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Review Article
Current Status and Prospect of Ultrasonographic Application in Buffaloes

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Abstract
Unlike cattle, there is a shortage of literature on ultrasonographic findings in both normal and diseased organs in buffaloes. Although ultrasonography has been used as a diagnostic imaging technique in small animal practice for several decades, ultrasound examination in buffaloes just attracted the attention of scientists in the last decade. Recently, various studies have been conducted in both normal and diseased buffaloes particularly those suffering from traumatic reticuloperitonitis, traumatic pericarditis, diaphragmatic hernia, omasal impaction, intestinal obstruction, endocarditis, thoracic, hepatic and abdominal abscesses, bronchopneumonia, lung consolidation, pleural effusion, pulmonary emphysema, pleuritis, mastitis and urolithiasis. Moreover, ultrasound is of tremendous value and provides much promise as a tool of dairy buffalo reproductive management and research. Several physiologic and pathologic conditions of the ovaries and uterus, not accurately diagnosed by rectal palpation, can easily be confirmed by ultrasound and consequently, appropriate therapies can be applied. Based on the results of the available studies, ultrasonography seems to be a valuable tool for determination of various physiologic and pathologic conditions in buffaloes. Additionally, this technique is a decision-making tool in surgery in buffaloes. Several ultrasonographic differences are recorded in buffaloes compared to cattle. Therefore, more studies should be conducted to evaluate the reliability of ultrasound in both normal and diseased uninvestigated organs in buffaloes particularly spleen, abomasum, kidneys, joints and tendons.

Key words: Buffalos, diaphragmatic hernia, mastitis, pericarditis, traumatic reticuloperitonitis, ultrasonography

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The buffaloes have been an important part of livestock agriculture in Asia since 5000 years, producing milk, meat, hides and draft power (Nanda and Nakao, 2003; Abu-Seida and Al-Abbadi, 2015). In several countries, the buffalo is the principal dairy animal and plays an important role in the socioeconomic development of rural Asia (Abu-Seida and Al-Abbadi, 2014; Al-Abbadi *et al*., 2014).

Ultrasound examination is a non-invasive, valuable diagnostic imaging technique commonly used in both small animals (Abu-Seida and Torad, 2012; Younis *et al*., 2014) and large animals practice (Fouad *et al*., 2001; Abu-Seida *et al*., 2012; Mostafa *et al*., 2014a, b).

Unlike cattle, the veterinary library is suffering from remarkable scarcity of comprehensive literature about ultrasonographic findings in both normal and diseased buffaloes. Thus, the current review describes the current practical applications and prospect of ultrasonography as a diagnostic imaging technique for the evaluation of different physiological and pathologic conditions in buffaloes.

Examination of the reproductive organs: Nowadays ultrasonography is a widely recognized key tool in bovine reproductive management and research due to its simplicity and reliability. Several ovarian and uterine pathologies, not accurately detected by rectal palpation, can easily be diagnosed with this technique. Moreover, the dramatic development of portable ultrasound machines has provided the veterinarians an additional tool for management of several reproductive conditions. However, continuing education programs to train veterinarians on the uses of ultrasound in different reproductive conditions remain a critical step toward rapid development of this technology in dairy buffaloes.

Mostly, the reproductive organs in buffaloes are scanned per rectum using real-time, B-mode ultrasound scanners connecting with a 5.0-7.5 MHz frequency linear-array transducer. However, a transvaginal approach using a sector transducer is applied for ovum pickup and follicle ablation.

Ultrasound is used for determination of ovarian status, onset of puberty, follicular status for diagnosis or pharmacological treatments (Fig. 1a), ovulatory follicles, ovulation time and anovulatory conditions, Corpus Luteum (CL) status, stages of the estrus cycle, optimal time for artificial insemination, oocytes recovery through ultrasound guided Ovum Pick-up and recipients testing for MOET programmes (Manik *et al*., 2002a; Karen and Darwish, 2010; Gimenes *et al*., 2011; Yindee *et al*., 2011). Ultrasonography reveals that ovarian follicular turnover during an unstimulated oestrous cycle in buffalo is similar to that observed in cattle and is characterized by waves of follicular recruitment, growth and regression. The number of follicular waves during an oestrous cycle can vary from 1-3 in buffaloes. Although, 4 waves were recorded in cattle, no report of 4 follicular waves was recorded in buffaloes. These follicular waves consist of a group of anechoic antral follicles begin to grow to 4 mm then one of them grows to a dominant follicle, while others become subordinate follicles and initiate a process of atresia. In a cycle with three waves, the waves emerge, on average, at days 1, 9 and 16 after ovulation. The maximum size of each dominant follicle is around 15 mm (Terzano, 2012). Wave-like patterns of follicular growth were imaged by ultrasonography, in pre-pubertal Mediterranean Italian buffaloes at 5-9 months of age, in primiparous and pluriparous Mediterranean Italian buffaloes postpartum and during pregnancy (Presicce *et al*., 2005).

Ovarian follicles appear as anechoic circular structures surrounded by echogenic ovarian tissue. Most veterinary grade ultrasound scanners can image the ovarian follicles with a diameter of 2-3 mm or greater. Ovulation is defined when the previously ultrasonic identified follicle (>9 mm) disappeared on a subsequent ultrasound scan and subsequently confirmed by the development of corpus luteum at the same spot.

A developing CL appears on the ultrasound image as a poorly defined, irregular, greyish-black structure with echogenic spots. A mid-cycle CL is a well defined granular, greyish echogenic structure with a demarcation line visible between it and the ovarian stroma. Regressing CL has faint demarcation line. Most of corpora lutea in early diestrus tend to have a fluid filled lumen versus the corpora lutea during late diestrus and advanced stages of pregnancy. Luteal size and echogenic characteristics assessed by ultrasound at specific times post breeding may improve accuracy of early pregnancy diagnosis in dairy buffaloes.

The ultrasonographic findings of the follicle, corpus leutum and tubular genitalia during different stages of estrous cycle in buffaloes (Vecchio *et al*., 2012; Yotov and Atanasov, 2013) are similar to those reported in cows by Pierson and Ginther (1988). However, ultrasound application reveals that the number of follicles recruited into a follicular wave is lower in buffaloes than in cattle. Buffalo heifers also tend to have a greater rate of follicle atresia (67%) relative to cattle (50%). The number of antral follicles in swamp buffaloes was reported to be only 20% of antral follicles in cattle. The average number of non-atretic follicles (>1.7 mm) was 3 for buffaloes and 22 for cattle (Presicce *et al*., 2005).

