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Laboratory and Ultrasonographic Diagnosis of Mastitis in Buffaloes

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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 mastitic buffaloes. 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. The results showed that the ultrasonography is a helpful tool in diagnosis of mastitis in buffaloes. All ultrasongraphic and laboratory findings were discussed in correlation to each other.

Keywords: Laboratory Diagnosis, Ultrasonography, Buffaloes, Mastitis.

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. The magnitude of these changes in individual animal varies with the severity and duration of the infection and the causative microorganisms. These microorganisms produce toxins that can directly damage milk-producing tissue of the mammary gland, and the presence of bacteria initiates inflammation within the mammary tissue in an attempt to eliminate the invading microorganisms (Szencziova and Strapak, 2012).

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. It is considered as a complex disease caused by the interaction of several factors as animals, the environment and microorganisms such bacterial contamination, mycotic infection and sometimes viral infection. These infections may be with one or more of different micro-organisms that affect both mammary epithelial cell and alveolar function followed by impair both the quantity and quality of milk causing high economic losses for the dairy industry and serious hazard for public health (Jai *et al.*, 2005).

California Mastitis Test (CMT) and Somatic cell count (SCC) are useful predictors of intra mammary infection (IMI) depending upon severity of inflammatory response to infection as mastitis increase migration of polymorphneucler cells in tissue injury and stress. Somatic cells are protective

for the animal body and fight infectious organisms. An elevated SCC in milk has a negative influence on the quality of raw milk (Sharma *et al.,* 2011).

The inflammation contributes to decrease milk production and is primarily responsible for the compositional changes observed in milk from infected quarters as well as increased costs for treatment and early culling of the animals (NMC, 2011).

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 tuberculosis mastitis (NMC, 2004).

Ultrasonography could be used as a helpful tool to diagnose pathological alterations in the udder such as inflammation, mucosal lesions, tissue proliferation, foreign bodies, milk stones, congenital changes, hematoma and abscess. Udder and teat scanning can be also performed for diagnosis of milk flow disturbances and different inner anatomical structures of the teat like teat canal length and diameter, teat cistern diameter, and teat wall thickness (Szencziova and Strapak, 2012).

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 higher frequency linear probe (at least 7.5 MHz) for good image quality (Szencziova and Strapak, 2012).

Final diagnosis of mastitis in buffaloes depends mainly upon laboratory diagnosis. The most common laboratory diagnosis of mastitis including CMT, SCC and microbiological diagnosis (NMC, 2001).

The majority of published studies about mastitis are related to etiology, control and prevention of mastitis (Seker *et al.,* 2009).

Therefore, the present study was focused to get the benefits of ultarasonographic and laboratory diagnosis to evaluate the alternation of the tissues damage due to mastitis in buffaloes that help in the decision of treatment or culling and replacement in dairy farms.

Material 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 Xlyazine HCL given intramuscularly at a dose of 0.1mg/Kg body weight.

Laboratory Diagnosis

Following thorough clinical examination by inspection and palpation, California Mastitis Test (CMT), Somatic Cell Count (SCC) and microbiological examination were carried out.

California Mastitis Test (CMT)

It was applied to milk samples after discarding the first three strips of fore milk to detect clinical and subclinical mastitis according to Schalm and Noorlander (1957).

Somatic Cell Count (SCC)

Milk samples were collected from all quarters according to (Andrews *et al*., 2004) for somatic cell counts (SCC) using Bently 150 infrared Milk Analyzer (Soma count, France). Animals were considered with subclinical mastitis when SCC was 250000-500000 cells/ml milk with positive pathogen isolation and considered as clinical mastitis when SCC above 500000 cells/ml milk (Djabri *et al.,* 2002). The samples containing flakes, clots or other unusual aspect were not used for SCC.

