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Original article

Ultrasonography as a diagnostic and prognostic approach in cattle and buffaloes with fatty infiltration of the liver

M. Tharwat

Department of Veterinary Medicine, College of Agriculture and Veterinary Medicine, Qassim University, Saudi Arabia
Permanent address: Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt

Abstract

The aim of the present study was to determine whether ultrasonographic evaluation of the hepatic parenchyma could be used as a diagnostic and prognostic approach in cows and buffaloes with hepatic lipidosis. For this purpose, cows (n=16) and buffaloes (n=10) with fatty infiltration of the liver were examined by ultrasonography. Treated cows and buffaloes were monitored for hepatic changes ultrasonographically, biochemically and histologically. Clinical findings were non-specific and included anorexia, recumbency, muzzle necrosis, and icteric mucosal membranes. Laboratory data revealed neutrophilia, hyper γ-globulinemia, elevated activities of aspartate aminotransferase, γ-glutamyl transpeptidase, creatine kinase and lactate dehydrogenase, and high concentrations of insulin, total bilirubin, non-esterified fatty acids and β-hydroxyl butyric acid. Laboratory results 7, and 21 days after treatment showed progressive improvement in the chemistry profile. On admission, ultrasonographic examination of the hepatic parenchyma in cows and buffaloes revealed either increased or decreased hepatic echogenicity; histologic examination revealed marked fatty infiltration of the hepatocytes. One week after treatment, the hepatic parenchyma was visualized easily, liver boundaries were clearly imaged, and histologic examination of hepatic specimen showed a moderate degree of fatty infiltration. Three weeks after treatment, the hepatic parenchyma was almost similar to normal, the hepatic and portal blood vessels could be easily imaged, and the histologic picture had greatly improved where the liver resembled the normal organ. Six cows and seven buffaloes made a full recovery while the remaining ten cows and three buffaloes were slaughtered and thoroughly examined postmortem. Ultrasonography showed a good correlation with histologic and laboratory findings.

Key words: cattle, buffaloes, fatty liver, diagnosis, prognosis, ultrasonography

Correspondence to: M. Tharwat, e-mail: mohamedtharwat129@gmail.com

Introduction

In dairy cows, fatty infiltration of the liver is an increasingly recognized condition that occurs mostly in fat cows, constituting a major cause of liver-related morbidity and mortality (Bobe et al. 2004). Negative energy balance (NEB), decreased dry matter intake (DMI) (especially during the final week before calving), stress, hormonal imbalance, and parturition are important risk factors for the development of hepatic lipidosis in dairy cattle (Mohamed et al. 2004a,b). As a consequence of NEB, cows mobilize fatty acids from adipose tissue causing an increase in the postpartum concentrations of circulating non-esterified fatty acids (NEFA) (Grummer 2008). Unrestricted access to feed in the dry period enhances the release of NEFA postpartum and increases the risk of developing fatty liver because the enhanced formation of triglycerides (TG) easily exceeds the low capacity for very-low density lipoproteins secretion (Oikawa et al. 2010). The severity of hepatic lipidosis can be measured by several methods. Currently, fatty liver can be detected reliably only by using liver biopsy followed by chemical or histological analysis, which is not practical in most on-farm situations. The severity of fatty liver disease can also be evaluated based on the liver function tests such as the serum activities of aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (GGT), and the concentration of total bilirubin.

In cows, ultrasound imaging has been used for detection of liver disorders such as abscesses, tumors, diffuse fatty liver, focal fatty liver, congestion, calcifications of the bile ducts, cholestasis, fibrosis, pneumobilia and thrombosis of the hepatic veins and caudal vena cava (Acorda et al. 1994, Bobe et al. 2008, Braun, 2009). On the other hand, no ultrasonographic studies were recorded in water buffaloes with hepatic dysfunction. The present study was therefore designed: (1) to evaluate hepatic parenchyma in cows and buffaloes with fatty liver, (2) to determine whether ultrasonographic evaluation of the hepatic parenchyma in cows and buffaloes with fatty liver could be used, rather than liver biopsy, as a diagnostic and prognostic indicator, and (3) to show the association between liver sonograms, chemistry profile and histologic examination of the hepatic specimens during treatment follow-up of cows and buffaloes with fatty infiltration of the liver.

Materials and Methods

Animals, history and physical examination

Sixteen cows and ten buffaloes were examined in this study. Nine cows and the ten buffaloes were examined at Zagazig University, Veterinary Teaching Hospital, Egypt from 2004 to 2007. Remaining seven cows were examined at Qassim University, Veterinary Teaching Hospital, Saudi Arabia from 2007 to 2011. Animals were 4 to 6 yr old, weighing 480-670 kg, lactating for the third to fifth season of lactation, had a body condition score between 3.5 and 4.5 based on the 5-point scale (Edmonson et al. 1989). Cows comprised ten Holstein, two Holstein-Friesian, two Friesian and two Braun Swiss. Animals had referred because of inappetance and/or anorexia and decreased milk production. According to the owners, duration of illness ranged from 5-15 days before admission. All animals underwent a thorough physical examination (Radostits 2000).

Hematological and biochemical analyses

Two blood samples were collected from each animal; one in EDTA and the other in plain tubes. Blood samples on EDTA tubes were used for determination of the hematocrit, hemoglobin, erythrocytes and total and differential leucocyte count using automated veterinary haematological analyzer (Vet Scan HM5, ABAXIS, Hungary). The blood samples in plain tubes were centrifuged at 3000 rpm for 10 minutes and serum samples were harvested and frozen at – 20°C for future analysis. Commercial kits were used to determine the concentrations of total protein, albumin, activities of AST, GGT, creatine kinase (CK) and lactate dehydrogenase (LDH) and its isozymes. Serum samples were also used for measuring total bilirubin, total cholesterol, calcium, phosphorus, magnesium, glucose, NFFA, β-hydroxyl butyric acid (BHBA), blood urea nitrogen (BUN), sodium, potassium and chloride. Automated biochemical analyzer (Biosystems A15, Spain) was used for measurement of all serum parameters. Insulin concentration was measured by radioimmunoassay (Itoh et al. 1997). A third venous blood sample was also collected for rapid determination of blood gases. Serum protein fractions were determined by electrophoresis.