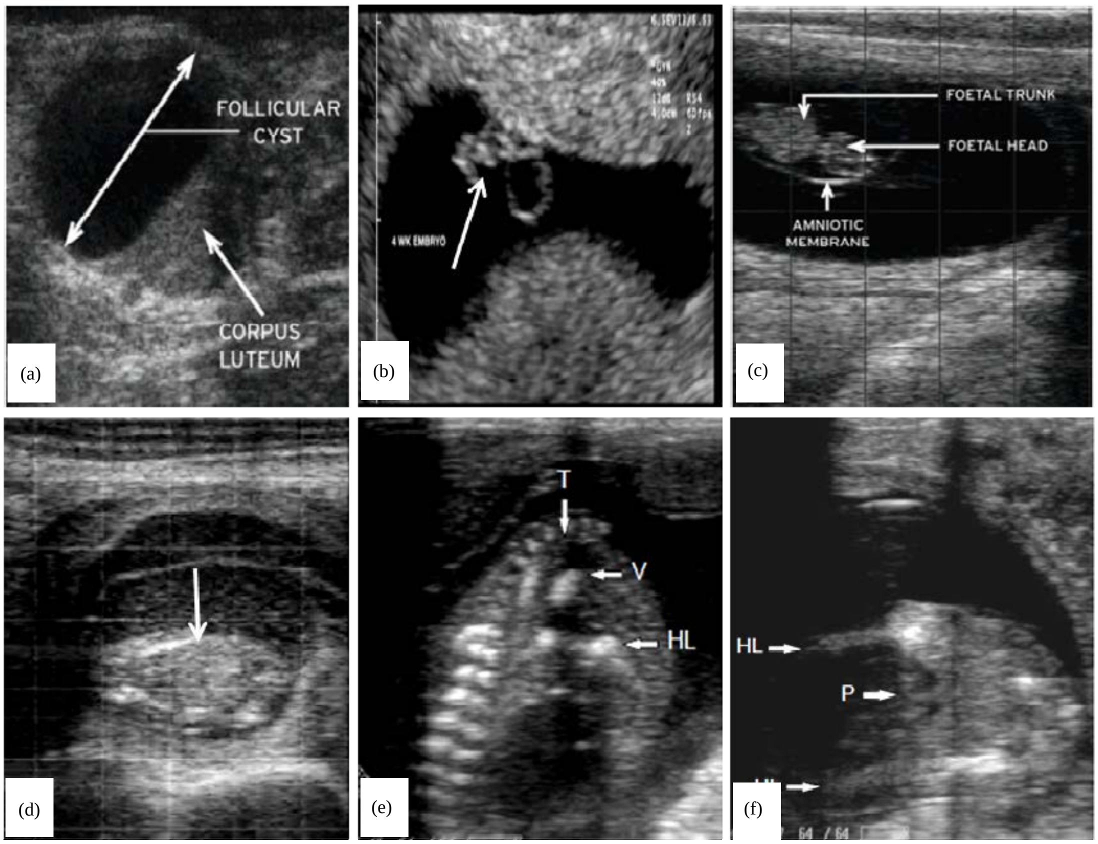

Fig. 1(a-f): (a) Ultrasonogram of ovary with a follicular cyst and corpus luteum (Palgrave and Cezon, 2010), (b) Ultrasonogram of a 26 day pregnancy showing embryo (arrow) (Terzano, 2012), (c) Ultrasonogram of a 38 day pregnancy (Terzano, 2012), (d) Uterine ultrasonogram showing flocculent material in the pregnancy fluids and dead fetus (arrow) (Terzano, 2012), (e) Ultrasonogram of a female foetus at 13th gestation week, HL: Hindlimb; V: Vulva; T: Tail (Yotov *et al*., 2011) and (f) Ultrasonogram of a male foetus at 12th gestation week, HL: Hindlimb; P: Penis (Yotov *et al*., 2011)

Differentiation between follicular and luteal cysts by rectal palpation is difficult; however, accuracy of diagnosis increases when transrectal ultrasonography with correct identification is used (Farin *et al*., 1990). Moreover, sector scan ultrasonography is more effective for diagnosing luteinized cysts and color Doppler sonography is superior to B-mode sonography for differentiating follicular and luteal cysts. The sonographic difference between the two types of cysts is the appearance of distinct hypoechoic wall (2.5 mm) of luteal tissue in luteal cyst. The diameters of ovarian cysts vary from 30- 48 mm. The sonographic features of cysts are simple round or ovoid anechoic fluid filled structures with thin walls. Sensitivity of ultrasound examination for correct diagnosis of ovarian follicular and luteinized cysts was 93.1 and 91.5%, respectively. The technique of Ovum Pick-Up (OPU) in buffalo, as in cattle, consists in the transvaginal recovery of the oocytes by follicular aspiration under ultrasound guide followed by *in vitro* fertilization and culture of the fertilized oocytes (Manik *et al.,* 2002b).

Ultrasonography is indicated for early pregnancy diagnosis (Fig. 1b and c), embryo growth characterization, foetal viability (Fig. 1d), sexing (Fig. 1e and f), number and age determination, assessment of post partum uterine involution and embryonic death rate (Beal *et al*., 1992; Singh *et al*., 1997; Ali and Fahmy, 2008; Terzano, 2012). There are several ultrasonographic indicators of foetal death including: lack of foetal heart beat/foetal movement, presence of flocculent material in the amniotic or chorioallantoic fluid and separation of the chorioallantois from the uterine wall (Terzano, 2012).

Several applications of transvaginal ultrasound-guided puncture include; *In Vitro* Embryo Production (IVEP) technologies, sex determination by aspiration of fetal fluids, hormonal and biochemical analysis; collection of uterine, luteal and follicular samples and injection of various substances into the ovaries, follicles and uterus (Manik *et al*., 2002b; Techakumphu *et al*., 2004). Transvaginal ultrasound-guided amniocentesis followed by PCR analysis of aspirated cell DNA can be used accurately to determine fetal sex in bovine at 70-100 days of gestation. This technique requires considerable skill and is not without some risk to fetal viability (Gupta *et al.,* 2006).

Scanning of the uterus for infection and pregnancy diagnosis is the most common application of ultrasound. In non-pregnant cycling buffalo cows, the uterus appears as a somewhat echogenic structure on the screen. Whereas the longitudinal section of uterus is less recognizable, a "rosette" structure is easily distinguished from other surrounding organs on the cross-sectional view of the uterus. In addition, a scoring system has been developed to describe the changes in uterine echogenicity during different stages of estrous cycle (Pierson and Ginther, 1988). Characteristic ultrasonographic uterine changes involve thickness of the uterine body, evidence of increased vascularity, edema and accumulations of mucus. The period of proestrus and estrous days -4 to -1 (day 0 = ovulation) is characterized by increasing thickness of the uterine body, 2) accumulation of luminal fluid in the uterus then in the cervix and vagina and minimal curl of the uterine horns. Conversely, diestrus, (day 3-16) is characterized by minimal thickness, minimal luminal fluid and maximal curl to the uterine horns. Heterogeneous endometrial echotexture is reflective of uterine edema and associated with impending estrus and ovulation (Ali *et al.,* 2009).

Ultrasound is a rapid diagnostic method for pregnancy diagnosis in buffaloes. The bovine fetus can be imaged beginning at 20 day post breeding and continuing throughout gestation. It is difficult to image fetus after about 90 days due to its movement beyond the penetration depth of the ultrasound waves. The sensitivity of transrectal ultrasonography in pregnancy diagnosis at days 19-24 is 44.4%, reaching 100% from day 31 after mating (Karen *et al*., 2007). There is great feasibility and value of ultrasonographic fetometry in buffaloes for evaluation of fetal development, age and sexing. Embryo and Amniotic Vesicle (AV) are well visualized by the fourth and fifth week of pregnancy, respectively. Organization and ossification are observed by the seventh and between eighth and 10th week, respectively (Ali and Fahmy, 2008).