Microbiological Examination

A total of 220 quarter milk samples were aseptically collected during morning milking for cultivation according to the National Mastitis Council (NMC, 1999). Briefly, from each sample, 0.1 ml of milk was plated on blood agar, Mannetol salt agar, Edwer's media, Macconky agar (one plate per buffalo) and incubated for 24- 48 hours at 37°C. Dextrose Sabaurd agar was used for isolation of mycotic infections at 25 ºC during one week.

A quarter was considered culture-positive, when growth of at least one colony was detected. Bacteria were identified based on 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, referred to as coagulase-negative staphylococci. 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 done by using Muller-Hinton agar media and antibiotic sensitivity discs (Oxoid). The types of antibiotic sensitivity discs were selected according to (Carter and Cole, 1990).

Ultrasonographic Examination

It was conducted with ECM–Novico, Exagyne (France) device connecting with linear 5-8 MHz transducer according to (Rambabu *et al.,* 2008) and (Szencziovaand Strapak, 2012). Before scanning, the udder and teats were cleaned thoroughly with warm water then ultrasound coupling gel (Ultrasound Gel, jaayveemeditech international, Pondicherry, India) was applied. Both sagittal and transverse planes were applied then the images were recorded on Polaroid paper with a thermal printer. Water bath method was applied to examine the teats by ultrasound according to (Fasulkov *et al.,* 2010). 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 (Fig. 1).

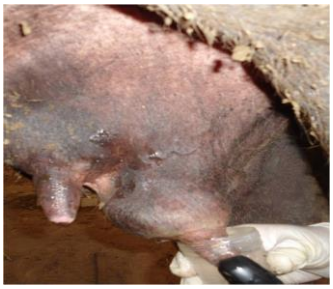

Fig. 1. Ultrasonography Water bath method for teat examination.

Results

Out of 55 examined buffaloes, 25 (45.5%) animals suffered clinical mastitis with severe clinical symptoms including: fever and hotness, tenderness, redness and swelling of the udder (Fig. 1).

Laboratory Findings

California Mastitis Test (CMT)

Out of 55 examined buffaloes, the results of CMT revealed 30 buffaloes suffering subclinical mastitis with different degrees of CMT.

Somatic Cell Count

Mean cell count from 30 subclinical mastitic buffaloes ranged between 320 000 and 476000 cell/ ml milk.

Microbiological Findings

Cultures with more than one species of bacterial isolates (mixed infections) were found in most samples (91%) while single infection was recorded in 9% of the collected samples (Table 1). The most common isolated pathogens were, S. aureus, environmental bacteria and Candida spp. Candida spp. could be isolated from clinical and subclinical samples with percentages of 33% and 38.5% respectively. Culling of buffaloes with mycotic mastitis, multiple udder abscessations or fibrosis was done due to unsuccessful treatment.

Sensitivity test

Results of the test were collected in Table 1. In the present study, Marbocil and Norfloxacin were the most sensitive antibiotics.

Ultrasonographic Findings

Ultrasonographic examination gave additional information on the status of the udder and teats. It showed specific findings for some causal agents. In buffaloes with subclinical mastitis, the teat canal and sinus showed irregular contour, the three layers of teat wall couldn't be clearly demarcated and the papillary duct and rosette of Furstenberg showed an overlapped pattern with clarity image of udder parenchyma and gland sinus (Fig. 2a).

The milk alveoli showed anechoic fluid with suspended hypoechoic dots before milking (Fig. 2b). After milking, the echogenicity of milk alveoli increased (Fig. 2c).

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	

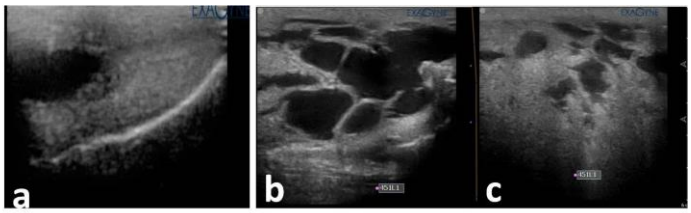

Fig. 2. Showing changes in the udder with subclinical mastitis before and after milking.

