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ECHO-ASSISTED PERCUTANEOUS RENAL BIOPSY COMPARED WITH BLIND RENAL BIOPSY IN CATTLE

(With One Table and 2 Figures)

By

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بزل الكلى عن طريق الجلد بالاسترشاد بصورة الموجات فوق الصوتية مقارنة
بالبذل بدون استخدام الموجات فوق الصوتية في الماشية

محمد ثروت عبدا لعال ، حاتم محمد سليم ، شين اويكاوا

أجرى هذا البحث باستخدام طريقتين لبذل الكلى لتقييم مدى سلامتهما وكفاءتهما اعتمادا على استخدام ٢٥ حالة. تم استخدام إبرة بذل لأخذ عينات من الكلية اليمنى وذلك بالاسترشاد بصورة الموجات فوق الصوتية بينما أخذت عينات من الكلية اليسرى بدون الاسترشاد بجهاز الموجات فوق الصوتية ثم قورنت نتائج الطريقتين ببعضهما. تم عمل البذل قبل الذبح مباشرة لعدد ٢٠ عجل لدراسة التأثير السريع لعملية البذل (مجموعه ١) بينما تم بذل ٥ عجول ثم تم تدوين الأعراض الاكلينيكية وفحص صورته الدم وتحليل مصل الدم يوميا لمدة ٩ أيام بعد البذل ثم تم ذبحها وفحصها (مجموعه ٢). بمقارنه الطريقتين لبذل الكلى وجد أن طريقة البذل باستخدام الموجات فوق الصوتية أدت إلى الحصول على نسيج كافي للفحص الهستولوجى بنسبة ٩٠% من محاولة واحدة للبذل مقارنة بنسبة ٥٠% بدون استخدام الموجات فوق الصوتية من ٢-٣ محاولة للبذل. البذل باستخدام الموجات فوق الصوتية لم يؤدي أي مضاعفات بينما وجدت حالتين بهما بول مدمم بعد عملية البذل مباشرة بدون استخدام الموجات فوق الصوتية. وجد البحث أن البذل من الكلية باستخدام الموجات فوق الصوتية هو طريقه آمنه وكان ذلك مؤكدا بعدم تغير ملحوظ في صورة الدم أو انزيمات الدم أو وظائف الكلى في خلال ٩ أيام بعد البذل قبل ذبح العجول. عند فحص الحيوانات المذبوحة وجد أن البذل بدون استخدام الموجات فوق الصوتية أدى إلى حدوث نزف كثيف في الكلى مقارنة بنزف طفيف جدا باستخدام الموجات فوق الصوتية. الخلاصة أن البذل بعناية وحرص باستخدام الموجات فوق الصوتية هو طريقه آمنه وسريعة واقتصادية وعملية ويمكن أن تستخدم بكفاءته عالية لتشخيص أمراض الكلى في الماشية.

SUMMARY

Two techniques for percutaneous renal biopsy were reviewed to assess their relative safety and efficacy. The safety was evaluated based on twenty-five consecutive procedures. Using a hand-held 14G spinal biopsy needle, right kidney was biopsied under real-time ultrasound control. Needle biopsy specimens were also obtained from the left kidney blindly and the procedure was compared with biopsy performed under ultrasound control. To assess the immediate effects of renal biopsy, twenty steers were slaughtered shortly after the procedure and examined. The remaining five animals were biopsied then examined daily for nine days and then slaughtered and examined. The echo-assisted technique was more reliable at retrieving an adequate specimen for histological examination (90%) than the blind biopsy technique (50%). Only one pass was made in each of the ultrasound-guided biopsies, as compared to 2-3 passes in the blindly biopsied steers. In the blindly sampled group, 2 (20%) steers developed macroscopic haematuria. No complications occurred post biopsy in the group biopsied under ultrasound guidance. Changes indicative of inflammation were not apparent in total and differential WBC counts and in albumin/globulin ratios. The activities of aspartate aminotransferase, γ -glutamyltransferase, creatinine kinase and lactate dehydrogenase, the percent of haematocrite and the serum concentrations of urea nitrogen, creatinine and haemoglobin remained within reference ranges. Unlike the ultrasound-guided biopsies where animals developed a steak of haematoma at the site of biopsy and six had a thin subcapsular haematoma (< 2cm), six of the ten steers used for the blind biopsy had subcapsular haematoma (< 5cm). On the cut surface, the needle used in the blind biopsy of the left kidney tended to penetrate deeper than that used in the echo-assisted biopsy of the right kidney. Changes in the peritoneum, omentum and bowel observed in slaughtered animals were negligible. It is concluded that percutaneous ultrasound-guided renal biopsy is safe, fast, cost-effective, and practical as long as it is performed properly. It is believed that this technique can be used in cattle with suspected renal disease for making an antemortem diagnosis.