The best window for fetal sexing is located between the 10th and 18th week of gestation, with an overall accuracy of 97.1% (Ali and Fahmy, 2008). The gender of foetuses can be detected by visualization of the morphology and location of the genital tubercle or the scrotum and mammary glands. The transducer must be manipulated within the rectum to provide a frontal, cross-sectional or sagittal image of the ventral surface of the fetus (Yotov *et al*., 2011). The tail and umbilicus serve as excellent landmarks when determining the location of the genital tubercle or the presence or absence of the scrotum. In the male, the genital tubercle is located adjacent to and caudal to the umbilicus, whereas the genital tubercle in the female is located just ventral to the tail. The scrotum is detectable between the hind legs of the male fetus. Both scrotum and genital tubercle are echogenic structures. There are no appreciable differences in the hyperechogenic image of the buffalo genital tubercle from the cattle genital tubercle (Terzano, 2012). There are two limitations that may inhibit the ability of fetal sexing including; the continued increase in the fetal size that makes the movement of transducer relative to the fetus to obtain the desired image difficult and descending of the gravid horn into the abdominal cavity in larger or older animals.

Fetal viability is accurately detected at 22 days of age when the heartbeat of a fetus can be assessed. Meanwhile, the number of fetuses can most accurately be detected at 49 and 55 days of gestation (Davis and Haibel, 1993).

Recently, use of transrectal Doppler ultrasongraphy has been introduced for assessment of the uterine blood flow during pregnancy in buffaloes. Thus hemodynamic changes in complicated pregnancy as abnormalities in uterine blood flow/placenta/fetus and its abnormalities have been confirmed (Varughese *et al*., 2013).

The Combined Thickness of Uterus and Placenta (CTUP), as a method to assess fetal development and/or placental function in bovine, is imaged by transrectal ultrasound in buffaloes (Atanasov *et al*., 2012). The CTUP increases monthly from 2.5 mm at the second month to 12 mm at the full term. The monthly increase in the CTUP is higher in last trimester than first and second trimesters (Zaher *et al*., 2012).

As regards uterine pathology, speedy diagnosis of several uterine abnormalities including; abscesses, tumors, adhesions, hydrometra, mucometra, unviable, abnormal uterine fluids and pyometra has been conducted by ultrasound. Distended uterus with echogenic uterine fluid is the main ultrasonographic finding in pyometra (Ali *et al*., 2009). In addition, ultrasound can be used to determine the type of intrauterine fluid, purulence or mucous and detect the little amount of fluid in subclinical metritis. Ultrasound can also identify the origin of vaginal discharges, either due to metritis or vaginitis.

Color Doppler sonography of middle uterine artery is helpful for accurate diagnosis of duration and degree of uterine torsion and concurrently predicting the viability of the fetus and dam due to high systolic flow, absence of early diastolic flow, poor uterine and placentomal blood perfusion (Hussein, 2013). Rarely, fetal anomalies such as multiple fetal heads, schistosomus reflexus, extreme arthrogryposis and fetal ascites have been recorded by ultrasonography.

Testicular ultrasonography can afford veterinarians the opportunity for more precise diagnosis and treatment of numerous infertility disorders (Abu-Seida *et al*., 2015). However, there are limited data regarding the use of ultrasound in buffalo's bulls. Ultrasonography has been used to differentiate between ruptured urethra with scrotal swelling, malignant sertoli cell neoplasm, hydrocele, testicular hypoplasia and scrotal hernia in bulls (Abu-Seida, 2012).

In the future, scientists may discover new practical applications of reproductive ultrasound that will enhance the scientific merit of research and improve the reproductive efficiency in buffaloes.

Examination of the digestive system: In last few years, ultrasongraphy has attracted the attention of scientists in the large animal practice as a diagnostic and prognostic imaging tool for various surgical affections of digestive system.

Transcutaneous ultrasonography of the reticulum can be performed in standing buffaloes without sedation at the area from 6-8th intercostal space with a 3.5-5 MHz transducer. Reticulum is imaged at the ventral aspect of the thorax on the left and right of the sternum as well as the left and right lateral thorax up to the level of elbow (Mostafa *et al*., 2015). Ultrasonographically, the normal reticulum of buffaloes has a smooth contour and appears as a half moon-shaped structure in 85% of the cases and crescent shaped in 15%. Sometimes, the mucosal folds of reticulum appear as irregular echogenic projections extending into the lumen (Mostafa *et al*., 2015). The different layers of the reticular wall usually cannot be imaged and the honeycomb-like structure of the mucosa is not often seen clearly (Abouelnasr *et al*., 2014).

In healthy buffaloes, several reticular features are assessed by ultrasound including; frequency of reticular contraction (4.95 ± 0.15 per 5 min), reticular wall thickness (0.45 ± 0.07 cm), distance between the reticulum and abdominal wall (2.08 ± 0.06 cm), duration of 1st, 2nd and total reticular contraction (2.00 ± 0.12, 3.90 ± 0.22 and 5.90 ± 0.25 sec, respectively), relaxation period of reticulum (60 ± 1.8 sec), amplitude of 1st and 2nd reticular contractions (5.46 ± 0.32 and 17.67 ± 0.32 cm, respectively) (Mostafa *et al*., 2015).

Additionally, ultrasonography is a good imaging technique for early diagnosis of traumatic reticuloperitonitis (TRP) especially in buffaloes because it provides exact information concerning the various sequelae of the disease such as local and diffuse peritonitis, reticular, thoracic and abdominal abscesses, traumatic pericarditis (TP), pleuropneumonia and diaphragmatic hernia (Mostafa *et al*., 2015). Moreover, it helps to determine the exact location and extent of the lesions and suitable site for abdomino and thoraco-centesis. However, few ultrasonographic differences between cattle and buffaloes with TRP have been recorded (Abdelaal *et al*., 2009). Cattle usually suffer from TP as a complication of hardware disease in which the reticulum appears as half moon shaped structure with inflammatory deposits on its serosal surface. Meanwhile, buffaloes are usually affected with acute and chronic local peritonitis and thoracic abscess as complications of TRP. Buffaloes with acute local peritonitis and thoracic abscess show a half-moon shaped reticulum with inflammatory deposits on its serosal surface while buffaloes with chronic local peritonits have a corrugated reticulum with loss of its normal shape. Unlike cattle, buffaloes with thoracic abscess show the same clinical signs of TP and ultrasound is essential for the differential diagnosis (Abdelaal *et al*., 2009). The classical ultrasonographic findings in TRP are reduced or absence of biphasic reticular contractions and deposition of inflammatory materials on serosal surface of the organ (Mohamed and Oikawa, 2007; Mostafa *et al*., 2015) (Fig. 2a).