In clinical mastitis, the affected buffaloes showed various ultrasonographic images according to the causative agents. Ultrasonographically, the teat wall was thick and lost its threefold layered appearance, complete obstruction of teat canal and disappearance of rosette of Furstenberg (Fig. 3a). The teat cistern had irregular lining and filled with homogenous hypoechoic milk (Fig. 3b). Milk alveoli in buffaloes suffered clinical mastitis caused by pyogenic bacteria, appeared as anechoic cavities filled with homogenous hypoechoic fluid (Fig. 3c). In case of clinical mastitis caused by *Staph.aureus* mixed with Candid spp., the milk alveoli had hypoechoic fluid with suspended hyperechoic flakes (Fig. 3d).

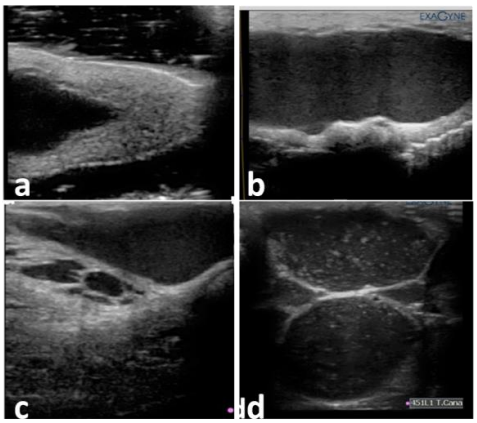

Fig. 3 a. Showing changes of the teat and teat cistern in clinical mastitis.
b. Milk alveoli in clinical mastitis.
c. Showing alveoli in case of pyogenic clinical mastitis.
d. Showing alveoli in case of clinical mastitis caused by *S. aureus* mixed with Candid spp.

Several buffaloes with clinical mastitis developed multiple parenchymatous abscesses (Fig. 4a). 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 (Fig. 4b) and the udder parenchyma showed multiple abscesses filled with hyperechoiccaseated pus and surrounded by hyperechoic thick capsules (Fig. 4c). The parenchymatous abscesses caused by Staph. aureus were less echogenic than that caused by *C. pyogen* (Fig. 4d).

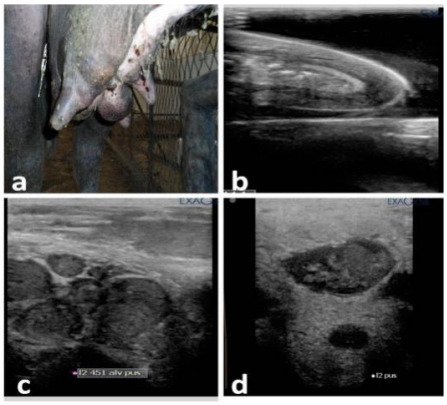

Fig. 4 a. showing multiple parenchymatous abscesses in affected buff aloe.
b. revealed complete obstruction of the teat canal and cistern with hypeechoic mass.
c. showing that The parenchymatous abscesses caused by *S. aureus* were less echogenic than that caused by the *C.pyogen* a.
d. the udder parenchyma showed multiple abscesses filled with hyperechoic caseated pus and surrounded by hyperechoic thick capsules in case of *C.pyogen* infection.

Udder atrophy and fibrosis was recorded in four quarters of three mastitic buffaloes (Fig. 5a) Ultrasonographically, the affected teat showed complete disappearance of teat canal and cistern and diffuse hyperechoic small cordial echoes were present (Fig. 5b) The affected udder parenchyma showed complete replacement of the milk alveoli with hyperechoic fibrous tissue (Fig. 5c) Ultrasonography of the other normal quarters showed homogenous and hyperechoic glandular tissue with anechoic milk alveoli.