Key words: Cattle, renal disease, needle biopsy, safety, ultrasound

INTRODUCTION

In human medicine, attempts at percutaneous renal biopsy were reported as early as 1944, although the first systematic study was not published until 1951 (Iversen and Brun, 1951). Reports published in both human and veterinary literature in the 1970s and 1980s demonstrated the impracticality of distinguishing between glomerular pathologies based exclusively on applied clinical and laboratory parameters (Tomura *et al.* 1985 and Minkus *et al.* 1994). Furthermore, it was demonstrated that diagnosis and therapy were modified in about 40% of cases following renal biopsy (Richard *et al.* 1994) and that the technique used altered clinical prognosis in about 50% of cases (Turner *et al.* 1986). Hence, renal biopsy is considered the diagnostic procedure of choice in many patients in whom renal disease is suspected (Hergesell *et al.* 1998). Renal biopsy is, however, not free of morbidity and is not always successful in obtaining a diagnostic specimen.

In dogs, renal biopsy is commonly performed to evaluate the type and severity of lesions in animals suspected to have renal disease, particularly dogs with diseases primarily involving the renal cortex, such as protein-losing glomerulopathy (Osborne *et al.* 1996). Other indications for biopsy of the renal cortex include nephritic syndrome without signs of systemic disease and acute renal failure for which the cause cannot be determined on the basis of history, physical examination findings, or laboratory test results (Squadrito and Coletta, 1991 and Bigge *et al.* 2001). However, biopsy of the renal medulla is seldom indicated clinically and is associated with a risk of injury to the deeper renal vessels (Leveille *et al.* 1993, Osborne *et al.* 1996 and Bigge *et al.* 2001). The procedure has been adopted widely as a diagnostic procedure and research tool. As the use of this technique has increased, indications and complications have been defined. Complications of renal biopsy result from penetration of large blood vessels and include microscopic and macroscopic haematuria, obstruction of the renal pelvis by blood clots resulting in hydronephrosis and severe renal, subcapsular or perirenal haemorrhage resulting in haemorrhagic shock (Leveille *et al.* 1993). Animals with peritonitis, intra-abdominal adhesions, abdominal hernia, coagulation defects and obesity were excluded from biopsy (Wise *et al.* 1989).

In cattle, ultrasonography of the kidneys and urinary system was originally reported by Braun (1991 & 1993). It has been used for the diagnosis of pyelonephritis (Rebhun *et al.* 1989 and Hayashi *et al.* 1994), nephrolithiasis (Divers *et al.* 1989) and hydronephrosis (Harrison

et al. 1992). Percutaneous ultrasound-guided tissue core biopsy and percutaneous ultrasound-guided fine-needle aspiration are minimally invasive and cost-effective methods of obtaining specimens for histologic evaluation and bacteriologic culture (Leveille *et al.* 1993). It is believed that the procedure is required for sampling of renal tissue in cows with renal diseases. Informations on the technique, success rate and safety of the use of this procedure in cattle is not available in English literature. Here our experience of the safety and efficacy of a free-hand technique for percutaneous ultrasound-guided biopsy of kidneys in cattle are reported with comparing our experience of ultrasound guided renal biopsies of the right kidney with blind biopsy of the left kidney are compared. The ultrasound-guided procedure is minimally invasive and cost-effective for obtaining specimens for histological evaluation and bacteriological culture that could be used in veterinary practice and also in research. With increasing use of ultrasonography and ultrasound-guided techniques, the authors believe that this technique may increase the early diagnosis of suspected renal diseases in cattle.

MATERIALS and METHODS

Ultrasonographic examination of the kidneys

Ultrasonography of the right and left kidneys was performed according to standard protocols (Braun 1991 and 1993). For examination of the right kidney, the areas over the intercostal spaces 10, 11 and 12, the lumbar region and paralumbar fossa on the right side were clipped and shaved. After application of a generous amount of alcohol to the skin, imaging of the right kidney was performed using an ultrasound scanner (Model RT 2600) and a 3.5 MHz linear transducer. Ultrasonographic examination of the left kidney was performed rectally with a 5.0 MHz linear transducer, although the left kidney was accessible from the right flank in some cows. Transmission gel was applied to the probe and it was placed in a plastic rectal glove before being introduced into the rectum.

