FULL PAPER *Internal Medicine*

Efficacy and Safety of Ultrasound-guided Percutaneous Biopsy of the Right Kidney in Cattle

Tharwat MOHAMED[1,2] and Shin OIKAWA[1)]*

[1)]*Department of Large Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069–8501, Japan and* [2)]*Department of Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Egypt*

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ABSTRACT. The purpose of the present study was to develop a technique for biopsy of the kidneys of cattle and to evaluate its usefulness and safety based on 25 consecutive biopsies. Using a handheld 14G spinal biopsy needle, the right kidney was biopsied under ultrasound control by one operator. To assess the immediate effects of renal biopsy, twenty cattle were necropsied shortly after the procedure and examined. The remaining five animals were biopsied and examined daily for nine days and then necropsied. One pass was made for biopsy of the right kidney. No immediate or delayed macroscopic hematuria was observed post-biopsy. Six animals had a thin subcapsular hematoma (less than 2 cm in diameter). We conclude that percutaneous ultrasound-guided biopsy of the right kidney is safe, fast, cost-effective and practical as long as it is performed properly.

KEY WORDS: cattle, kidney, needle biopsy, safety, ultrasound.

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In human medicine, attempts at percutaneous renal biopsy have been reported as early as 1944, although the first systematic study was not published until 1951 [17]. Reports published in both human and veterinary literature in the 1970s and 1980s demonstrate the impracticality of distinguishing between glomerular pathologies based exclusively on applied clinical and laboratory parameters [26]. Furthermore, it has been demonstrated that diagnosis and therapy are modified in about 40% of cases following renal biopsy [36] and that the technique used alters the clinical prognosis in about 50% of cases [40]. Therefore, renal biopsy is considered to be the diagnostic procedure of choice for many patients in whom renal disease is suspected [14].

In dogs, renal biopsy is commonly performed to evaluate the type and severity of lesions in animals suspected to have renal disease, particularly in dogs with diseases primarily involving the renal cortex, such as protein-losing glomerulopathy [34]. 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 [1, 37]. However, biopsy of the renal medulla is seldom indicated clinically and is associated with a risk of injury to the deeper renal vessels [1, 23, 34]. As the use of this technique has increased, its indications and complications have been defined. Complications of renal biopsy result from penetration of large blood vessels and include microscopic and macroscopic hematuria, obstruction of the renal pelvis by blood clots resulting in hydronephrosis and severe renal, subcapsular or perirenal hemorrhage resulting in hemorrhagic shock [23].

In cattle, ultrasonography of the kidneys and urinary system has been reported [2, 3] and has been used for diagnosis of pyelonephritis [13, 35], nephrolithiasis [6] and hydronephrosis [12]. Percutaneous ultrasound-guided tissue core biopsy and fine-needle aspiration are minimally invasive and cost-effective methods of obtaining specimens for histologic evaluation and bacteriologic culture in dogs and cats [23]. Because of their anatomy, the right and left kidneys cannot be examined in cattle by the same ultrasonographic method [2]. In the current study, the right kidney was biopsied under ultrasound guidance because it is situated too far cranially and close to the body surface, making it accessible to transcutaneous ultrasonographic examination [2]. However, because of the far distance to the left kidney from the body surface, which makes it too far away from the ultrasonographic field [3], the left kidney could not be biopsied under ultrasound visualization. At present, there are no reports related to ultrasound-guided renal biopsy and its safety in cattle. The purpose of this study was to develop and verify the usefulness of a safe ultrasound-guided technique for biopsy of the right kidneys of cattle that can be used in veterinary practice and research.

MATERIALS AND METHODS

Biopsy procedure: Fifty non-pregnant, non-lactating and clinically healthy Holstein cattle were used. A normal platelet count, prothrombin time and activated partial thromboplastin time (APTT) were prerequisites for enrollment into the renal biopsy experiment. In addition, animals with systemic venous congestion, mostly due to right-sided heart failure, were excluded from the study [28–32]. To obtain adequate restraint, the cattle were slightly sedated with xylazine (0.07 mg/kg body weight, intravenously), and the region was infiltrated with 10 m*l* of 2% procaine hydrochlo-

* CORRESPONDENCE TO: OIKAWA, S., Department of Large Animal Clinical Sciences, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyodai-Midorimachi, Ebetsu, Hokkaido 069–8501, Japan.
e-mail: oishin@rakuno.ac.jp

ride. Approval for conduct of this study was obtained from the Animal Welfare Regulation Committee of Rakuno Gakuen University, whose regulations are in conformity with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health in the U.S.A. (NIH publication No. 86–23, revised 1996).

