Diagnostic quality of percutaneous fine-needle aspirates and laparoscopic biopsy specimens of the liver in rabbits (Oryctolagus cuniculus)

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Objective—To evaluate diagnostic quality of liver percutaneous ultrasound-guided fine-needle aspirates and laparoscopic biopsy specimens of rabbits (Oryctolagus cuniculus).

Design—Prospective descriptive study.

Animals—7 healthy adult rabbits.

Procedures—3 to 5 liver fine-needle aspirates were obtained with a 22-gauge needle under ultrasound guidance in anesthetized rabbits. Liver biopsy specimens were also obtained with 1.7-mm (n = 2) or 3.0-mm (1) biopsy forceps by direct laparoscopic observation. Fine-needle aspirates were cytologically evaluated on a scale from 0 (suboptimal specimen) to 3 (optimal specimen) for cellularity, cell distribution, cell preservation, cell morphology, and blood contamination. Biopsy specimens were histologically evaluated on a scale from 0 (optimal specimen) to 5 (suboptimal specimen) for artifactual changes; numbers of portal triads and central veins were quantified.

Results—Aspirates were moderately to highly cellular (mean, 2.54) with good cell distribution (mean, 2.56), good cell preservation (mean, 2.20), and moderate blood contamination (mean, 1.04). The 1.7-mm biopsy specimens had a mean score of 1.3 for artifactual changes, and contained a mean of 0.6 portal triads and 1.6 central veins/biopsy specimen. The 3.0-mm liver biopsy specimens had a mean score of 2.7 for artifactual changes, with a mean of 4.0 portal triads and 14.14 central veins/biopsy specimen. All but one 3.0-mm liver biopsy specimen had ≥ 1 portal triad suitable for histologic evaluation, and all had ≥ 1 central vein; in contrast, only half of the 1.7-mm liver biopsy specimens had a discernible portal triad or central vein.

Conclusions and Clinical Relevance—For histologic evaluation, advantages of obtaining 3.0-mm liver biopsy specimens, compared with 1.7-mm liver biopsy specimens or fine-needle aspirates, should be considered in rabbits with suspected liver disease. (J Am Vet Med Assoc 2015;246:313–320)
In companion zoological mammal medicine, the diagnostic approach to liver disease is similar to that of dogs and cats, and microscopic evaluation of tissue morphology is typically required for a definitive diagnosis.\(^6\) Reference range values for common liver biochemical analytes measured in domestic animals, such as pre- and postprandial bile acids and ammonia concentrations, are not available for rabbits, complicating the biochemical evaluation of liver function.

Laparoscopic techniques have been safely performed and described in rabbits (Oryctolagus cuniculus),\(^3,11\) but studies evaluating the safety and quality of different methods in acquiring liver specimens have not been performed. The most appropriate method of liver specimen collection varies depending on the differential diagnoses. Animal safety and diagnostic capability may be mutually exclusive.\(^9\)

Acknowledging the advantages and disadvantages of various techniques for acquiring liver specimens will help clinicians make informed decisions when investigating liver disease in rabbits. Therefore, the objective of the study reported here was to evaluate the diagnostic quality of liver percutaneous ultrasound-guided fine-needle aspirates and laparoscopic biopsy specimens obtained with 1.7- and 3.0-mm biopsy forceps in healthy rabbits.

Materials and Methods

Animals—The study was approved by the University of Georgia Institutional Animal Care and Use Committee (A2012 07-018-Y2-A0). Seven healthy, approximately 6-month-old sexually intact female New Zealand rabbits were used for this prospective study and maintained in conditions approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Rabbits were physically examined on arrival and acclimated to the research facilities for 7 days prior to the start of the study, when CBCs were performed. Food was withheld for 12 hours prior to anesthesia, although access to water was maintained.