Biopsy procedure

All animals enrolled into the study were evaluated ultrasonographically for haemorrhage immediately prior to the procedure as we recently demonstrated (Mohamed *et al.* 2002a&b, 2003 and 2004a&b). Animals with systemic venous congestion, mostly due to right-sided heart failure, were excluded from the study. In all steers, the concentrations of serum urea and creatinine were within normal ranges.

To obtain adequate restraint, cows were slightly sedated with xylazine (0.07 mg/kg body weight, intravenously) and the region was infiltrated with 10 ml of 2% procaine hydrochloride. During biopsy, care was taken not to exceed three attempts for each animal.

Echo-assisted percutaneous technique for biopsy of the right kidney

The right kidney was imaged to determine the best path of the needle (Fig. 1). The needle path was chosen to avoid penetration of the liver, great blood vessels and the bowel. The above shaved abdominal area was sterilized using standard surgical disinfection techniques. 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×150mm spinal biopsy needle (Kurita Co., Ltd, Tokyo, Japan) was advanced through the tissues under direct ultrasound control and its relation to the kidney was assessed either from identifying the needle directly or, in few cases, by assessing the movement of the tissues as the needle is passing through them. Under visualization by ultrasound, the needle was advanced until the capsule of the right kidney has been reached. The needle was directed obliquely in an attempt to sample cortical tissue only to avoid renal medulla, renal pelvis and hilar and renal vessels. When the needle is considered to be in the correct position, touching the renal capsule, the plain stylet was withdrawn and a notched part inserted and advanced 1cm into the renal cortex beyond the renal capsule. Often the needle can be identified on ultrasound within the cortex while the specimen is being obtained, thus confirming the location of biopsy (Fig. 2). Both the needle and forked stylet were then removed with a sample of renal tissue. A single operator performed the biopsy procedure. All animals subjected to biopsy were evaluated ultrasonographically for complications such as haemorrhage immediately after biopsy. Tissues were fixed in neutral-buffered 10% formalin, processed routinely and embedded in paraffin. Sections of 4 micron thick were cut and stained with H&E, and examined by light microscopy.

Blind percutaneous technique for biopsy of the left kidney

The skin over the left flank was surgically prepared and infiltrated with a local anesthetic agent. After manual rectal examination, the left kidney was firmly hanged by the right hand and pushed toward the left abdominal wall. At the same time, the skin over the left flank was incised by the tip of a scalpel blade just over the left kidney. While the left kidney was in contact to the skin of the left flank, the biopsy

needle was advanced transcutaneously to the left kidney and the organ was blindly biopsied by a second operator.

Short-term effect of renal biopsy

In this part of the experiment, the analysis concerned twenty renal biopsies that were performed in male cattle weighing between 360 and 720 kg. The animals were submitted to the quarantine of El-Tal El-Keber Military Slaughter House. Biopsy was carried out immediately before slaughter. The right kidney was biopsied in ten animals with the help of portable ultrasound machine. In the other ten steers, the left kidney was sampled by localising the kidney by rectal palpation. Animals were examined post-slaughter for possible procedure-associated pathological changes in the peritoneum, omentum, bowel and kidneys.

Long-term effect of renal biopsy

The purpose of this part of the study was to determine whether renal biopsy in examined animals is a safe procedure.

Five healthy steers weighing between 500 and 620 kg were used. All of them were free of alimentary tract diseases, as shown by the history and by physical and laboratory examinations. Prior to biopsy, the steers were subjected to preliminary examination that included a thorough physical examination, and tests for a complete blood count (CBC), albumin/globulin ratio, and urea nitrogen (UN), and creatinine concentrations. The right kidney was then biopsied. At the end of the procedure, each animal was monitored for several hours and all urine passed was carefully examined for macroscopic haematuria. After biopsy, steers were monitored for a nine-day observation period. Daily evaluation included assessment of general appearance, activity, and appetite; determination of rectal temperature, pulse rate, respiratory rate, and intestinal and ruminal motility; and auscultation of the thorax and heart. In addition, the following blood determinations were measured before biopsy and for the following nine days: CBC, serum activities of aspartate aminotransferase (AST), γ -glutamyltransferase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH) and serum concentrations of UN and creatinine. At the end of the observation period, the cows were euthanased and the peritoneum, omentum, bowel and kidneys were examined.