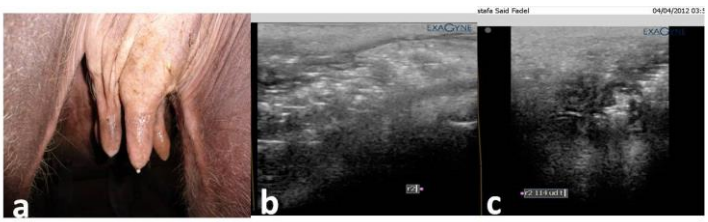

Fig. 5. showing Udder atrophy and fibrosis

Discussion

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.

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.,* 2004, Santos *et al.,* 2004 ,Gungor *et al.,* 2005 and Riekerink *et al.,* 2007). According to (Reneau, 1986) this variation is associated with differences in the magnitude of cellular response and duration of intra mammary infection.

In the present study, some contagious micro-organisms were isolated from mastitic buffaloes as *S.aureus* and *C. pyogen* either alone or mixed with some environmental micro-organisms which indicate bad hygiene. These results were in agreement with that recorded by (Karyak *et al.*, 2011).

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 is a helpful tool to diagnose pathologic alterations of the udder such as inflammation, mucosal lesions, hematoma, and abscess. In addition, ultrasonography of the teat allows the localization and demarcation of the extent of pathologic changes that help in the decision of treatment or culling of affected animals in dairy farms (Franz *et al.,* 2009, Fasulkov *et al.,* 2010 and Maki *et al.,* 2011)

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. Similar findings were mentioned by (Cartee *et al.,* 1986, Bruckmaier & Blum, 1992, Rambabu *et al.,* 2008 and Szencziova & Strapak, 2012). Physiological teat canal sonograms were presented as a thin, white, hyperechoic line circumscribed on each side by parallel hypo- to anechoic bands as mentioned by (Franz *et al.,* 2001).

In subclinical mastitis, the teat canal and sinus showed irregular contour, the three layers of teat wall couldn't be clearly demarcated and the papillary duct and rosette of Furstenberg showed an overlapped pattern with clarity image of udder parenchyma and gland sinus. This could be explained by slight inflammation of these structures. This is in agreement with the results mentioned by (Dinc *et al.,* 2000).

It was noticed that the echogenicity of milk alveoli in buffaloes suffered subclinical mastitis increased after milking. 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. In the present study, ultrasound examination of mastitis caused by pyogenic bacteria showed diffuse hypoechoic fluid inside the milk alveoli due to formation of pus. Although, in case of mastitic buffaloes caused by *Staph. aureus* and Candida spp., the milk alveoli filled with hypoechoic fluid with suspededhyperechoic flakes representing the mycotic hyphae.. Histopathologically, (Thompson *et al.,* 1978) recorded similar finding in udder with mycotic mastitis.

The properties of each udder abscess (concentration of pus and formation of the abscess wall) could be evaluated by ultrasonography. In several cases, clinically non-palpable abscesses due to their small-sized or deep localization were able to be 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 appeared ultrasonographically as hyperechoic cordial bands representing the fibrous tissues that replacing the glandular tissues whereas, the other normal quarters appeared as homogenous and hyperechoic with anechoic alveoli. This is in agreement with (Rambabu *et al.,* 2008).

Conclusion

Ultrasonography gives additional information on the status of the udder and teat that help in decision of culling and replacement infected animals or treatments. All forms of mastitis require microbiological confirmation for final diagnosis. Periodic examination for detection new cases of mastitis is recommended.