Anesthesia—Rabbits were weighed and received midazolam (1 mg/kg [0.45 mg/lb], SC) and butorphanol (0.5 mg/kg [0.22 mg/lb], SC) 30 minutes prior to cephalic vein catheterization. Anesthetic induction and maintenance were achieved with isoflurane in oxygen, delivered by facial mask, to allow for specimen collection via ultrasound-guided fine-needle aspiration. Immediately subsequent to fine-needle aspiration, rabbits were allowed to recover from anesthesia and were transported to the surgery facility, where a second dose of butorphanol (0.5 mg/kg, SC) was administered, and anesthesia induction was accomplished with ketamine (20 mg/kg [9 mg/lb], IV). Following intubation with an appropriately sized cuffed endotracheal tube and a fiberscope, anesthesia was maintained with isoflurane in oxygen, adjusted to individual requirements, and delivered via a pressure-cycle ventilator.\(^5\) Anesthesia depth was monitored by evaluating reflexes (palpebral and corneal), heart and respiratory rates, ECG, end-tidal carbon dioxide concentration, peripheral pulse (radial artery), oxygen saturation, and body temperature via a multiparameter

Ultrasonic-guided fine-needle aspiration—Ultrasoundographic images\(^9\) were acquired with patients in dorsal recumbency. A variable-frequency, 13- to 18-MHz linear transducer\(^7\) with a trapezoid field of view was used. Quantitative measurements of the liver were made in the sagittal and transverse planes with electronic calipers. Ultrasonographic examination of the liver was performed by positioning the transducer caudal to the xiphoid process and angling the probe craniodorsally. Subjective assessment of the liver was made qualitatively on the basis of echogenicity and echotexture, margination, and the presence of typical hepatic and portal vascular markings. Multiple liver fine-needle aspirates (3 to 5) for cytologic evaluation were collected from each rabbit by use of 22-gauge hypodermic needles under ultrasound guidance. All liver ultrasonographic examinations and fine-needle aspirations were performed by the same authors (DS and AS).

Endoscopic equipment and sterilization—For endoscopy, a 30° rigid telescope (27 mm × 18 cm),\(^1\) operating sheath,\(^7\) endoscopic camera,\(^8\) xenon light source,\(^6\) imaging capture system,\(^7\) monitor, endoscopic inflation device,\(^1\) and 3-mm endoscopic instrument\(^8\) were used.
All equipment was cleaned with neutral pH enzymatic detergent and initially gas sterilized with hydrogen peroxide. If multiple surgeries were performed on the same day, the equipment was cold sterilized with 2% glutaraldehyde\(^6\) for 30 minutes and rinsed with sterile water before repeated use.

Surgery—Each rabbit was placed in dorsal recumbency on a heated surgery table\(^6\) maintained at 28° to 30°C (82.4° to 86°F). Following aseptic preparation of the ventral aspect of the abdomen (from xiphoid process to pubis), an approximately 1-cm-long skin incision was made on the midline, 1 to 2 cm caudal to the umbilicus, exposing the linea alba. The musculature was elevated with the use of thumb forceps to lift the body wall away from the viscera, and the linea alba was incised with a No. 15 scalpel blade. Then, the sheathed 30° 2.7-mm-diameter telescope was inserted into the peritoneal cavity toward the right cranial quadrant. According to the Hasson technique, a mattress suture with 2-0 nylon was placed around the sheath to create an airtight seal. Carbon dioxide insufflation was started via the cannula port at 1 to 2 L/min to a pressure of 8 to 10 mm Hg.

After appreciation of the abdominal cavity and observation of the liver, a 1.7-mm biopsy forceps\(^8\) (Figure 1) was inserted down the instrument channel of the operating sheath. Two 1.7-mm liver biopsy specimens were collected from a caudal edge of the liver in each rabbit, avoiding previous aspiration sites (Figure 2). The biopsy site was observed for signs of persistent hemorrhage.

