonogram in a buffalo with clinical mastitis showing; thickened teat wall, loss of the three layered appearance of the teat wall, complete obstruction of teat canal and disappearance of rosette of Furstenberg. (b): Teat ultrasonogram in a buffalo with clinical mastitis showing irregular lining of the teat cistern and presence of homogenous hypoechoic milk. (c): Ultrasonogram of milk alveoli in a buffaloes with clinical mastitis caused by pyogenic bacteria showing anechoic cavities filled with homogenous hypoechoic fluid. (d): Ultrasongram of an udder with clinical mastitis caused by *Staph. aureus* mixed with *Candida spp.,* showing milk alveoli filled with hypoechoic fluid and suspended hyperechoic flakes.

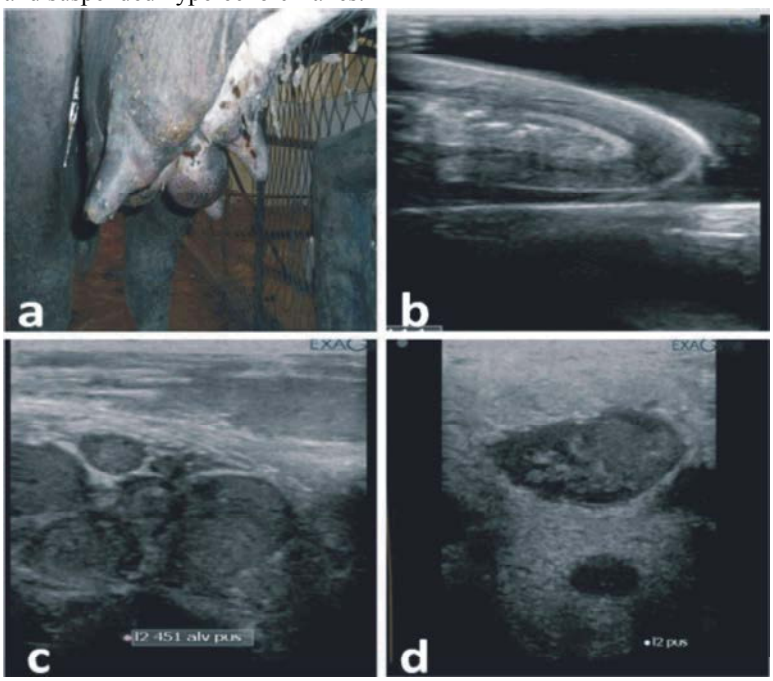

Fig. 3: (a): A six-year-old buffalo showing multiple parenchymatous udder abscesses caused by *C. pyogen*. (b): Ultrasonogram of the same udder showing complete obstruction of the teat canal and cistern with hyperechoic caseated materials. (c & d): Ultrasongrams of the same udder showing multiple parenchymatous abscesses filled with hyperechoic caseated pus and surrounded by hyperechoic thick capsule.

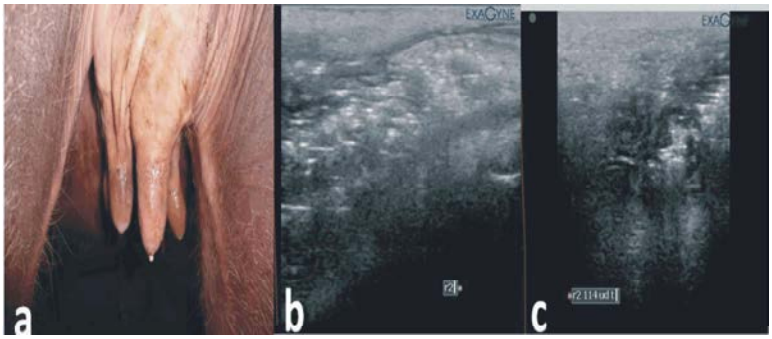

Fig. 4: (a): Udder atrophy and fibrosis of the right hind quarter in an 8-year-old buffalo. (b): Ultrasonogram of the same quarter showing disappearance of teat canal and cistern and diffuse hyperechoic small cordial echoes. (c): Ultrasonogram of the same quarter showing complete replacement of milk alveoli with hyperechoic fibrous tissue.

Table 1: Results of culture and sensitivity tests in clinical and subclinical mastitis in the examined buffaloes.