Ultrasonography of the hepatic parenchyma and liver biopsy

Liver ultrasonograms were acquired with a real-time ultrasound machines (Pie Medical 240 Parus, The Netherlands and SSD-500, Aloka, Tokyo, Japan) equipped with 3.5 MHz linear and sector transducers. The area between the 5th to 12th ribs was clipped and shaved. The transducer was placed parallel to the ribs and between the dorsal and middle third of the

12, 11, 10, 9, 8, 7, 6 and 5th intercostal spaces. The liver was examined caudocranially and dorsoventrally (Mohamed et al. 2004a,b). Ultrasonic coupling gel was liberally applied to the wet skin to ensure good contact, and the transducer was held firmly against the body wall.

Under ultrasound guidance, liver biopsy was carried out as previously described (Mohamed et al. 2002). Briefly, the procedure is as follows: the areas over the intercostal spaces 7 to 12 on the right side were surgically prepared. All animals were evaluated ultrasonographically for hemorrhage immediately prior to the procedure (Mohamed et al. 2004a,b). Biopsy site was then infiltrated with 10 ml of 2% procaine hydrochloride. Prior to biopsy, a small incision was made immediately adjacent to the transducer through the skin and abdominal wall with the point of a scalpel blade. With a free-hand technique, a 14G × 150 mm spinal biopsy needle (Kurita Co., Ltd, Tokyo, Japan) was advanced through the hepatic parenchyma under direct ultrasound control. When the needle was considered to be in the correct position, the plain stylet was withdrawn and a notched part inserted and advanced. Often the needle can be identified on ultrasound within the hepatic parenchyma while the specimen is being obtained, thus confirming the location of biopsy.

Treatment and follow-up

Starting on the same day of admission, treatment consisted of parenteral glucose injection (500 ml 40% glucose twice a day for 5 successive days), oral propylene glycol administration (250 ml Ketosaid®, Norbrook, Norway, Northern Ireland, twice a day for 5 successive days) and one dose of dexamethasone (20 mg intramuscularly, Dexasone®, Riyadh, KSA). The appetite had greatly improved one week after treatment. The degree of response to treatment was evaluated at three parallel levels: repeated examinations of hepatic ultrasonograms, laboratory tests and histological examination of liver specimens. Liver parenchyma was biopsied (Mohamed et al. 2004b) and specimens were fixed in neutral buffered 10% formalin and processed routinely for staining with hematoxylin and eosin (H&E). The condition of six cows and seven buffaloes has improved gradually and steadily. However, the response of the other ten cows and three buffaloes was frustrated, and therefore the owners preferred slaughter where the animals were thoroughly examined and findings were recorded.

Statistical analysis

Data were presented as means ± SD and the analysis was conducted using SPSS program, version 16.0 (2007). Hematological and biochemical data were evaluated in diseased cows and buffaloes using ANOVA. The level of significance was set at $P < 0.05$.

Results

Data of clinical examination are described in Table 1. Clinical findings were non-specific and included anorexia, recumbency, photosensitization, and icteric mucosal membranes (Fig. 1). Two of the buffaloes had presternal edema (Fig. 2). Results of hematological and biochemical analyses are described in Table 2. Neutrophilia was the only hematological abnormal finding ($P < 0.05$). Other laboratory abnormalities included hyper γ-globulinemia ($P < 0.05$), elevated activities of AST, GGT ($P < 0.01$), CK, LDH ($P < 0.001$) and insulin ($P < 0.05$). Elevated concentrations of total bilirubin, NEFA ($P < 0.01$) and BHBA ($P < 0.001$) were also detected in blood samples. Hypochloremia ($P < 0.05$) was an additional laboratory finding. Laboratory data recorded seven, and twenty-one days after treatment showed progressive improvement in the chemistry profile (Table 2).

Table 1. Clinical, ultrasonographic and postmortem findings in 16 cows and 10 water buffaloes with fatty infiltration of the liver.

Parameters	Number (%) of affected animals
Clinical findings (n=26)	
Inappetance	26 (100)
Reduced ruminal motility	23 (88)
Decreased milk production	21 (81)
Recumbency	12 (46)
Icteric mucus membranes	9 (35)
Scanty feces	7 (27)
Presternal edema	5 (19)
Systemic reactions*	3 (12)
Lameness	2 (8)
Necrosis of the muzzle	1(4)
Ultrasonographic findings (n=26)	
Anechoic hepatic parenchyma	14 (54)
White hepatic parenchyma	12 (46)
Invisible portal and hepatic veins	18 (69)
Decreased diameter of portal and hepatic veins	8 (31)
Dilated gallbladder	10 (38)
Postmortem findings (n=13)	
Hepatomegaly	13 (100)
Distended gallbladder	8 (62)
Icteric carcass	9 (69)
Peritoneal fluids	3 (23)
Omasal ulcer	1 (8)

* Systemic reactions include moderate rise in rectal temperature (39.0-40°C), respiratory rate (35-44/min) and pulse rate (95-108/min).