A second 3.0-mm-long skin incision was made 2 to 3 cm cranial and 4 to 5 cm lateral to the first incision, parallel to the linea alba. A 3.5-mm endoscopic threaded imaging port cannula\(^6\) with a trocar\(^6\) was inserted through the skin incision. The telescope was used to
view the entry of the cannula. The trocar was removed and replaced by 3.0-mm biopsy forceps (Figure 1). One liver biopsy specimen was collected from a caudal edge of the liver, taking care to avoid previous biopsy sites (Figure 3). To avoid tissue damage, all tissue specimens were gently shaken from the forceps into a sterile tube containing sterile saline (0.9% NaCl) solution. The liver was observed for signs of persistent hemorrhage.

After the biopsy specimens were taken, the carbon dioxide from insufflation was removed from the peritoneal cavity by manual expression. The telescope, cannulae, and mattress suture were removed, and the muscle and skin were closed in 2 layers in a simple interrupted and subcuticular pattern, respectively, with 3-0 poliglecaprone 25. All surgeries were performed together by 2 of the authors (LMP and SJD).

Postoperative care—All rabbits received lactated Ringer’s solution (100 mL/kg [45.5 mL/lb], SC) immediately after surgery and meloxicam (0.2 mg/kg [0.09 mg/lb], PO, q 24 h) for 3 days. No Elizabethan collars were used after surgery. Surgery sites, general behavior, and food intake were monitored for 7 days. Any rabbit not defecating was administered additional fluid therapy SC on a daily basis and received assisted feedings via syringe until improvement was observed. Postoperative PCV assessment was performed 48 hours after surgery.

Cytologic evaluation of fine-needle aspirates—Immediately following aspirate collection, slides for cytologic evaluation were prepared and allowed to air-dry and then stained with modified Wright stain. All cytologic specimens were evaluated by a single clinical pathologist (MC). Slides were assessed on a semiquantitative scoring system (scale from 0 to 3) for 5 criteria: cellularity (0 = acellular, containing no hepatocytes; 3 = highly cellular), cell distribution (0 = unusable because of cellular clumping; 3 = well distributed), cell preservation (0 = unusable because of virtually all broken cells; 3 = virtually all cells intact), cell morphology (0 = unusable because of poor cellular detail; 3 = optimal), and blood contamination (0 = abundant blood contamination; 3 = no blood contamination).

Histologic evaluation of biopsy specimens—Immediately following collection, liver biopsy specimens were transferred from saline solution to cassettes and fixed in neutral-buffered 10% formalin for ≥ 24
All formalin-fixed biopsy specimens were measured and placed directly into cassettes without trimming because of their small size. Thereafter, specimens were processed routinely and embedded in paraffin, and 4-µm-thick sections were stained with H&E.

Each biopsy specimen (1 section each) was evaluated by a single pathologist (NN) at 40X and 100X magnification for artifactual changes (eg, tissue fragmenta-
tion or loss), 100X for quantification of portal triads and central veins, and 400X for cellular detail. Microscopic lesions, if present, were noted.

The numbers of discernible central veins and portal triads were counted for each biopsy specimen. Central veins and portal triads that were partially cut or distorted by tissue crush artifact near biopsy edges were not counted because they could not be adequately evaluated. Mean numbers of central veins and portal triads were calculated across all rabbits for each of the 2 sizes of biopsy specimens.

Artifacts were graded for each biopsy on the basis of the amount of tissue affected by tissue crush artifact (associated with collection during surgery) and tissue fragmenta-
tion or loss (associated with tissue processing after fixation). The grading scheme ranged from 0 to 5 as follows: 0 = no observable tissue artifact; 1 = tissue minimally affected (approx < 10%); 2 = 10% to 25% of tissue affected; 3 = > 25% to 50% of tissue affected; 4 = > 50% to 75% of tissue affected; and 5 = > 75% to 100% of tissue affected. Mean artifact scores were calculated across all rabbits for both sizes of biopsy specimens.