	Bacterial isolates	%	Sensitivity test
Clinical Mastitis	*Coagulase –ve Staph + Coliform*	38	Marbocil, Gentamycin, Norfoxacin and Ciprofloxacin
	Strept spp. + Coliform	22	Amoxicillin, Gentamycin and Cholamphincol
	S. aureus + Strep.spp	16%	Marbocil, Norfoxacin and Ciprofloxacin
	C. pyogen + Strep.spp + Coliform	15%	Marbocil, Gentamycin
	S. aureus	8%	Marbocil and Norfoxacin
	C. pyogen	1%	Gentamycin
	Total	100	
Subclinical Mastitis	*S. aureus + Strep. spp*	20%	Marbocil, Norfoxacin and Ciprofloxacin
	Coagulase negative Staph+ Strep.spp + Coliform	80%	Amoxicillin, Gentamycin and Cholamphincol
	Total	100	

infection was recorded in 9.1% of the collected samples (Table 1). The most common isolated pathogens were; *S. aureus*, environmental bacteria and *Candida spp*. *Candida spp.* could be isolated as a secondary infection from clinical and subclinical mastitic milk samples with percentages of 33% and 38.5% respectively.

Sensitivity Test: Results of the test were illustrated in Table 1. In the present study, Marbocil and Norfloxacin were the most sensitive antibiotics.

DISCUSSION

Diseases of the udder and teat especially mastitis are common in buffaloes worldwide. Mastitis is the inflammatory condition of the udder irrespective of the cause. Mastitis makes up negative economic effect and may be lead to transmission of some zoonotic diseases through consumptions of infected low quality milk and milk products. Early and correct diagnosis of mastitis helps in the decision of treatment or culling and replacement program in dairy farms. In this respect, culling of buffaloes with mycotic mastitis, multiple udder abscessations or fibrosis was done in this study.

In the present study, mammary quarters infected by major pathogens showed SCC scores greater than those infected by minor pathogens. This is in agreement with the results of Hoquem *et al*. [15], Santos *et al*. [16], Gungor *et al*. [17] and Riekerink *et al*. [18]. Somatic cells are protective for the animal body and fight infectious organisms therefore it is used to evaluate the alternation of the tissues damage due to mastitis [19]. An elevated SCC in milk has a negative influence on the quality of raw milk. In this work, a great variation was noticed between SCC in subclinical and clinical mastitis in buffaloes. This variation was attributed to the differences in the magnitude of cellular response and duration of intra mammary infection.

Concerning the causative pathogens of mastitis in the examined buffaloes, some contagious micro-organisms as *S. aureus* and *C. pyogen* were isolated either alone or mixed with some environmental micro-organisms which indicate bad hygiene. These results agree with that recorded before [20].

Isolation of *Candida spp*. from clinical and subclinical mastitis with high incidence indicates its importance as a causative agent of mastitis in buffaloes and further studies are required to study its prevention and treatment.

Ultrasonography showed characteristic findings in the examined buffaloes, therefore it is considered as a helpful tool to diagnose pathologic alterations of the udder and teats. Similar findings were previously mentioned in cattle [21-23].

The application of water bath method for teat ultrasonography increases the acoustic impedance difference between the teat wall and the surrounding medium. The presence of milk in the teat sinus acted similarly as a window of acoustic impedance for imaging the deeper structures and far wall of the teat. This is in agreement with previous studied [2, 6].

Slight inflammation of udder and teats subclinical mastitis results in clear ultrasonographic findings as; irregular contour the teat canal and sinus, absence of the three layered appearance of teat, overlapped papillary duct and rosette of Furstenberg and clear image of udder parenchyma and gland sinus. This is in agreement with the results mentioned before by Dinc and Sendag [24].

It is worthy to mention that the echogenicity of milk alveoli was increased after milking the buffaloes suffered subclinical mastitis. This is due to the concentration of the somatic cells in the residue of milk after milking.

Ultrasound examination of clinical mastitis showed various images according to the causative agent. This variation depends upon the pathological alterations induced by the causative pathogens. In the present study, milk alveoli had diffuse hypoechoic fluid in buffaloes with mastitis caused by pyogenic bacteria. This could be attributed to the formation of pus inside the affected alveoli. On the other hand, buffaloes with clinical mastitis caused by *Staph. aureus* and *Candida spp.* had milk alveoli filled with hypoechoic fluid and suspended hyperechoic flakes representing the mycotic hyphae. These ultrasonographic findings are more or less similar to the pathological findings of cows' udder with mycotic mastitis [25].

In addition, ultrasonography had a useful role in the detection of the site and properties of each udder abscess. In several examined buffaloes, clinically non-palpable abscesses due to their small-sized or deep localization were easily visualized by ultrasound. It was noticed that the parenchymatous abscesses caused by *Staph. aureus* were less echogenic than that caused by *C. pyogen.* This could be explained by the caseated nature of pus formed by *C. pyogen*.

Udder fibrosis and atrophy due to chronic mastitis had hyperechoic cordial bands representing the fibrous tissues which replace the glandular tissues. This is in agreement with the previous results [6].

In conclusion, ultrasonography and laboratory diagnosis are complementary to each other in correct diagnosis of mastitis in buffaloes.

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