In buffaloes suffering from local peritonitis, echogenic strands interspersed with anechoic material representing the inflammatory reaction are the main ultrasonographic findings. Moreover, reticular abscesses with different shapes and sizes appear as anechoic to hypoechoic center surrounded by echogenic wall (Fig. 2b). Sometimes, the reticulum moves beyond the penetration depth of the ultrasound waves due to the accumulation of large amount of inflammatory exudates as in diffuse peritonitis (Mostafa *et al*., 2015).

Reduced frequency of reticular contractions and increase of reticular wall thickness are seen in buffaloes with local peritonitis, abdominal and thoracic abscesses, traumatic pericarditis and pleuropnemonia. Meanwhile, the distance between reticulum and abdominal wall was significantly increased in hardware diseased buffaloes due to accumulation of large amount of hypoechoic exudates interspersed by hyperechoic fibrin strands (Fig. 2c). Also the durations of 1st, 2nd and total reticular contractions and the relaxation period are significantly longer in buffaloes with local peritonitis, traumatic pericarditis (except the 1st contraction), abdominal and thoracic abscesses than normal buffaloes. Buffaloes with traumatic pericarditis, local peritonitis, abdominal and thoracic abscesses have significantly lower amplitude of 1st reticular contraction than the normal. Buffaloes with all recorded complications of TRP had significantly lower amplitude of 2nd reticular contraction than healthy buffaloes (Mostafa *et al*., 2015).

Interestingly, the ingested metallic foreign bodies are detected by ultrasound as hyperechogenic structures with

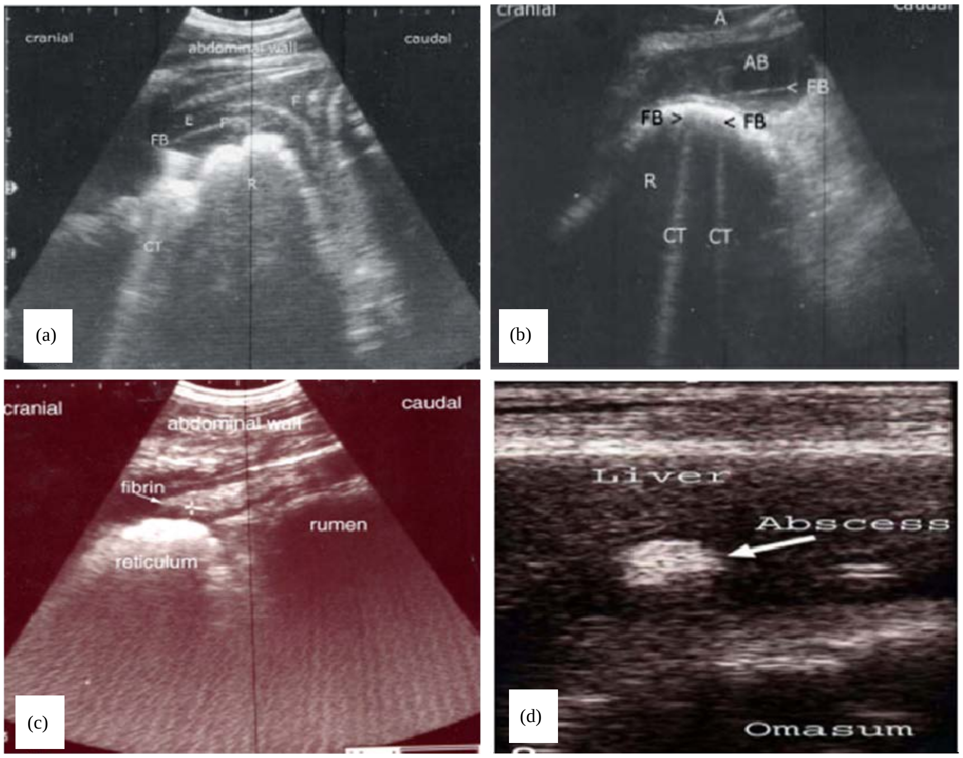

Fig. 2(a-d): (a) Ultrasonogram of the Reticulum (R) of a buffalo with hardware disease showing corrugated reticular wall, hyperechoic Foreign Body (FB) perforating the wall with comet tail artifacts (CT) and hypoechoic Exudates (E) interspersed with hyperechoic fibrin strands (F) inbetween the reticulum and abdominal wall, (b) Ultrasonogram of the Reticulum (R) of a buffalo with hardware disease showing cross sections of two hyperechoic FB perforating the reticular wall with CT and longitudinal section of a hyperechoic foreign body inside a hypoechoic abscess (AB) in between the reticulum, abomasum and abdominal wall (A) (Abdelaal and Floeck, 2015), (c) Ultrasonogram of a buffalo with acute local peritonitis showing the half moon shaped reticulum and echogenic fibrinous strands between the reticulum, rumen and abdominal wall (Abdelaal *et al*., 2009) and (d) Hepatic ultrasonogram showing a single circumscribed hyperechoic abscess (arrow) (Abdelaal *et al*., 2014a)

comet tail artifact or with acoustic shadows in hardware diseased buffaloes (Fig. 2b). Abdelaal and Floeck (2015) reported acoustic shadows only in foreign bodies located near the abdominal wall.

Omasal ultrasonography is conducted in both normal and diseased buffaloes (Buczinski, 2008; Mohindroo *et al*., 2008) using a 3.5 MHz microconvex transducer. Ultrasonographically, in healthy buffaloes, omasum is seen as a round or oval structure with thick echogenic wall and echogenic leaves that can be imaged at right 8-9th intercostal spaces. Also, gradual slow movements of omasal leaves can be seen in real-time B-mode ultrasound. The omasum appears clear, large and close to the transducer at the start of the omasal contraction and as the contraction progresses, the omasum retracts away from the transducer and becomes small (Mohindroo *et al*., 2008). In contrast, cattle have a crescent-shaped omasum with an echogenic wall, the omasal contents and leaves cannot be visualized and omasal contractility isn't as prominent as in buffaloes. Ultrasonography is a helpful tool to diagnose omasal impaction but its severity cannot be evaluated. In buffaloes, the impacted omasum appears non-motile with invisible omasal leaves and a clear distal acoustic shadow (Mohindroo *et al*., 2008). However, the diagnosis of omasal impaction in cows depends mainly upon the appearance of non-motile omasum covering a large area on the right side of abdomen.

Hepatic ultrasonography has been used for diagnosis of hepatic lipidosis, abscesses and fascioliasis in buffaloes. Ultrasonographically, hepatic lipidosis shows either increased or decreased hepatic echogenicity with unclear liver boundaries (Tharwat, 2012a). However, hepatic abscess appears as hypoechoic to echogenic circumscribed mass, pinpoint to 10 cm in diameter, with or without echogenic wall

(Fig. 2d). These abscesses are presented either intra-hepatic or peri-hepatic in feedlot buffaloes (Abdelaal *et al*., 2014a). Moreover, the main ultrasonographic findings in buffaloes and cattle with chronic hepatic fascioliasis include; bile duct mineralization, various degrees of edema of the gallbladder wall, distended gallbladders with homogenous contents, heterogeneous and hyperechogenic hepatic parenchymas with multiple echogenic foci. Other ultrasonographic findings such as peritoneal, pleural and pericardial effusions have also been imaged in diseased animals (Tharwat, 2012b).