Results

Surgery—Mean preoperative body weight of the 7 rabbits was 2.45 kg (5.39 lb), ranging from 2.22 (4.88 lb) to 2.68 kg (5.90 lb). Results of preoperative physical examination and CBC revealed no abnormalities. Mean preoperative Hct was 37.9% (range, 34.7% to 40.5%). All surgeries were uneventful with no appreciated difficulties or complications. Mild hemoabdomen was observed in all rabbits and attributed to the fine-needle aspiration.

All but 1 rabbit recovered from anesthesia. The single rabbit that died went into cardiac arrest moments after extubation. Necropsy findings were consistent with acute pulmonary aspiration during anesthetic recovery. No evidence of surgical complications was observed, and the death was considered anesthesia related.

All remaining rabbits were observed eating and defecating within 24 hours after surgery, except for 1 rabbit, which was observed defecating within 48 hours. On day 1 after surgery, the midline suture of 1 rabbit was missing, believed to have been removed by the rabbit. No evisceration, discharge, or hemorrhage was observed. The site was vigorously flushed with sterile saline solution, and suture glue was applied to promote healing. One rabbit developed a small (approx 1 X 1 cm) seroma in the area of the midline incision, which was resolved without additional intervention within a few days. Postoperative mean PCV of the remaining 6 rabbits was 37.7% (range, 34% to 42%).

Ultrasonography—On the basis of the subjective criteria, livers of all rabbits were deemed to be ultrasonographically unremarkable. The mean ± SD midline sagittal dorsoventral liver thickness measurement was 23.8 ± 5.9 mm. The mean midline transverse dorsoventral liver thickness measurement was 21.5 ± 4.7 mm. To the authors’ knowledge, no previous reference ranges for ultrasonographic measurements of livers for rabbits have been established.

Cytologic evaluation—Twenty-five fine-needle aspirate slides were examined (Figure 4). The mean and median scores for cellularity were 1.88 (range, 0 to 3) and 2.00, respectively. This mean was lowered by the fact that 3 slides contained no hepatocytes and were assigned a score of 0. The mean and median scores for cell distribution were 2.56 (range, 2 to 3) and 3.00, re-
The mean and median scores for cell preservation were 2.20 (range, 0 to 3) and 3.00, respectively. The mean and median scores for cell morphology were 2.24 (range, 0 to 3) and 2.00, respectively. This mean value, however, is likely inaccurately lowered as a result of the inclusion of the 3 slides lacking hepatocytes, which were assigned a morphology score of 0. If these 3 specimens are excluded, the mean score for cellularity was 2.54 (range, 2 to 3). The mean and median scores for blood contamination were 1.04 (range, 0 to 2) and 1.00, respectively.

Histologic evaluation—Formalin-fixed 1.7-mm biopsy specimens were uniform in shape (round cylinder) and generally between 1.5 and 2.0 mm, and 2 of the 14 specimens were slightly smaller (i.e., 1.0 mm) along 1 edge. The 3.0-mm biopsy specimens varied in shape (ovoid, disk-shaped, rectangular, and wedge) and size (from 4.0 × 2.0 × 2.0 mm to 8.0 × 7.0 × 2.0 mm).

The range in the number of portal triads per 1.7-mm biopsy specimen was 0 to 2 (mean, 0.6; median, 0.50), with 0 to 8 central veins (mean, 1.6; median, 1.00). Seven of fourteen 1.7-mm biopsy specimens had no discernible portal triads, and 5 of 14 had no discernible central veins; the observation of 8 central veins was rare and was only observed in one 1.7-mm biopsy specimen. For the 3.0-mm biopsy specimens, the range of portal triads was 0 to 10 (mean, 4.0; median, 5.0), with 1 to 9 central veins (mean, 4.1; median, 3.0). All but one 3.0-mm biopsy had ≥1 portal triad suitable for evaluation.