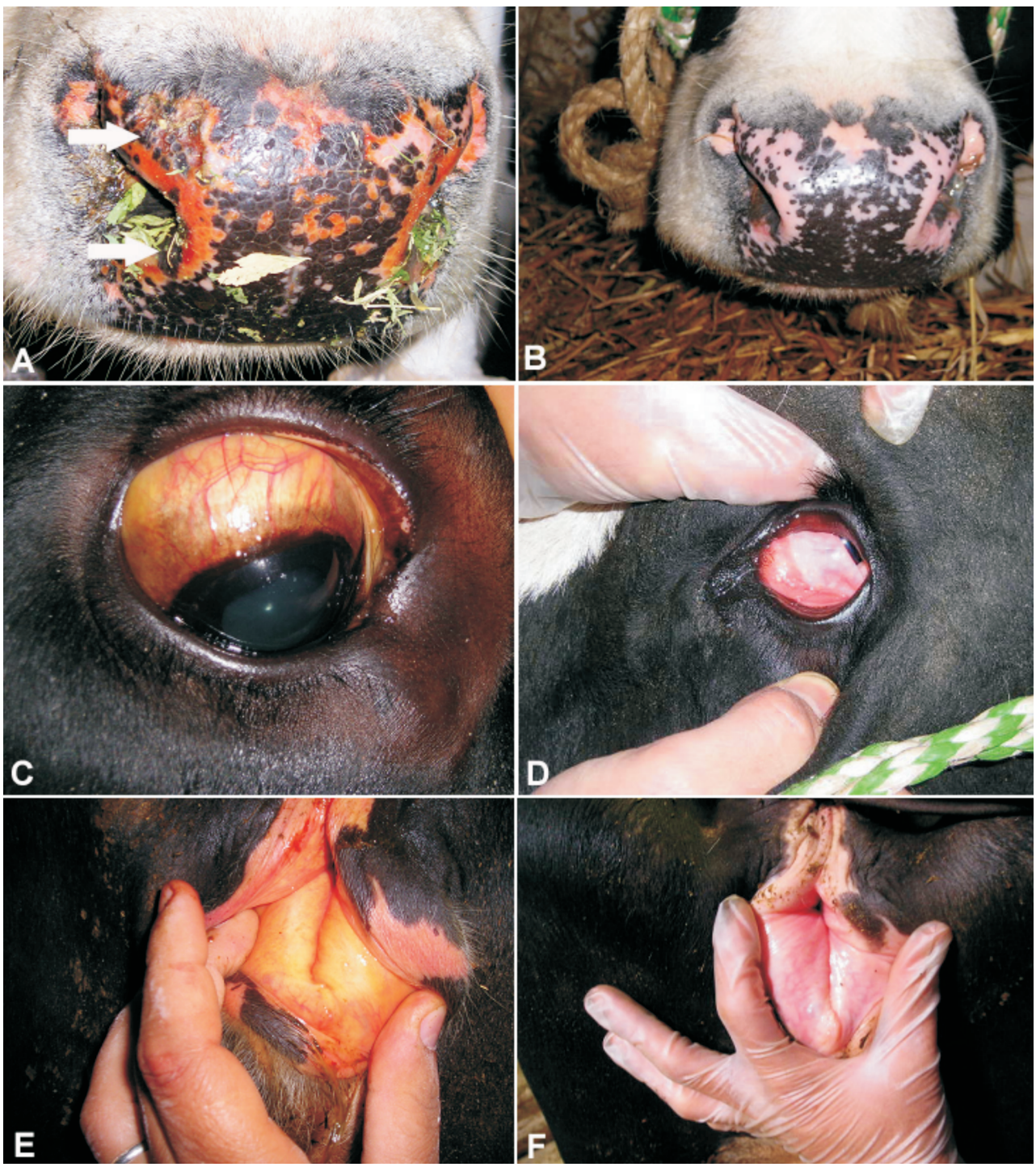

Fig. 1. Presentation and recovery of a cow with fatty infiltration of the liver. **A**: photosensitization the muzzle region as a symptom of hepatic dysfunction. **B**: return of the muzzle to the normal condition after treatment. **C**: icteric sclera and conjunctival mucous membrane as a manifestation of fatty liver. **D**: normal coloration of the conjunctival mucous membrane after therapy. **E**: Yellowish vulvar mucous membrane as a clinical sign of fatty liver. **F**: return to normal color of the vulva after treatment.