Echo-assisted percutaneous technique for biopsy of the right kidney: 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, Yokogawa Medical Systems, Tokyo, Japan) equipped with a 3.5 MHz linear transducer. The right kidney was scanned to determine the best path for the needle [2]. The needle path was chosen to avoid the liver, large blood vessels and bowel. The 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. Using a free-hand technique, a 14G × 150 mm 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 by identifying the needle directly or, in a few cases, by assessing the movement of tissues as the needle passed through them. Under visualization by ultrasound, the needle was advanced until the capsule of the right kidney was reached. The needle was directed obliquely in an attempt to sample cortical tissue only and avoid the renal medulla, renal pelvis and hilar and renal vessels. When the needle was considered to be in the correct position, that is, touching the renal capsule, the plain stylet was withdrawn and a notched part was inserted and advanced 1 cm into the renal cortex beyond the renal capsule. Often the needle could be identified on the ultrasound within the cortex while the specimen was being obtained, thus confirming the location of the biopsy. 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 immediate or delayed (12 hr later) hemorrhage after biopsy.

Immediate effect of renal biopsy: In this part of the study, analysis was conducted for 45 renal biopsies performed on cattle weighing between 360 and 720 kg. The right kidney was biopsied with the help of the ultrasound-guided technique. The animals were necropsied within 2 hr after the procedure to evaluate possible biopsy-associated pathological changes in the peritoneum, omentum, bowel and kidneys.

Safety of ultrasound-guided biopsy of the right kidney: Five non-pregnant, non-lactating and clinically healthy Holstein cows weighing between 500 and 620 kg were used. All animals were free of alimentary tract disease, as shown by their histories and physical and laboratory examinations. Prior to biopsy, the cows were subjected to preliminary examinations that included a thorough physical examination and tests for complete blood count (CBC), albumin/globulin ratio and the urea nitrogen (UN) and creatinine concentrations. The right kidney was then biopsied. At the end of the procedure, each cow was monitored for several hours, and all urine passed was carefully examined for macroscopic hematuria. After biopsy, the cows were monitored during 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 performed before biopsy and for the following nine days: CBC; the serum activities of aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), creatine kinase (CK), lactate dehydrogenase (LDH); and the 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: The platelet count, prothrombin time and APTT were determined at Kishimoto Clinical Laboratory, Sapporo, Japan. Hemograms were measured using a Particle counter (Model F-820, Sysmex Inc., Tokyo, Japan). Clinical chemistry kits (Wako Pure Chemical Industries, Osaka, Japan) were used to analyze other parameters.

Statistical analysis: The data are expressed as mean values ± SD. All statistical analyses were performed using STATISTICA v 5.5 for Windows (StatSoft, Inc., Tulsa, OK, U.S.A.). The data were tested for normal distribution using the Kolmogarov-Smirnov test. When the data exhibited a normal distribution, the homogeneity of variances was verified using Levene's test. Data were then subjected to one-way analysis of variance (ANOVA) using time as the independent factor and blood variables as dependent factors. Post hoc comparisons (Duncan's multiplerange 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 high in the 12th intercostal space in 18 cattle (90%) and in the right dorsal flank in the remaining 2 animals (10%). The renal medulla appeared to be less echogenic than the rest of the parenchyma. Compared with the liver, the hypoechoic renal parenchyma was well differentiated from the hyperechogenic sinus.

Echo-assisted biopsy of the right kidney allowed us to sample all animals used on the first attempt. Ninety-five percent of the biopsies yielded adequate tissue for histopathological examination. The average length of time for echo-assisted biopsy of the right kidney (from preparing the animal to finishing the procedure) was 25 min, with a range of 15–35 min. No evidence of immediate or delayed (after 12 hr) macroscopic hematuria was observed post-biopsy. At necropsy, six cows had a thin insignificant subcapsular (less than 2 cm) hematoma. All needle tracks were limited to the cortex.

At no time during the nine days of observation of the

cows post-biopsy were appetite, general behavior, rectal temperature or attitude abnormal in any of the animals. In addition, no changes indicative of inflammation were apparent in the total and differential WBC counts or in the albumin/globulin ratio; these results were confirmed at necropsy. Moreover, the hematocrit and hemoglobin values were within the normal ranges and did not differ significantly compared with the pre-biopsy values. The activities of AST, GGT, CK and LDH remained within the reference ranges for all cows throughout the observation period. There were no significant differences in mean serum UN or creatinine post-biopsy (Table 1). No gross changes indicative of peritonitis were seen in any of the cows observed for nine days after biopsy. A small scar was detected on the surface of the kidney at the biopsy site, but it healed without any gross changes.