On a scale of 0 to 5, with 0 indicative of no discernible tissue artifacts to 5 having >75% to 100% of tissue affected owing to disruption of architecture from multifocal fragmentation and separation of tissue, the mean and median artifact scores for 1.7-mm biopsy specimens were 1.3 (range, 0 to 3) and 1.00, respectively. The mean and median artifact scores for 3.0-mm biopsy specimens were 2.7 (range, 1 to 4) and 3.00, respectively. Despite the higher mean artifact score, all 3.0-mm biopsy specimens had adequate hepatocellular detail. Hepatocellular detail was subjectively distorted in some of the 1.7-mm biopsy specimens, attributed to

![Figure 4](image1.png)  
**Figure 4**—Representative photomicrographs of cytologically normal fine-needle aspirates from the liver of a rabbit. **A**—A large cluster of well-differentiated hepatocytes is present among a background of peripheral blood contamination. Occasional binucleated hepatocytes are evident. Modified Wright stain; bar = 50 µm. **B**—Closer magnification image of the same specimen, in which the nuclear and cytoplasmic details of the well-differentiated hepatocytes are more apparent. Modified Wright stain; bar = 25 µm.

![Figure 5](image2.png)  
**Figure 5**—Representative photomicrographs of liver biopsy specimens from the same rabbit as in Figure 4 at the same magnification for size comparison. **A**—Photomicrograph of a 1.7-mm liver biopsy specimen demonstrating specimen collection artifacts, including fragmented tissue (arrowheads) and crushed tissue (arrows) along the edge of the specimen. **B**—Photomicrograph of a 3.0-mm liver biopsy specimen demonstrating multiple postfixation artifacts, including fragmented tissue (arrowheads). H&E stain; bar = 200 µm.
crush artifact. Three of the fourteen 1.7-mm biopsy specimens had severe crush artifact, consistent with tissue collection. The artifact consisted of moderately compressed sinusoids and gave the false appearance of lipid vacuoles. One of these specimens also had distorted hepatic cords. Photomicrographs of 1.7- and 3.0-mm liver biopsy specimens were obtained to demonstrate the type of postfixation artifacts between the 2 sizes of biopsy specimens (Figure 5).

Mild subcapsular fibrosis was seen in the 3.0-mm biopsy specimens of 2 of the rabbits, and 1 of these as well as 1 additional rabbit also had mild, multifocal periportal fibrosis that also evident in the 3.0-mm biopsy specimens, but not the 1.7-mm biopsy specimens. Many hepatocytes in both the 1.7- and 3.0-mm biopsy specimens from another rabbit had hepatocellular, intracytoplasmic vacuoles that were both clear and round (suggestive of lipid) and irregular and flocculent (suggestive of glycogen). Microscopic lesions found in the rabbits of the present study were not considered clinically relevant.

Discussion

The combined goal of the clinician and pathologist is to perform a minimally invasive procedure that is diagnostically rewarding. The most appropriate diagnostic specimen varies depending on the differential diagnoses. In the most extreme cases, animal safety and diagnostic capability may be mutually exclusive. However, in general, safe collection methods can be used to obtain useful specimens for cytologic or histologic evaluation to arrive at the most accurate diagnosis and prognosis possible.

In the present study, ultrasound-guided fine-needle aspiration proved to be a safe method of liver specimen collection in rabbits with no evident clinical disease, requiring mild sedation and short-term general anesthesia. Mild hemoabdomen was observed after liver fine-needle aspiration in all rabbits, suggesting that despite the advantages of being fast and minimally invasive, the procedure is not completely innocuous and hemorrhage might represent a complication when coagulopathies or liver disease is present.

Although information can be gained from fine-needle aspirates of the liver, limitations of this diagnostic modality should be recognized. The usefulness of fine-needle aspiration for cytologic evaluation is extremely location dependent. When evaluating a large organ, such as the liver, it is possible to miss focal or multifocal disease (eg, an abscess or neoplasm) by aspiration of microscopically unremarkable tissue adjacent to the lesion. Cytologic evaluation is typically better suited for diagnosis of diffuse diseases (eg, hepatic lipidosis). It is also possible that the cells in aspirated tissue do not exfoliate well and are subsequently absent in the associated slides.1 3 Suboptimal exfoliation typically occurs with aspiration of mesenchymal tissues, but it can occur with epithelial cells, as evidenced in the present study by the 3 aspirates that contained no hepatocytes, in which aspiration of the liver could not be confirmed and liver evaluation was not possible.