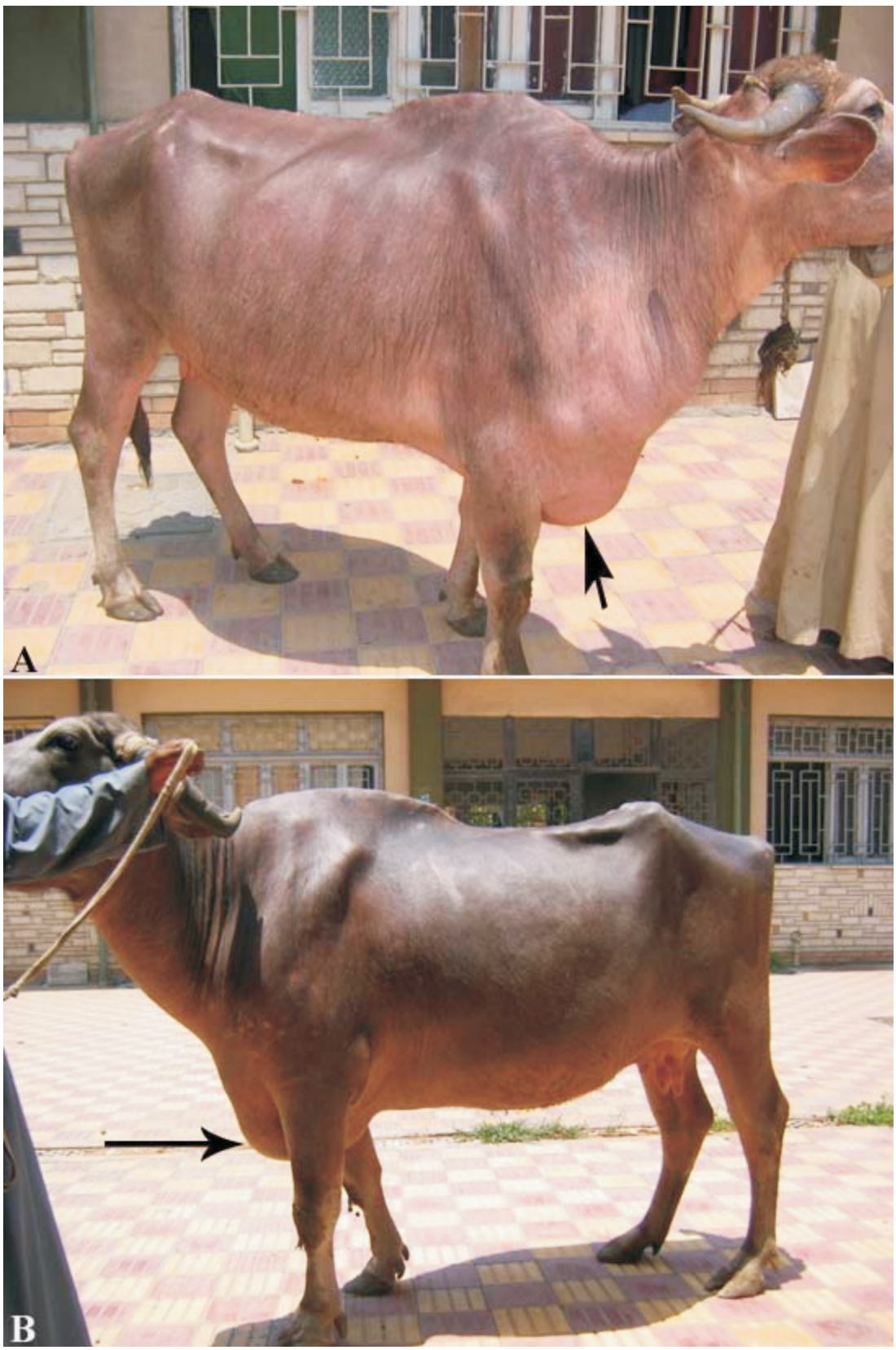

Fig. 2. Presternal edema in two water buffaloes with fatty infiltration of the liver.

Table 2. Serial hematological and biochemical findings during treatment follow up in 16 cows and 10 buffaloes with fatty infiltration of the liver.

Parameters	Finding at admission	Finding 7 days after treatment	Finding 21 days after treatment	Reference values#
Hematocrit (%)	33 4	28 ± 7	32 ± 6	24-46
Hemoglobin (g/dL)	13 ± 3	11 ± 5	10 ± 4	8.0-15.0
Erythrocytes × 10^4/μL	695 ± 49	580 ± 113	605 ± 91	500-1000
Leukocyte count (/μL)	7900 ± 2850	9850 ± 3889	9500 ± 2560	4000-12000
Segmented neutrophils (/μL)	5200 ± 2300*	4900 ± 1557*	5800 ± 1557*	600-4000
Lymphocytes (/μL)	3200 ± 2500	4950 ± 7500	4250 ± 1485	2500-7500
Total protein (g/dL)	7.0 ± 1.1	7.1 ± 0.7	6.5 ± 1.5	5.7-8.1
Albumin (g/dL)	2.52 ± 0.2	2.34 ± 0.4	2.75 ± 0.5	2.1-3.6
α-globulin (g/dL)	0.64 ± 0.3	0.60 ± 0.6	0.79 ± 0.8	0.75-0.88
β-globulin (g/dL)	0.89 ± 0.8	0.91 ± 0.5	0.55 ± 0.6	0.8-1.1
γ-globulin (g/dL)	2.91 ± 1.9*	2.95 ± 2.0*	2.81 ± 1.9*	1.69-2.27
A/G ratio	0.57 ± 0.06	0.52 ± 0.09	0.66 ± 0.13	0.80-0.90
Aspartate aminotransferase (U/L)	473 ± 276**	293 ± 125**	155 ± 88*	43-127
γ-glutamyl transferase (U/L)	160 ± 211**	109 ± 183*	87 ± 113*	15-39
Total bilirubin (mg/dL)	2.4 ± 0.9**	1.2 ± 1.1*	0.75 ± 0.7*	0.01-0.47
Calcium (mg/dL)	9.0 ± 2.9	8.9 ± 1.9	11 ± 2.3	9.7-12.4
Phosphorus (mg/dL)	5.0 ± 2.8	3.2 ± 5	5 ± 1.3	3.9-9.2
Magnesium (mg/dL)	2.9 ± 0.6	3.1 ± 0.3	2.7 ± 0.4	1.8-2.3
Glucose (mg/dL)	67 ± 24	48 ± 12	53 ± 20	45-75
Blood urea nitrogen ((mg/dL)	33 ± 35	19 ± 11	12 ± 25	6.0-27
Free fatty acids (mEq/L)	1.19 ± 0.80**	1.02 ± 0.30**	0.29 ± 0.11	0.07-0.15
Total cholesterol (mg/dL)	89 ± 61	91 ± 47	116 ± 30	65-220
β-hydroxy butyric acid (μmol/L)	3119 ± 1854***	1973 ± 1280**	537 ± 264	100-400
Insulin (μU/L)	20 ± 26*	13 ± 3.6	9 ± 7	0-5
Sodium (mmol/L)	142 ± 8	140 ± 6	139 ± 11	132-152
Potassium (mmol/L)	4.3 ± 0.60	3.7 ± 0.52	4.0 ± 0.24	3.9-5.8
Chloride (mmol/L)	82 ± 18*	98 ± 9	90 ± 10	95-110
Creatine kinase (U/L)	3497 ± 2319***	1958 ± 2549**	698 ± 1567*	35-280
Lactate dehydrogenase (LDH) (U/L)	10930 ± 3297***	6133 ± 2300**	1244 ± 1538*	692-1445
LDH-1 (%)	37 ± 11	28.6 ±13	48.9 ±10	39.8-63.5
LDH-2 (%)	27 ± 5	21.6 ±4	29.9 ±2	19.7-34.8
LDH-3 (%)	18 ± 4	17.4 ±7	15.3 ±5	11.7-18.1
LDH-4 (%)	7 ± 3	10.1 ±2	3.4 ±4	0.0-8.8
LDH-5 (%)	11 ± 10	22.3 ±9	2.5 ±6	0.0-12.4
Venous blood gas analysis pH	7.315 ± 0.03*	7.404 ± 0.08	7.432 ± 0.06	7.35-7.50
pCO_2 (mmHg)	41.2 ± 7.1*	45.8 ± 9*	44.4 ± 10*	20-30
pO_2 (mmHg)	31.1 ± 6.9	29.2 ± 5.5	49.5 ± 8	34-45
HCO_3(mmol/L)	26.7 ± 8.33	27.8 ± 11	28.3 ± 9	20-30