DISCUSSION

Ultrasound-guided needle biopsy of the kidney has proven to be a valuable aid in clinical evaluation of humans with many renal disorders [9, 25, 38]. Similarly, percutaneous needle aspiration and ultrasound-guided renal biopsy have proven effective in the dog [10, 15, 39] and horse [27]. In cattle, the clinical signs of urinary tract disease may include abnormal urination or non-specific signs associated with infection or azotemia [8]. For this reason, ultrasonographic evaluation of the kidneys and urinary tracts of cattle is valuable in diagnosis of renal disorders. Specific application of ultrasonography has been described for several conditions of the urinary tract in large animals [4–6, 12, 13, 16, 18, 20–22, 33], although early diagnosis of renal diseases requires sampling of the renal tissue.

In the present study, real-time ultrasound-guidance, which provides more accurate localization of the needle in relation to the kidney and subsequent biopsy site in the renal cortex, seemed to be the reason for the low complication rate in biopsy of the right kidney. Moreover, direct ultrasound control allows correction of the needle position at any time during the biopsy procedure [7]. If the needle cannot be seen during ultrasonography, moving the transducer slightly into the path of the needle, gently agitating the needle or injecting air or microbubbles in saline solution through the needle usually allows the needle's position to be determined [24].

Large blood vessels in the kidney increase the likelihood of hemorrhage after percutaneous renal biopsy [11, 19]. Coagulation parameters should be determined before biopsy; however, even with these precautions, complications developed in a study of dogs and cats [1]. The signs of hemorrhage include ultrasonographic evidence of a fluid-filled area, hematuria and a decrease in hematocrit. In the present study, few post-biopsy complications were encountered for echo-assisted biopsy. A small hematoma of no clinical significance was noted at the site of biopsy in all successful biopsies. The low complication rate may have resulted, at least in part, from the fact that ultrasonography allowed highly vascularized parts to be avoided. Knowledge of 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. According to our unpublished data, blind biopsy of the left kidney produced a sufficient amount of materials in only 12 of 25 cases (48%). In addition, there was evidence of hematuria attributable to the biopsy procedure in 4 cases (16%).

Biopsy of the kidney is not an innocuous procedure and should not be used indiscriminately. The fact that serious iatrogenic complications can occur dictates its clinical use only when the information obtained is likely to benefit decisions about patient management. We emphasize that in order to minimize the risks, it is not only important to adopt an adequate biopsy technique, but it is also imperative to exclude high-risk cattle. Performing biopsy of the kidneys is contraindicated in cattle if the animal has a high risk of bleeding. Scanning of the liver may detect abnormalities; however, the animal should also be evaluated for hemostatic disorders prior to biopsy because distention of the hepatic

Table 1. Hematological and biochemical measurements for five cows during the nine-day observation period

Parameters		Days after renal biopsy (day)									
		0	1	2	3	4	5	6	7	8	9
WBC	$\times 10^2/\mu l$	62±13	65±5	65±15	64±11	51±6	58±12	58±12	63±13	62±7	65±12
M Neu	$\times 10^2/\mu l$	24±5	30±6	25±9	20±10	22±8	20±5	16±9	16±11	22±5	18±6
Lym	$\times 10^2/\mu l$	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	mg/d*l*	11.6±2.3	11.1±1.1	11.0±1.2	11.5±2.0	10.5±1.3	11.2±1.1	11.3±0.8	11.9±2.7	10.6±0.9	11.9±1.2
A/G	ratio	0.80±0.06	0.80±0.07	0.80±0.08	0.90±0.03	0.80±0.04	0.80±0.07	0.70±0.01	0.90±0.18	0.80±0.01	0.90±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*	2,463±506	2,480±746	2,280±410	2,493±296	2,285±445	2,517±635	2,340±636	2,280±300	2,430±255	2,363±341
UN	mg/d*l*	10.5±3.1	8.4±1.9	11.2±3.1	9.4±2.5	11.1±5.1	9.3±2.7	9.0±2.6	11.6±5.3	9.8±4.2	9.2±3.5
Cre	mg/d*l*	0.85±0.13	0.85±0.13	0.80±0.01	0.90±0.08	0.80±0.04	0.90±0.22	0.77±0.12	0.90±0.08	0.83±0.12	0.90±0.14

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

veins is a clear indication of enlargement of the liver, which mostly arises from right-sided heart failure. In addition, obstruction of the caudal vena cava by a thrombus or mass is also possible [28, 32].

The results of this study document show that the risk of serious biopsy-induced complications is extremely low when ultrasound visualization is used. Utilizing the ultrasound-guided biopsy technique, biopsy of the right kidney is safe, practical and reliable and requires only one operator. With increased use of ultrasonography and ultrasound-guided techniques, the authors believe that this technique may increase early antemortem diagnosis of suspected renal diseases in cattle. Further study is, however, required to verify differences in the shape of the probe and other biopsy tools.

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