Analytical methods

Haemograms, the activities of AST, GGT, CK and LDH, and the concentrations of UN and creatinine were measured using an automatic biochemical analyzer (Hitachi 911; Roche Diagnostics).

Statistical analysis

Data are expressed as mean values \pm SD. All statistical analyses were performed using STATISTICA v 5.5 for windows (StatSoft, Inc., Tulsa, OK, USA). Data were tested for normal distribution using Komogorov-Smirnov test. When the data had a normal distribution, homogeneity of variances was verified using Levene's test. Data were then subjected to one-way analysis of variance (ANOVA) using time as independent factor and blood variables as a dependent factor. Post hoc comparisons (Duncan's multiple-range test and critical ranges) were used to test the significance among variables. For all analyses, a value of $P < 0.05$ was considered significant.

RESULTS

The right kidney was clearly imaged upper in the 12th intercostal space in most of the steers and in the right dorsal flank in few animals. Ultrasonography of the kidneys revealed less clear differentiation between the cortex and medulla. Renal medulla was the only exception and appeared less echogenic than the rest of the parenchyma. Compared to liver, the hypoechoic renal parenchyma was well differentiated from the hyperechogenic sinus. The right kidney was also well visible through the so-called liver window.

Echo-assisted biopsy of the right kidney allowed us to sample all animals used in the short and long-term study on the first endeavor. The number of passes made was only one in each ultrasound-guided biopsy. However, blind biopsy of the left kidney was successful on the first attempt in only 20% of steers (2/10), second attempt in 50 % of steers (5/10) and on the third attempt in 30% of animals (3/10). Ninety percent (9/10) of the ultrasound-guided biopsies yielded adequate tissue for examination, whereas the blind biopsy revealed sufficient material in 50% of steers (5/10) and did not yield enough tissue in the remaining biopsies. The average length of time for the echo-assisted biopsy of the right kidney (from preparing the animal to finishing the procedure) was 40 min (average 25-50 min). This included an initial ultrasound examination of the animal's kidneys to assess their parenchyma. No accurate figures are available for the blind biopsy of the left kidney but our overall impression is that the time taken is considerably longer. In the ultrasound-biopsied animals, no immediate or delayed macroscopic haematuria was observed post biopsy. However, of the 10 steers used for blind biopsy of the left kidney, there was an evidence of haematuria attributable to the biopsy procedure in 2 steers (20%).

At no time during the nine days of observation of the animals post biopsy were appetite, general behavior, rectal temperature, and attitude abnormal in any of the steers. In addition, changes indicative of inflammation were not apparent in total and differential WBC counts and in albumin/globulin ratio. These results were confirmed after slaughter. Moreover, haematocrite and haemoglobin were within normal ranges and none of them differed significantly when compared with pre biopsy values. The activities of AST, GGT, CK, and LDH remained within reference ranges in all cows throughout the observation period. There were no significant differences in mean serum urea nitrogen (UN) and creatinine post-biopsy (Table 1).

At slaughter, all ultrasound-guided biopsies developed a steak of haematoma at the site of biopsy and six of them had a thin subcapsular haematoma, which was clinically insignificant (< 2cm). Six of the 10 steers used for the blind biopsy of the left kidney had subcapsular haematoma (< 5cm). On the cut surface, the needle used in the blind biopsy of the left kidney tended to penetrate deeper than that used in the echo-assisted biopsy of the right kidney. Gross changes indicative of peritonitis were not seen in any of the animals observed for nine days after the biopsy and only minor infiltration with inflammatory cells around the biopsy site was noted on histological examination of the kidneys. On the kidney, a small scar on the surface at the biopsy site was detected, but had healed without gross changes.

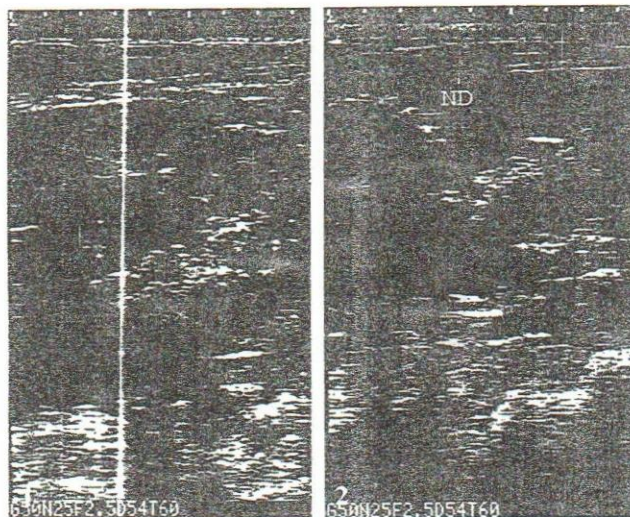


Fig. 1: Ultrasonogram of the longitudinal axis of the right kidney.