Data are expressed as mean ± SD. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$

Ultrasonographic findings are summarized in Table 1. Ultrasonographic examination of the liver revealed a hyperechogenic hepatic parenchyma in twelve cases (Figs. 3, 4). In the remaining fourteen animals, the hepatic parenchyma was completely anechoic where the hepatic parenchyma could not be visualized at all and liver borders were only determined through percussion (Fig. 5). Histological examination revealed marked fatty infiltration of the hepatocytes. The hepatic cells had large and clear intracytoplasmic vacuoles. These vacuoles displaced the nuclei to the periphery of the cells flattening them against the cytoplasmic membrane and giving these cells a signet-ring appearance. The histological picture of the liver specimen was compatible with a diagnosis of severe fatty liver. One week after treatment, the hepatic parenchyma was visualized easily and liver boundaries were clearly imaged. The ultrasound image of the hepatic parenchyma was characterized by mild echo intensity. Histopathology showed a moderate degree of fatty liver. Three weeks after treatment, the hepatic parenchyma was almost similar to normal and the hepatic blood vessels could be easily evaluated. The histologic picture had greatly improved and the liver resembled the normal organ (Fig. 5). Recovered cases were discharged from the hospital and

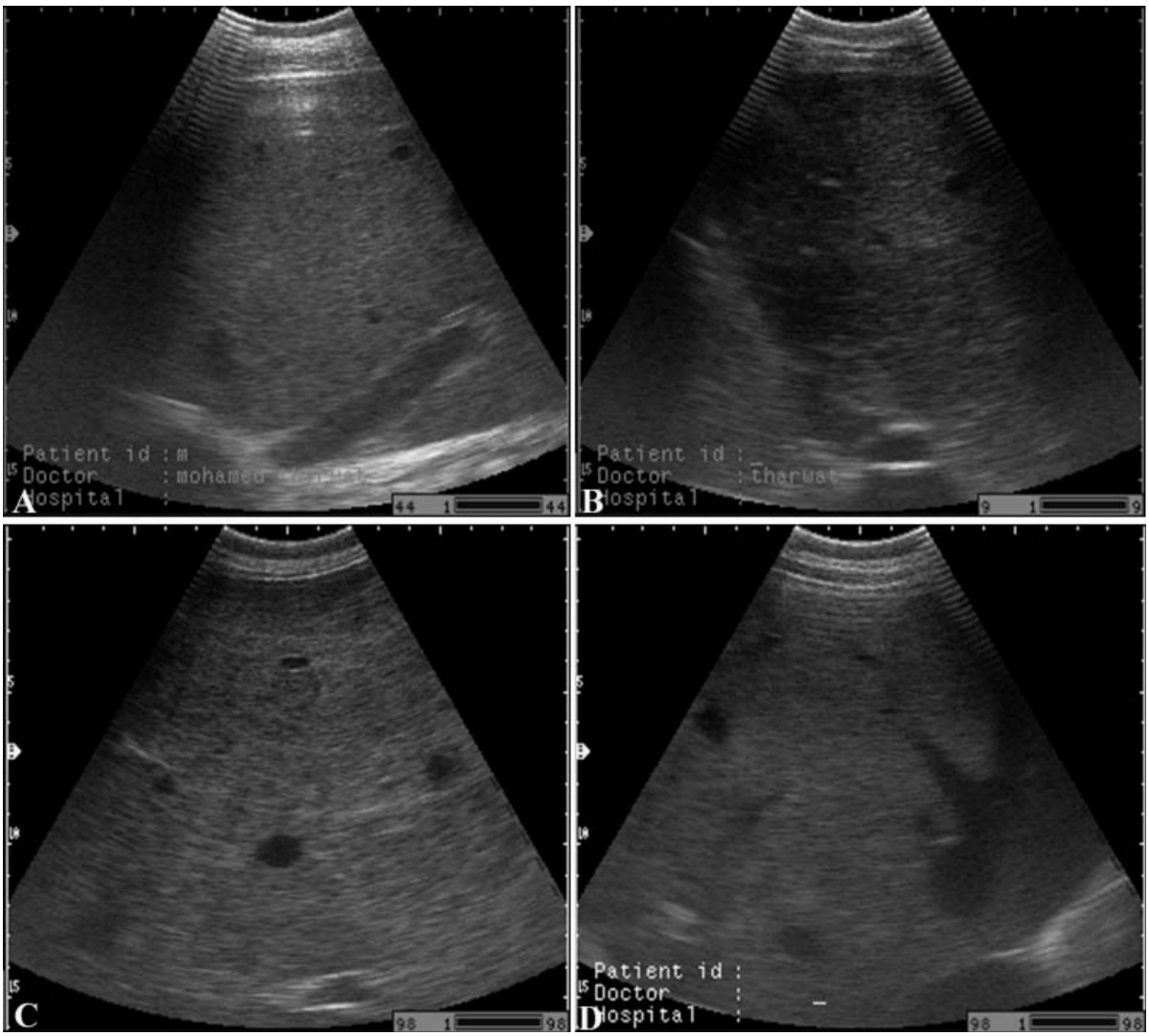

Fig. 3. Hepatic ultrasonograms in four water buffaloes affected with hepatic lipidosis. Images were taken from the 11th intercostal space on the right side using 3.5 MHz sector transducers. The hepatic parenchyma appeared hyperechogenic and the hepatic and portal blood vessels were narrowed and difficult to be differentiated.

a telephone follow-up one month later revealed that all animals had returned to full production and experienced no complications.