Ultrasound can be used as a decision-making tool in cattle and buffaloes with intestinal obstruction. On ultrasonographic examination of intussusception, the invaginated intestinal wall is swollen and the affected area of intestine appears hyperechogenic in cross section. On longitudinal view, a ''sandwich'' configuration of the affected intestine is seen. Other ultrasonographic findings include; increased intestinal diameter, decreased intestinal motility, anechoic intestinal contents and accumulation of hypoechogenic fluid between the dilated intestinal loops (Tharwat, 2011).

Examination of the heart: Echocardiography is performed in standing non-sedated buffaloes properly controlled in a stanchion. Examination is made after 15 min so that the animal is acclimatized to this environment. The examination is performed at a small area just caudal to the elbow (4-5th intercostal spaces) on both right and left hemithorax using 2-4 MHz sector transducer. The two-dimensional B-mode is suitable for assessment of the spatial relationships of cardiac structures, however, M-mode is a good tool for investigating cardiac dimensions and function as well as valve, chamber and septum movements. Brisket edema and distended jugular veins have been reported in both cattle and buffaloes with traumatic pericarditis (TP) and thoracic abscesses. Therefore, ultrasonography is a useful technique to differentiate between these disorders (Abdelaal *et al*., 2009).

Ultrasonographic examination of buffaloes with TP reveals pericardial effusions that appear either as uniform echogenic pus or anechoic to hypoechoic fluid with or without fibrin deposition in the pericardial sac (Fig. 3a-d). Other ultrasonographic findings such as marked hepatomegaly, dilated caudal vena cava and portal vein, displaced gall bladder with edematous and double walled appearance are also seen in buffaloes with TP (Kumar *et al*., 2012). Moreover, perireticular and mediastinal abscesses, moderate to severe corrugation of the reticular wall, deposits of hyperechoic fibrinous tissue interspersed with anechoic fluid between the reticulum, rumen and diaphragm, pleural effusions, ascites and vegetations of the tricuspid, mitral and pulmonary valves are also imaged (Mohamed, 2010).

In addition, ultrasonography is a helpful tool in the evaluation of congestive heart failure in buffaloes. Ultrasonographic examination shows accumulation of massive anechoic fluids in the pericardial sacs, peritoneum and pleura (Mohamed, 2010).

Buffaloes with endocarditis have hyperechogenic valves with nodular or proliferative vegetative lesions (Fig. 3e and f). The most commonly affected valves are the tricuspid, then mitral and pulmonary valves. Additionally, hepatomegaly, perireticular abscesses, dilated caudal vena cava and lung consolidations can also be imaged in these buffaloes (Hussein and Staufenbiel, 2014).

Examination of the urinary organs: Ultrasonography is a valuable diagnostic and prognostic tool for various urinary disorders in buffaloes (Khan *et al*., 2013; Saharan *et al*., 2013). Urinary tract ultrasonography either by transcutaneous technique using a 3.5-5.0 MHz convex transducer or transrectal technique using 6-8 MHz lineal transducer allows assessment of urethra, urinary bladder and renal morphology. Transcutaneous examination is usually carried out at the right inguinal region to avoid the ruminal gases at left side. Ultrasonography was a helpful tool in buffalo calves suffered from urine retention with and without urinary bladder rupture, hydronephrosis, nephrolithiasis and urethrolithiasis (Abdelaal *et al.,* 2016).

In ruptured urethra of buffalos bulls, the urethral calculi can be imaged as hyperechoic masses with distant shadowing (Fig. 4a). In addition, the site of urethral rupture is also visualized as disruption in the continuity of the urethral lumen with distant anechoic area (Fig. 4b). The prepuce, scrotum and inguinal areas show cellulitis in the form of diffuse anechoic areas separated by hyperechoic fibrin threads (Fig. 3c). Other ultrasonographic findings include; thick hyperechoic scrotum with normal testicular echo texture (Abu-Seida, 2012).

Uroperitoneum with either intact (Fig. 4d) or ruptured urinary bladder (Fig. 4e) is evident as anechoic fluid accumulation in the abdomen with floating internal organs (Fig. 4f) (Tharwat and El-Deeb, 2015). Moreover, hydronephrosis appears with a significant increase in the size of affected kidney and anechoic appearance of renal sinus and medullary pyramid (Harrison *et al.,* 1992). Interestingly, renal and urethral calculi are observed as hyperechogenic dotes with acoustic shadowing in buffalo calves (Abdelaal *et al.,* 2016).

Examination of the udder and teats: The ultrasound examination of the udder parenchyma is mainly performed