Fig. 2: A biopsy needle (ND) is penetrated into the cortex of the right kidney along the longitudinal axis. The image was taken in the right 12th intercostal space.

Table 1: Haematological and biochemical measurements in five cows during the nine-day observation period.

Parameter	Time after renal biopsy (day)										
	0	1	2	3	4	5	6	7	8	9	
TWBC	$\times 10^7/mL$	62±13	65±5	65±15	64±11	51±6	58±12	58±12	63±13	62±7	65±12
Neu (M)	$\times 10^7/mL$	24±5	30±6	25±9	20±10	22±8	20±5	16±9	16±11	22±5	18±6
Lym	$\times 10^7/mL$	32±7	31±11	37±10	38±10	36±11	36±7	38±12	42±12	38±11	43±6
Ht	%	31±7	28±3	28±4	30±5	27±2	29±3	29±2	31±7	28±3	30±3
Hb	g/L	116±23	111±11	110±12	115±20	105±13	112±11	113±8	119±27	106±9	119±12
A/G	ratio	0.8±0.06	0.8±0.07	0.8±0.08	0.9±0.03	0.8±0.04	0.8±0.07	0.7±0.01	0.9±0.18	0.8±0.01	0.9±0.19
AST	U/L	76±14	82±21	78±23	83±15	79±21	83±21	78±29	76±22	82±23	76±20
GGT	U/L	27±4	30±7	26±5	31±3	34±11	27±4	27±5	28±4	32±12	29±6
CK	U/L	180±34	185±102	190±71	176±51	201±35	166±56	183±54	149±56	167±56	144±46
LDH	U/L	2463±506	2480±746	2280±410	2493±296	2285±445	2517±635	2340±636	2280±300	2430±255	2363±341
UN	mmol/L	1.9±0.5	1.5±0.3	2.2±0.1	1.8±0.3	2.3±0.6	1.7±0.2	1.7±0.3	2.2±0.9	2.1±0.1	1.7±0.5
Cr	$\mu\text{mol/L}$	77±18	74±13	71±10	80±9	72±5	80±23	71±13	80±9	71±13	83±13

WBC: white blood cells; M Neu: mature neutrophils; Lym: lymphocytes; Ht: haematocrite; Hb: haemoglobin; A/G: albumin/globulin ratio; AST: aspartate aminotransferase; GGT: γ -glutamyltransferase; CK: creatine kinase; LDH: lactate dehydrogenase; UN: urea nitrogen; Cr: creatinine.

DISCUSSION

Ultrasound-guided needle biopsy of the kidney has proved to be a valuable aid to clinical evaluation of humans with many renal disorders (Gotti *et al.* 1985, Mendelsohn and Cole 1995 and Stiles *et al.* 2000). Similarly, percutaneous needle aspiration and ultrasound-guided renal biopsy has proved effective in dog (Szatmari *et al.* 2001, Hess and Ilan 2003 and Groman *et al.* 2004) and in horse (Modransky, 1986). In cattle, clinical signs of urinary tract disease may include abnormal urination or non-specific signs associated with infection or azotaemia (Fetcher, 1986). For this reason, ultrasonographic evaluation of the kidneys and urinary tract in the cattle is valuable in the diagnosis of renal disorders. Specific application of ultrasonography has been described for several conditions of the urinary tract in large animals (Koyama *et al.* 1984; Divers, 1988; Karcher *et al.* 1988; Byars *et al.* 1989; Divers *et al.* 1989; Hope *et al.* 1989; Kiper *et al.* 1990; Harrison *et al.* 1993; Hayashi *et al.* 1994; Kisthardt *et al.* 1999 and Mueller *et al.* 1999). Early diagnosis of renal diseases requires sampling of the renal tissue. We are not aware of any clinical study in cattle document renal biopsy and its safety. Thus, our interest was to provide practitioners with a safe methodology for sampling of renal tissue that could be used in veterinary practice and also in research. The aim was also to compare blind percutaneous and echo assisted renal biopsies in cattle.