At postmortem examination, the liver in the thirteen examined animals was enlarged (24 ± 6 kg), friable with distended gallbladder (Fig. 6). Other postmortem findings included icteric carcasses, abdominal fluid, excessive amounts of perirenal fat and omasal ulceration (Table 1).

Discussion

In the present study, cows and buffaloes with fatty liver experienced no specific signs. Inappetance was encountered in all animals examined in this study. This could be explained by the fact that increased adipose mass is associated with increased adipocyte cell size and increased adipose sensitivity to glucocorticoids and decreased sensitivity to BHBA, glucose, and insulin (Herdt 2000). In addition, bovine adipocytes secrete hormone-like compounds (Chelikani et al. 2003), most likely the pro-inflammatory cytokine, that decrease feed intake and insulin sensitivity and increase hepatic lipogenesis, catabolism, and inflammation (Ohtsuka et al. 2001).

Fatty liver in the present study was associated, in cows and buffaloes, with elevated concentrations of NEFA and BHBA in blood, both of which can be cytotoxic at high concentrations. Elevated concentrations of NEFA and BHBA can decrease the physiological functions of organs because of their toxicity at high concentrations and because of their metabolic effects. Elevated concentrations of NEFA increase

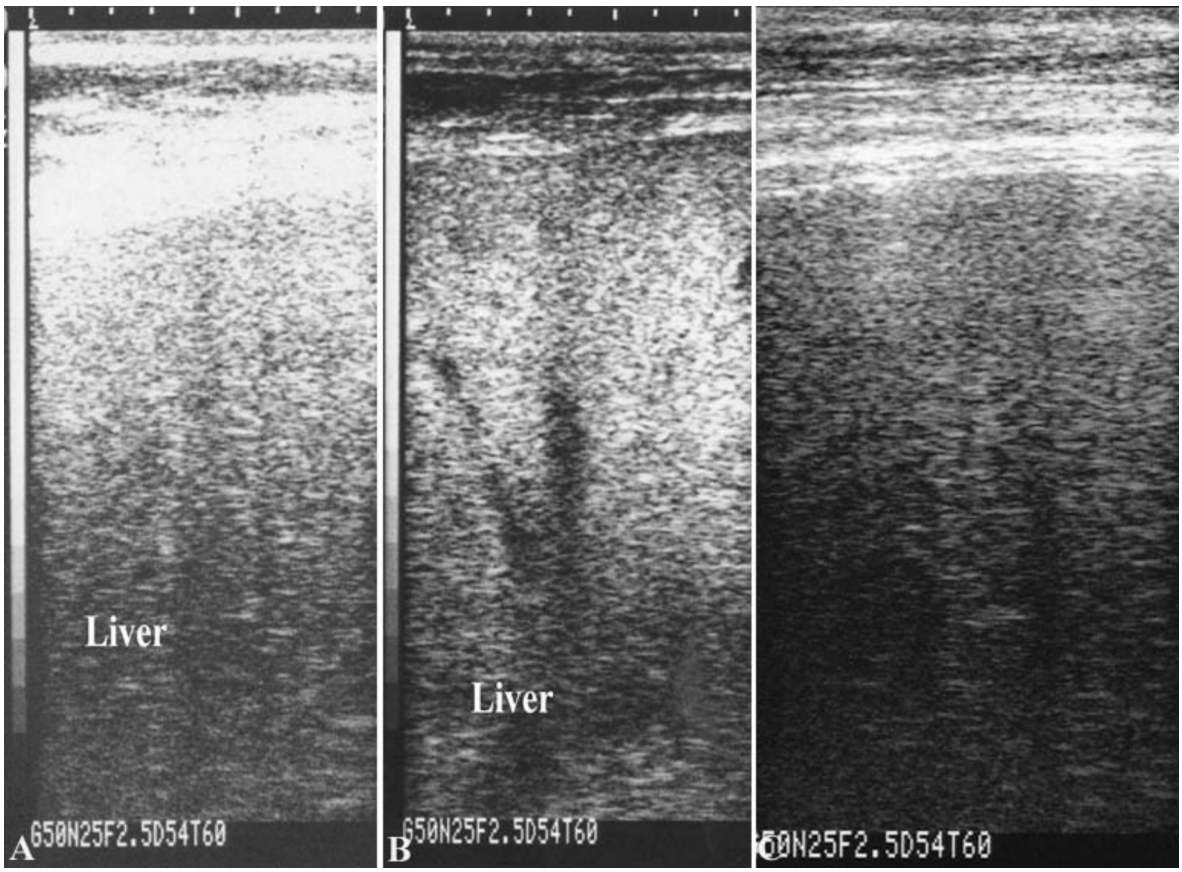

Fig. 4. Hepatic ultrasonograms in three cows with fatty infiltration of the liver. Images were taken from the 11th intercostal space on the right side using 3.5 MHz linear transducers. The hepatic parenchyma appeared hyperechogenic and the hepatic and portal blood vessels were difficult to be imaged.

lipogenesis and ketogenesis in hepatocytes (Cadorniga-Valino et al. 1997) and high concentrations of BHBA decrease rate of gluconeogenesis. In this study increased concentrations of bilirubin indicate that bile flow was decreased in the cows with fatty liver. In humans, high concentrations of bile are toxic and increase the production of free radicals in the liver, which can cause inflammation and tissue damage (Ljubuncic et al. 2000). Increased activities of AST and GGT in cows and buffaloes with fatty liver have also been reported (Ghanem and El-Deeb 2010) and this is a real result of hepatic infiltration by lipids. The elevated activities of CK and GGT recorded here could be explained due to long-term recumbency in some cows before admission for examination.