Acknowledgment

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تشخيص التهاب الضرع في الجاموس بالفحص المعملي والموجات الصوتية

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التهاب الضرع من الامرض التي تسبب خصارة اقتصادية كبيرة لمزارع الانتاج الحيواني وهو غالبا ينتج عن غزو ميكروبي للضرع يسبب تغيرات باثولوجية في الغدة اللبنية مما يؤثر علي كمية ونوعية اللبن الذي قد يكون له تأثير علي صحة الأنسان وانتشار الأمراض المشتركة وقد تمت دراسات كثيرة عن اسباب و طرق تشخيص التهاب االضرع في المزرعة والمعمل لم تتناول امكانية استخدام الموجات الصوتية كطريقة سهلة وسريعة تبين التغيرات الباثولوجية المستديمة او المؤقتة التي تستجيب للعلاج ومن ثم فان هذه الدراسة تلقي الضوء علي امكانية استخدام الموجات الصوتية مع الأختبارت الاخرى مثل الفحص البكتريولوجي لتقييم مدي التغيرات الباثولوجية المستديمة أو المؤقتة التي تحدث للضرع نتيجة الاصابة ومدي فعالية العلاج حيث يمكن فحص الضرع قبل وبعد العلاج مما يساعد في برامج الأحلال والاستبدال علي اسس علمية صحيحة .

تمت هذه الدراسة علي ٥٥ من الجاموس الحلاب منها ٢٥ مصابة بالتهاب الضرع الظاهرى ٣٠ مصابة بالتهاب الضرع الغير ظاهرى حيث فحصت في المزرعة فحصا ظاهريا واجري اختبار الكاليفورنيا وتم اخذ عينات للفحص البكتريولوجي وعد الخلايا الجسيمية .اظهرت هذه النتائج عزل اكثر من ميكروب في معظم العينات حيث تم عزل الميكروب العنقودى مع ميكروبات الكولي فورم بنسبة ٣٨ ٪ وقد كانت اكثر حساسية للمضاد الحيوية ماربوسيل ونورفلوكساسين والجنتاميسين و الميكروبات السبحية والكولي فورم بنسبة ٢٢ ٪ وقد كانت اكثر حساسية للمضاد الحيوية الاموكسيللين و الجنتاميسين والكلورمفنيكول والميكروب العنقوى الذهبي مع الميكروب السبحي بنسبة ١٦٪ وقد كانت اكثر حساسية للمضاد الحيوية ماربوسيل ونورفلوكساسين وسيبروفلوكساسين والميكروب الكوريني بيوجين مع الكولي فورم والميكروبات السبحية بنسبة ١٥٪ وقد كانت اكثر حساسية للمضاد الحيوية ماربوسيل و الجنتاميسين والميكروب العنقودى الذهبي بنسبة ٨٪ وقد كانت اكثر حساسية للمضاد الحيوية ماربوسيل و النورفلوكساسين والميكروب الكوريني بيوجين بنسبة ١٪ وقد كانت اكثر حساسية للمضاد الحيوية الجنتاميسين من عينات حالات التهاب الضرع الظاهرى.

اما حالات التهاب الضرع الغير ظاهري فقد تم عزل الميكروب العنقودى الذهبي مع الميكروبات السبحية بنسبة ٢٠٪ وقد كانت اكثر حساسية للمضاد الحيوية ماربوسيل ونورفلوكساسين وسيبروفلوكساسين بمتوسط عد للخلايا الجسيمية ٣٢٠٠٠٠ خلية /مل وكذلك تم عزل الميكروبات البيئية وكانت اكثر حساسية للمضاد الحيوية اموكسيللين والجنتاميسين و الكلوامفينكول بمتوسط عد للخلايا الجسيمية ٤٧٦٠٠٠ خلية /مل. اشتركت حالات التهاب الضرع الظاهرى والغير ظاهرى في وجود الكانديدا بنسبة ٣٣٪ و ٣٨,٨٪ علي التوالي.

استخدم جهاز الموجات الصوتية في فحص الحلمات ونسيج الضرع للحيوانات تحت الدراسة المصابة بالتهاب الضرع حيث اظهر التغيرات المؤقتة و المستديمة التي حدثت بالانسجة مما ساعد علي اتخاذ القرار بالعلاج او الاستبعاد .