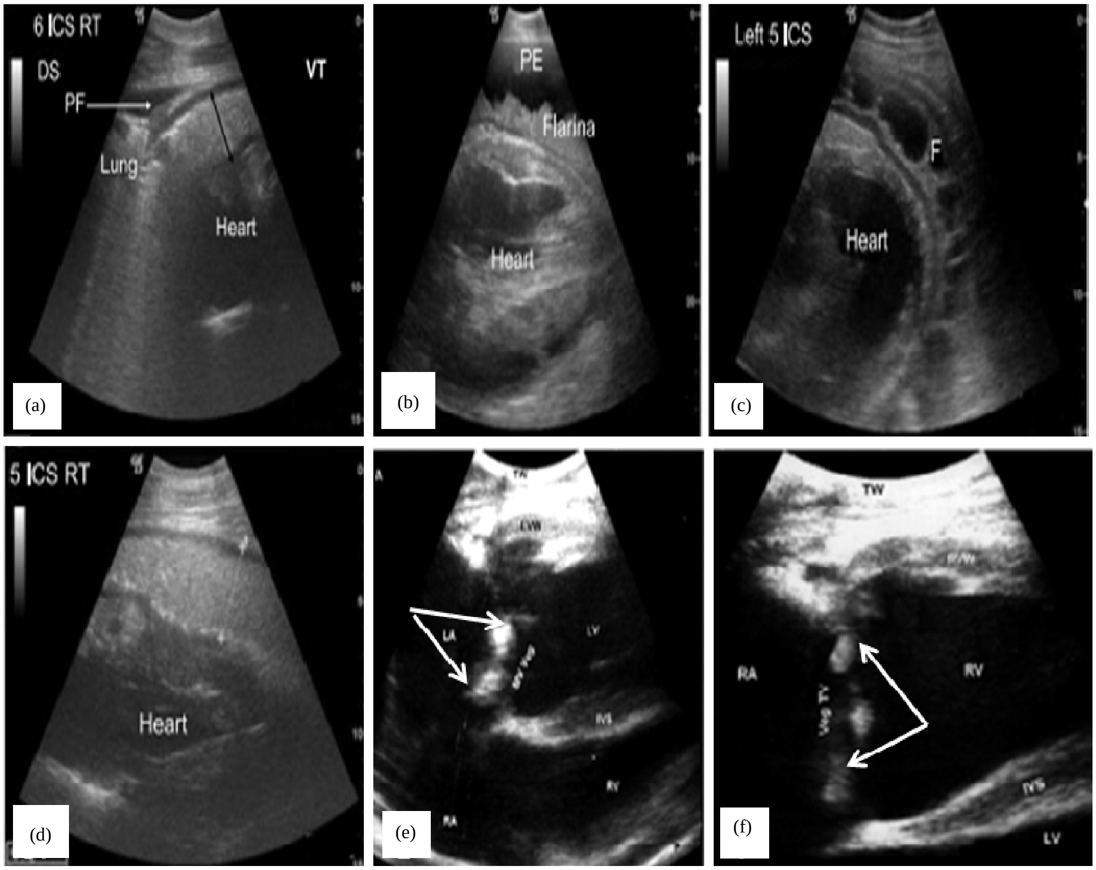

Fig. 3(a-f): (a) Ultrasonogram differentiating Pleural Fluid (PF) from pericardial fluid in a buffalo. DS (Dorsal), VT (Ventral), (b) Echocardiogram in a buffalo with traumatic pericarditis showing anechoic Pericardial Effusions (PE) with hyperechoic fibrin deposition at epicardium, (c) Echocardiogram in a buffalo with traumatic pericarditis showing echogenic fibrin strands connecting epicardium and pericardium with scanty amount of anechoic fluid, (d) Echocardiogram in a buffalo with traumatic pericarditis showing homogenous echogenic pus in the pericadial sac (double arrow) (Kumar *et al*., 2012), (e) Echocardiogram in a buffalo with vegetative endocarditis showing hyperechoic vegetative lesions at the mitral (arrows) and tricuspid valves (arrows) (f) LA: Left atrium, LV: Left ventricle, RA: Right atrium, RV: Right ventricle, IVS: Interventricular septum, LVW: Left ventricular wall, RVW: Right ventricular wall, MV Veg: Mitral valve vegetative lesions, Veg TV: Tricuspid valve vegetative lesions (Hussein and Staufenbiel, 2014)

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 (Rambadu *et al*., 2008). In the water bath method, the udder is cleaned with mild antiseptic solution then dipped in a polyethylene bag filled with water and the transducer is applied in vertical/horizontal planes of the outer wall of the polyethylene bag. A teat is dipped in a water filled condom before the ultrasound examination. Other techniques used for udder ultrasonography in buffaloes including; direct contact, gel application and standoff methods.

Murrah buffaloes have longer hind teat canals than fore teats. Additionally, there is a close correlation between the ultrasound cisternal area and cisternal milk yield. In the ultrasound cross sections, an increase in teat length and circumference at milk ejection is evident (Thomas *et al*., 2004).

Udder and teat ultrasonography of buffaloes with subclinical mastitis show irregular contour of teat canal and sinus, overlapped papillary duct and rosette of Furstenberg, loss of the three layered appearance of the affected teat wall, clarity image of udder parenchyma and gland sinus (Fig. 5a). However, in clinical mastitis; loss of the three layered appearance of the teat wall, thickened teat wall, complete

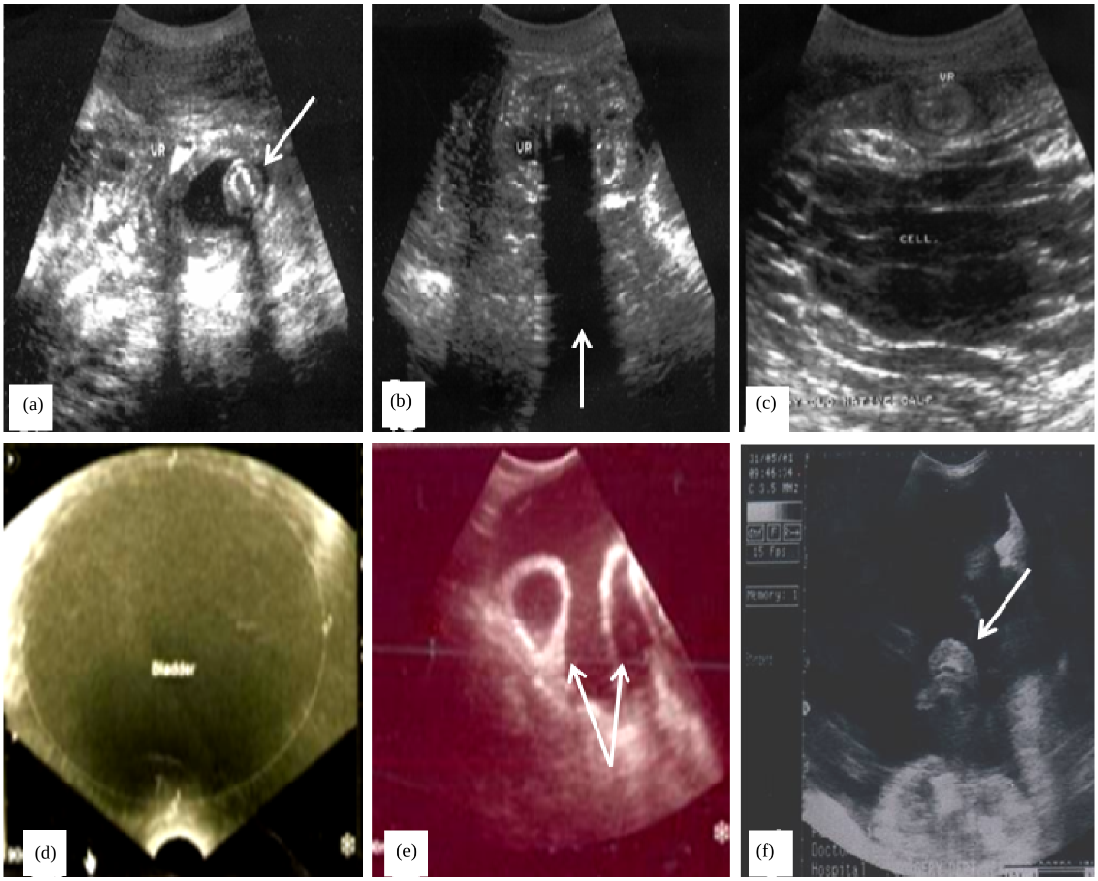

Fig. 4(a-f): (a): Ultrasonogram showing rounded, smooth, hyperechoic urethral calculus (arrow) with distant shadowing, (b) Ultrasonogram showing the site of urethral rupture with distant anechoic area (arrow), (c) Ruptured urethra in a buffalo calf showing diffuse anechoic areas separated by hyperechoic threads at the surrounding tissue. UR: urethra, Cell: cellulitis (Abu-Seida, 2012), (d) Trans-cutaneous ultrasonography of urinary bladder showing anechoic urine with hyperechoic sediments inside the intact urinary bladder (Khan *et al*., 2013), (e) Trans-cutaneous ultrasonogram showing the site of urinary bladder rupture (arrows) with anechoic uroperitoneum (Khan *et al*., 2013) and (f) Abdominal ultrasonogram showing anechoic uroperitoneum with floating intestines (arrow)

obstruction of teat canal (Fig. 5b and c), irregular teat cistern filled with homogenous hypoechoic milk, disappearance of rosette of Furstenberg, anechoic milk alveoli filled with hypoechoic fluid were seen (Kotb *et al*., 2014).