In the normal bovine, hepatic ultrasonogram consists of numerous weak echoes distributed homogeneously over the entire area of the liver. There is a gradual attenuation of the echo beam as it passes through the normal liver tissue. The portal and hepatic veins can be seen within the normal echotexture, and the parenchymal edges are normally visible (Mohamed et al. 2004a,b). In the present study, the echogenicity of hepatic parenchyma was either increased or decreased. In the first group (anechoic hepatic parenchyma), decreased echogenicity could be attributed to weakness of the echoes as the distance from the abdominal wall increases because the fat-containing hepatocytes enhance the acoustic impedance. The result is that the region near the abdominal wall was hyperechogenic, whereas areas more distant were hypoechogenic or could not be imaged at all. In the second group (white liver parenchyma), high echogenicity could be attributed to increases in the number and intensity of the internal echoes where the liver appears white on ultrasonograms and is difficult to differentiate from surrounding tissue. The contrast between the liver and vessels was also decreased. Often only large vessels were seen; small vessels were poorly imaged or not seen at all. This is because the small vessels are compressed by swollen hepatic tissue (Mohamed et al. 2004, Braun 2009).

During treatment follow-up, hepatic ultrasonograms improved steadily from hyperechoic or anechoic to normal images. The hepatic parenchymal ultrasound imaging differs when hepatocytes are

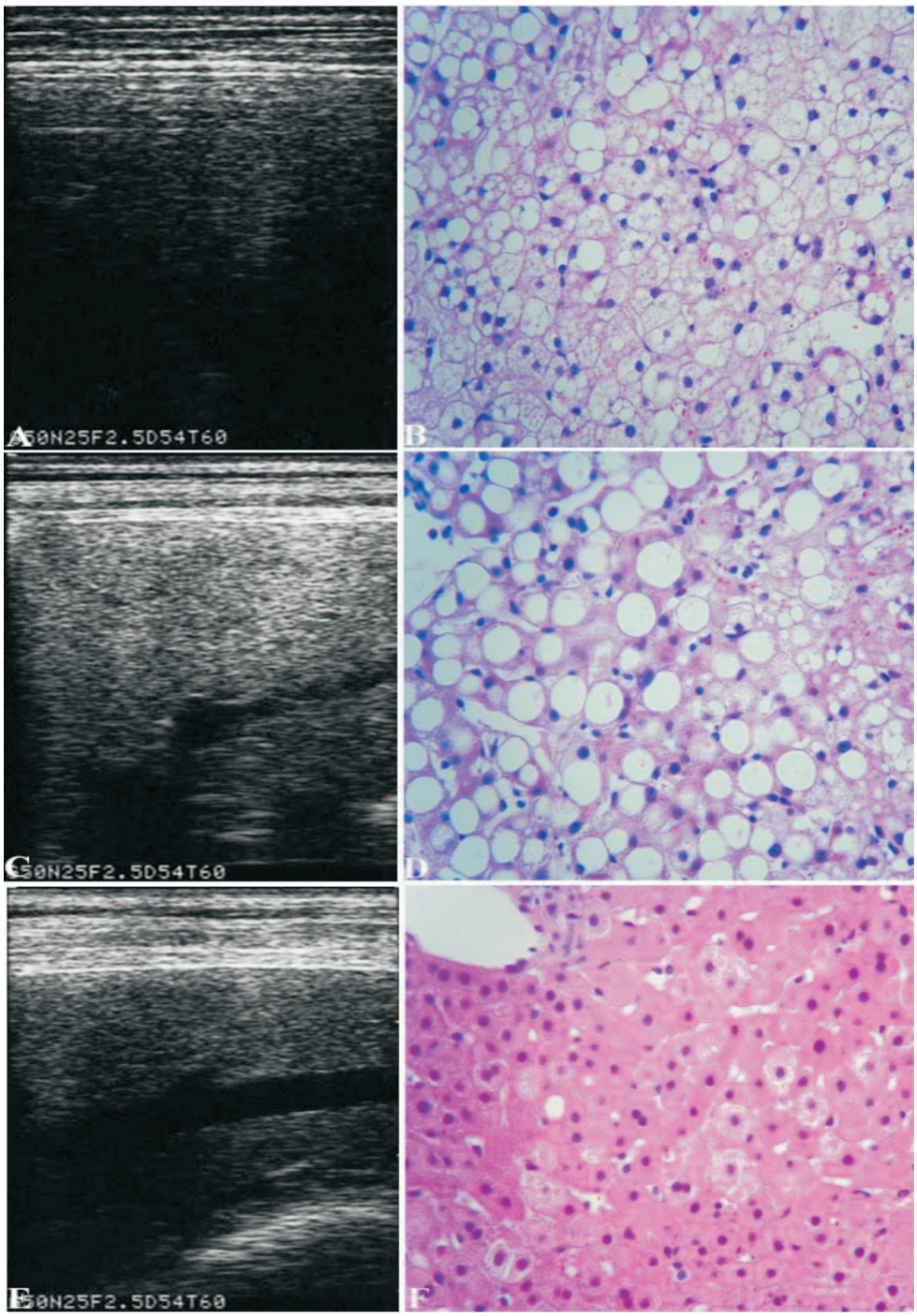

Fig. 5. Hepatic ultrasonograms and histopathologic follow-up in a cow with fatty liver. At admission, the liver parenchyma was totally anechoic **(A)**, with histologic evidence of severe fatty infiltration of the liver (**B**). On day 7 after treatment, the hepatic parenchyma was moderately echogenic **(C)** and histopathology showed a moderate degree of fatty liver (**D**). At day 21, the hepatic parenchyma was similar to normal liver image and the blood vessels could be easily evaluated **(E)** and the histopathologic picture of the liver had greatly improved and resembled the normal organ (**F**). **(B, D, F:** H&E stain**)**.