When we started using echo-assisted technique for renal biopsy we hoped to confirm, as has been shown with renal biopsy in human and small animal, that adequate tissue would be obtained and to show that the complication rate would be reduced compared with a blind renal biopsy technique. This study has found that adequate renal tissue is obtained using the ultrasound-guided technique compared to the blind biopsy technique. Real-time ultrasound-guidance, giving more accurate localization of the needle in relation to the kidney and subsequent biopsy site in the renal cortex, would seem to be the reason for this. Moreover, direct ultrasound control allows the correction of the needle position at any time during the biopsy procedure (Donovan *et al.* 1991). Therefore, it is easier to be certain that the area of interest is sampled. There was a definite learning curve, and once mastered the technique was relatively straightforward. This may explain that the yield of material obtained by echo-assisted biopsy was almost always sufficient for an adequate histopathological analysis. If, during ultrasonography, the needle cannot be seen, slightly moving the transducer into the path of the needle,

gently agitating the needle, or injecting air or microbubbles in saline solution through the needle will usually allow the needle's position to be determined (McGahan, 1990). The needle may be more easily seen when the free-hand technique is used because the needle can be positioned more perpendicular to the ultrasound beam than it can be when a biopsy guide is attached to the transducer is used.

Large blood vessels in the kidney increase the likelihood of haemorrhage after percutaneous renal biopsy (Hager *et al.* 1985 and Kerr, 1988). For this reason, we restricted biopsy attempts in this study to three. Coagulation parameters should be determined before biopsy, but even with these precautions, complication developed in a study in dogs and cats (Bigge *et al.* 2001). Signs of haemorrhage include ultrasonographic evidence of a fluid-filled area, haematuria and decrease in haematocrite. In the present study, the post biopsy complication encountered in echo-assisted biopsy were low comparable with the blind biopsy. A small haematoma of no clinical significance was noted at the site of biopsy in all our successful biopsies used in the long-term study. A repeat scan after seven days showed complete resolution in all cases. Severe life-threatening haemorrhage or fatality did not occur after ultrasound-guided biopsy. The low complication rate in the present study may be, at least in part, because highly vascularized parts were not biopsied. When the blind biopsy was performed on the left kidney, 2 steers had haematuria. The exact location of the needle in the cortex prevents deep penetration into the medulla. In contrast, manual insertion of the needle in the blind biopsy technique leads to uncontrolled penetration and often traumatizes the kidney. The results of this study document that the risk of serious biopsy-induced complications is extremely low when ultrasound control is used.

Biopsy of the kidney is not an innocuous procedure and should not be used indiscriminately. The fact that serious iatrogenic complications can occur dictates their clinical use only when information obtained would be likely to benefit decisions about patient management. We emphasize that in order to minimize risks, it is not only important to adopt an adequate biopsy technique, but it is also imperative to exclude high-risk cattle. Animals at high-risk should be excluded from renal biopsy particularly those with coagulation disorders and cattle with heart failure. It should be emphasized that performing percutaneous ultrasound-guided biopsy of the kidney in cattle is contraindicated if the animal has a high risk of bleeding. Distention of the hepatic veins is a clear indication of enlargement of the liver that

mostly arises from right-sided heart failure, but obstruction of the caudal vena cava by a thrombus or masses is also possible (Mohamed *et al.* 2000a and 2004b). It should also be stressed that scanning of the liver may detect such abnormalities, but the animal should also be evaluated for haemostatic disorders prior to biopsy. We feel that the almost total absence of severe complication when biopsy with ultrasound control results from the very strict inclusion criteria.

Compared with the blind biopsy procedure, results of our study offer evidence that obtaining a renal biopsy under ultrasound guidance improve the quality of the biopsy specimen and decrease the potential post biopsy complication rate. With the ultrasound-guided renal biopsy technique, renal biopsy has become safe, more practical and reliable. We believe that the accuracy and safety of ultrasound-guided renal biopsy can be improved by continually observing the needle with real-time ultrasound while the kidney is sampled, by identifying the tissue to be sampled before biopsy and by performing a complete ultrasonographic examination of the area prior to biopsy to identify a safe needle path. In the present study, the results have been impressive, and it is therefore not surprising that the present study, although limited in scope, has yielded encouraging results. We recommend use of renal biopsy guided by real-time ultrasound as the method of choice for percutaneous renal biopsy in cattle.

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