Buffaloes with parenchymatous abscesses show complete obstruction of the teat canal and cistern with hyperechoic materials, multiple abscesses filled with hypoechoic (Fig. 5d) or hyperechoic caseated pus and surrounded by hyperechoic thick capsules (Fig. 5e). However, udder fibrosis reveals disappearance of teat canal and cistern, diffuse hyperechoic small cordial echoes and complete replacement of milk alveoli with hyperechoic fibrous tissues (Fig. 5f) (Kotb *et al*., 2014).

Examination of the respiratory organs: Lungs and pleura could be imaged at both sides of thorax between 3rd and 11th intercostal spaces using 3.5-5 MHz convex transducer. Both pleural leaves appear as a broad, smooth, hyperechoic line between the surface of the lungs and the musculature of the thoracic wall (Fig. 6a) and move synchronously with respiration. It is difficult to differentiate the parietal and visceral pleura. The lung parenchyma could not be evaluated due to its air content. Instead, numerous reverberation artifacts running regularly and parallel to the pleura are characteristic of normal lung (Fig. 6a). The nonventilated lung has a liver parenchyma-like echotexture. Sporadically, fluid bronchograms and anechoic vascular structures are discernible in the hepatized lung areas (Tharwat and Oikawa, 2011).

In buffaloes, thoracic ultrasonography appears to be suitable screening method for diagnosis of pulmonary

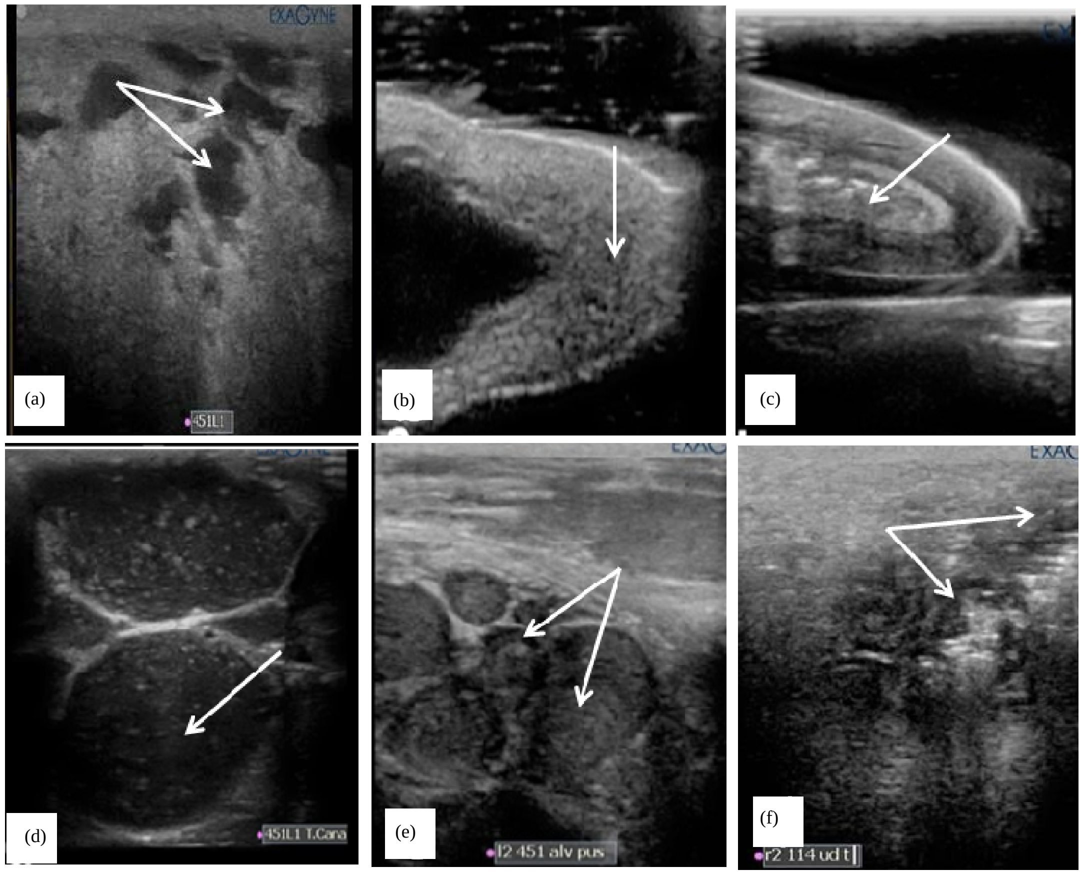

Fig. 5(a-f): (a) Udder ultrasonogram in a buffalo with subclinical mastitis showing increased echogenicity of the fluid inside milk alveoli (arrows), (b) Teat ultrasonogram in a buffalo with clinical mastitis showing; thickened teat wall, loss of the three layered appearance of the teat wall, complete obstruction of teat canal (arrow) and disappearance of rosette of Furstenberg, (c) Teat ultrasonogram in a buffalo with clinical mastitis showing complete obstruction of the teat canal and cistern with hyperechoic caseated materials (arrow), (d) Udder ultrasongram in a buffalo with clinical mastitis showing milk alveoli filled with hypoechoic fluid and suspended hyperechoic flakes (arrow), (e) Udder ultrasongram in a buffalo with clinical mastitis showing multiple parenchymatous abscesses (arrows) and (f) Udder ultrasonogram in a buffalo with udder fibrosis showing complete replacement of milk alveoli with hyperechoic fibrous tissue (arrows) (Kotb *et al*., 2014)

affections near the pleura such as; thoracic abscess, pleuritis, pulmonary emphysema, bronchopneumonia, pleural effusion, lung consolidation and pleuropneumonia. Buffaloes with pulmonary emphysema show numerous comet-tail artifacts in the form of bright, closely situated echo bands starting at the lung surface and running perpendicular to the pleura in the lung tissue (Fig. 6b). Bronchopneumonia is characterized by disseminated round and wedge shaped hypoechoic zones on the surface of the lungs with comet-tail artifacts (Fig. 6c). Severe pneumonia with consolidation could be identified by the weak, defined and blurry reverberation artifacts (Fig. 6d). Ultrasonographic diagnosis of lung abscess is only possible when it locates near the pleura. The pleura appears broadened and hyperechogenic while the lung tissue has medium echogenicity resembling liver parenchyma. In the compressed lung parenchyma, relatively well-defined abscesses could be seen in the form of round to ovoid anechoic areas with a slender reflective capsule (Fig. 6e). In buffaloes with pleuritis and pleural effusion, hypoechoic fluid (Fig. 6f) with echogenic bands is imaged in the pleural cavity (Tharwat and Oikawa, 2011).