occupied by fat. In the present cases on admission, because of the considerable amount of the intracellular fat, attenuation of the ultrasound in the subcapsular area was produced, leading to a hyperechogenic or anechoic pattern of the parenchyma. With advancement of treatment and at day seven, fat was removed partially from the hepatic cells producing moderate reflective interfaces giving a moderate echogenic parenchyma. At day twenty-one, the liver parenchyma was mostly normal where differentiation between the portal and hepatic blood vessels was clearly made. It is therefore assumed that ultrasonography can be per-

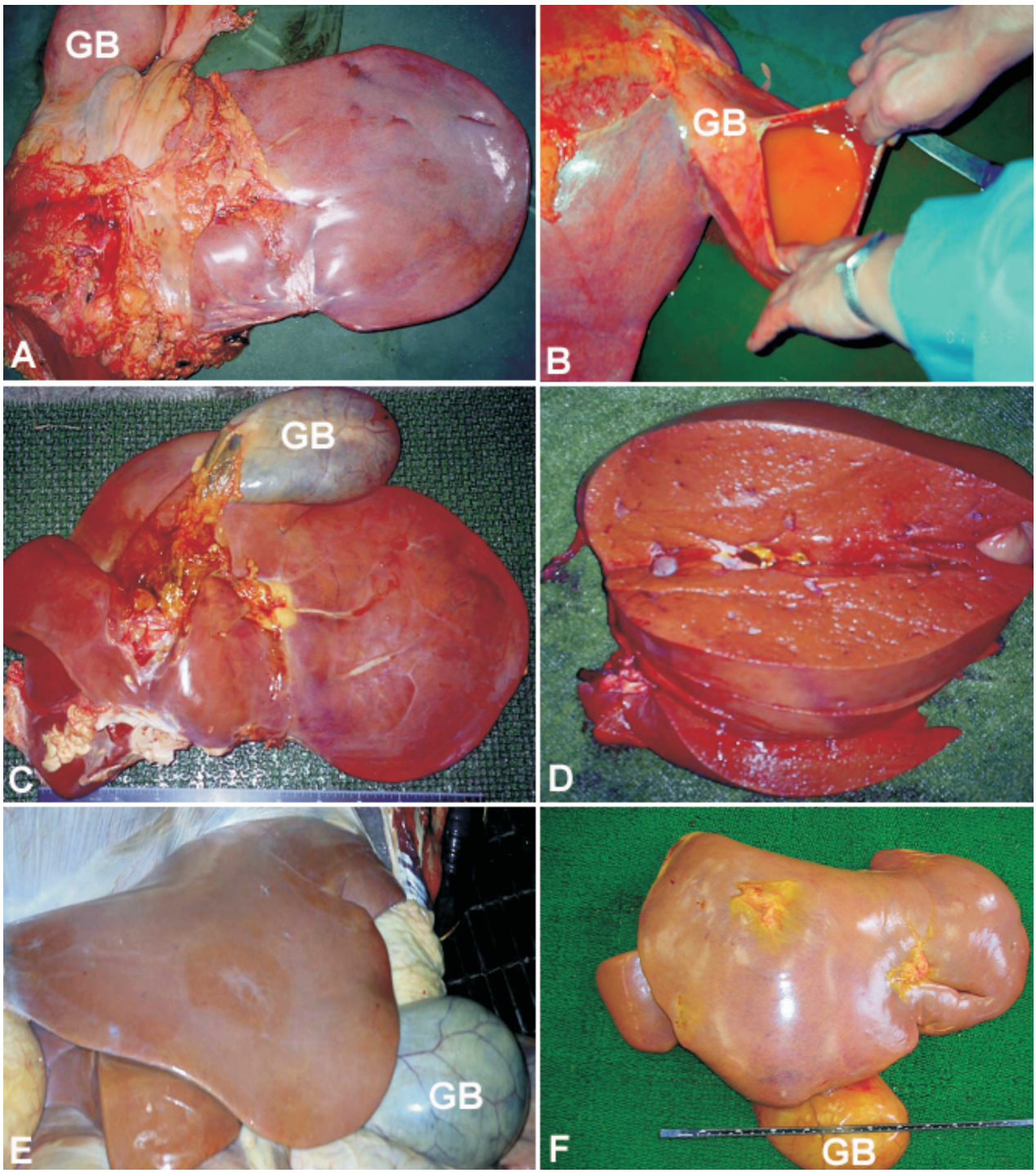

Fig. 6. Postmortem findings in cows and buffaloes with fatty liver. **A**: represent an enlarged liver (26 Kg) with distended gallbladder and **B**: opened gallbladder with yellow-orange bile. **C**: liver enlargement (24 Kg) with distended gallbladder and **D**: cut section through hepatic parenchyma where narrowing of the hepatic blood vessels is evident. **E**: enlarged liver (20 Kg) with distended gallbladder and **F**: enlarged liver (18 Kg) with distended gallbladder.

formed before more invasive procedures, such as liver biopsy, to determine the extent and characteristics of changes in the hepatic parenchyma. It can also be used to complement biochemical examination in the diagnosis of fatty infiltration of the liver in cows and buffaloes.

In conclusion, the present study shows a strong positive association between the severity of fatty liver and the ultrasonographic picture. These findings indicate that the features of ultrasonograms obtained during imaging of the liver on treatment follow-up can be used as an indicator for the prognosis in cows and

buffaloes with fatty liver. We cannot exclude the benefit of liver biopsy in quantification of fat volume in animals with fatty infiltration of the liver. However, in the present study, ultrasonography demonstrated a good correlation with histologic and laboratory findings of hepatic lipidosis and therefore prognosis of the animals. A non-invasive sonographic examination of the liver is therefore promising in this regard. Because of the convenience, noninvasiveness of ultrasound, it is suggested that hepatic needle biopsy can be replaced, at least partially, by diagnostic ultrasound to be used as an accepted alternative follow-up imaging approach, in cows and buffaloes with fatty infiltration of the liver.

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