Thoracic abscesses are imaged in the left third and fourth intercostal spaces with either echogenic or anechoic contents. These abscesses can be of various sizes (5-15 cm in diameter) and their contents may be partitioned by echogenic septae. The diagnosis can be confirmed by centesis and aspiration of the abscess under the ultrasonographic guidance (Mohamed and Oikawa, 2007).

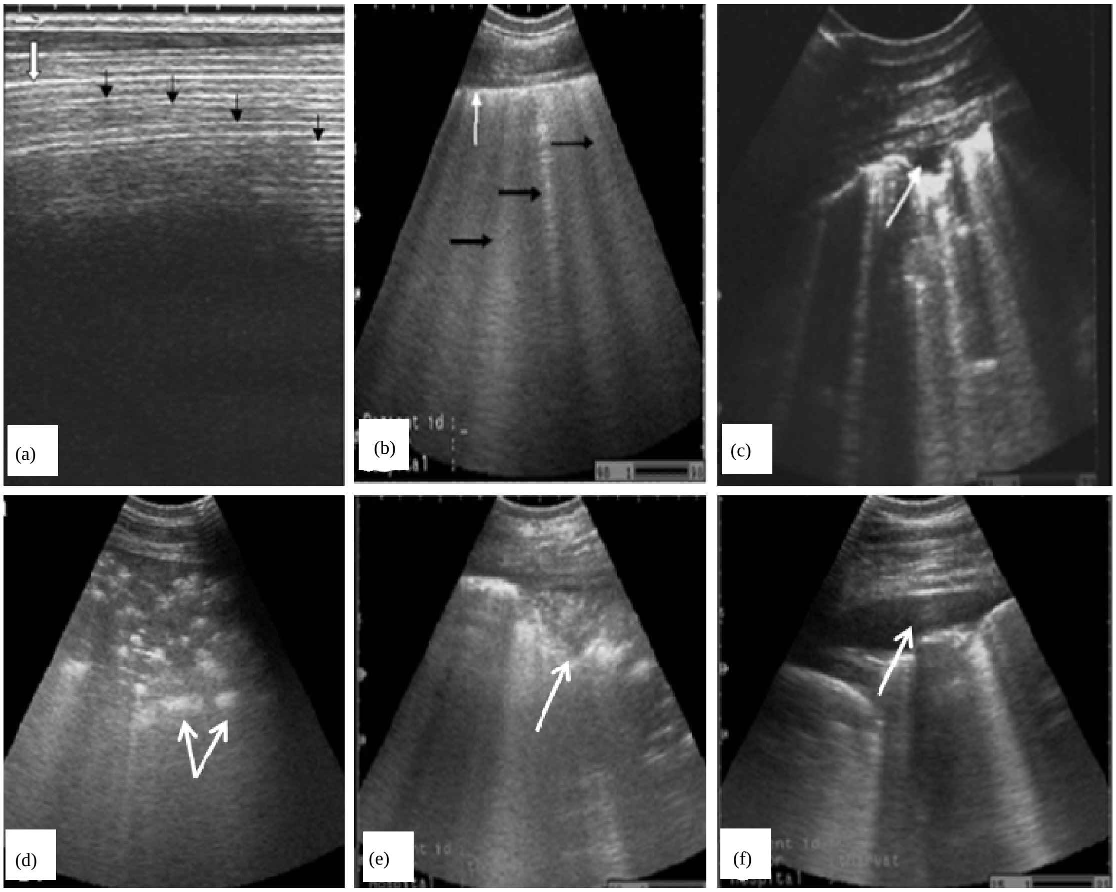

Fig. 6(a-f): (a) Ultrasonogram of the surface of a normal lung showing echogenic line (white arrow) representing the visceral and parietal pleura and echogenic lines running parallel to the pulmonary surface (black arrows) representing the reverberation artifacts, (b) Ultrasonogram of pulmonary emphysema in a buffalo showing numerous echogenic bands (black arrows) from the lung surface representing comet-tail artifacts and pleura (white arrow), (c) Ultrasonogram of bronchopneumonia showing small hypoechoic zones on the surface of the lungs with comet-tail artifact (arrow) "superficial fluid alveolograms", (d) Ultrasonogram of severe pneumonia showing consolidated lung with small hyperechoic pockets of gas remaining and an acoustic shadow (arrows), (e) Ultrasonogram of lung abscess in a buffalo showing a hypoechoic area immediately below the chest wall and bordered by a broad hyperechoic line (arrow) and (f) Ultrasonogram of pleural effusion showing accumulation of anechoic fluid inside the pleural sac (arrow) (Tharwat and Oikawa, 2011)

Examination of the diaphragm: Ultrasonographically, the diaphragm appears as echogenic line between abdominal and thoracic cavities. Diaphragmatic Hernia (DH) is a fatal thoracoabdominal disorder in buffaloes. In the past, the diagnosis of DH was mainly reached at post mortem examination. But now-a-days, both radiography and ultrasonography have made the diagnosis of such affection more accurate and easy (Mohindroo *et al.*, 2007; Athar *et al.*, 2010). Ultrasonographically, reticular motility is evident at the level of 4th/5th intercostal spaces in all buffaloes with diaphragmatic hernia (Kumar and Saini, 2011; Abdelaal *et al.*, 2014b). The reticular contraction is either biphasic, monophasic with reduced contraction frequency or absent. Also, the herniated reticulum is imaged beneath lung or heart with hypoechoic inflammatory adhesion between them (Abdelaal *et al.*, 2014b).

Examination of the eye: The ultrasonographic appearance of the buffalo eye is similar to that in other animal species (Assadnassab and Fartashvand, 2013). The buffaloes, eyes are imaged by transpalpebral technique using a 10 MHz transducer in both horizontal and vertical imaging planes. Ocular ultrasonography is performed with the animal restrained, without the use of sedation or anesthesia/analgesia. Transpalpebral ultrasonographic images are obtained through the skin of the upper eyelid after application of ultrasound coupling gel.

The aqueous humour and vitreous body of the buffalo eyes appear anechoic. Meanwhile, the cornea, iris, anterior and posterior lens capsule appear hyperechoic. Ocular measurements as axial length, vitreous chamber depth and corneal, lens and scleroretinal rim thickness increase with the advance of age (Kassab, 2012).

LIMITATIONS OF ULTRASONOGRAPHIC EXAMINATION

The effectiveness and speed of ultrasonographic scanning depends upon the quality of the ultrasound unit and training available to the operator. Moreover, ultrasonographic application in buffaloes is still limited under field conditions due to the need of external power source, the relatively expensive ultrasound machines and risk of damage of the device by uncontrolled animals. Recently, several cheaper, smaller, battery operated and portable ultrasound machines have been developed.

CONCLUSIONS

Ultrasonography is a useful diagnostic and prognostic tool in diseased buffaloes. Moreover, ultrasound helps in deciding whether the animal should undergo surgical or medical treatment or be slaughtered especially those with traumatic reticuloperitonitis, congestive heart failure, endocarditis and intestinal obstruction. Ultrasound also is an excellent diagnostic imaging tool for identification of hepatic, thoracic and abdominal abscesses, diaphragmatic hernia, various types of mastitis and urolithiasis.

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