Cytologic interpretation also depends heavily on well-distributed and well-stained specimens. Highly cellular specimens with poor cell distribution can be largely unusable, as the pathologist may be unable to discern cellular details, such as individual cell morphology. Similarly, if cells are broken during aspiration or spread, they cannot be interpreted, given that broken cells often imbibe water, obscuring cellular details. Additionally, if abundant blood contamination occurs secondary to the aspiration procedure, erythrocytes can obscure the tissue and preclude accurate interpretation. Finally, the presence of leukocytes must be interpreted with consideration of peripheral leukocyte counts, to prevent an inaccurate diagnosis of inflammation.1 3

In the present study, the overall quality of the liver aspirates in which liver cells were obtained was good. Slides were typically moderately to highly cellular with good cell distribution and minimal cell breakage. However, there was often a moderate amount of blood contamination that made morphological evaluation more difficult. This potentially could have been minimized by use of a smaller gauge needle for aspiration, recognizing that this would likely have yielded less cellular specimens. As a general rule, blood contamination worsens with each aspiration. Therefore, evaluation of the first of a series of aspirates may be most useful when multiple aspirates are submitted.

Despite the widespread use of ultrasound-guided fine-needle aspiration of the liver, cytologic evaluation alone can lead to a misdiagnosis for certain primary liver diseases, compared with histologic evaluation of liver biopsy specimens from dogs and cats. Results of a retrospective study1 with 56 dogs and 41 cats with signs of liver disease showed that findings on cytologic evaluation of aspirates versus findings on histologic evaluation of biopsy specimens agreed in only 17 of 56 (30.4%) dogs and 21 of 41 (51.2%) cats. Vascular hepatopathy was the most frequent misdiagnosis via cytologic evaluation in cats. A consistent cytologic diagnosis of diffuse neoplasia was limited to dogs with carcinoma and cats with lymphoma.1 Results of another comparative retrospective study3 including 56 dogs and cats showed that complete agreement between cytologic and histologic evaluation occurred in only 34 of 56 (61%) animals. Disagreement between evaluation techniques involved the disease conditions of fibrosis, amyloidosis, hemangiosarcoma, and lymphoma.3

In the present study, laparoscopy proved to be a safe and effective technique for collection of liver biopsy specimens in healthy rabbits. No surgical complications were encountered, and all necessary specimens were safely collected. Minor to mild, self-limiting bleeding occurred after obtaining 1.7- and 3.0-mm biopsy specimens. All the rabbits were healthy and had no evidence of coagulopathies (eg, thrombocytopenia) or severe manifestations of liver disease. Laparoscopic liver biopsy was shown to be safe in a retrospective study8 of 80 dogs with suspected liver disease. The technique resulted in low morbidity and mortality rates (4/80), and none of the dogs had hemorrhage sufficient to require conversion to laparotomy. Instead, when needed, hemorrhage was controlled by applying pressure with a blunt probe or applying absorbable gelatin material.8

Although not required in the present study, conversion from a laparoscopy to a laparotomy may be nec-
thetic recovery, recovered quickly from surgery, required no additional treatment and for preventing further complications such as evisceration. The suture removal was easily managed because another rabbit developed a seroma on the midline incision, and was zero in some cases. However, as expected, the number of portal triads and central veins for both sizes of biopsy specimens varied widely.

On the basis of histologic evaluation of biopsy specimens with quantification of architectural landmarks and subjective analyses, numerous conclusions can be drawn about the diagnostic usefulness of liver biopsy specimens and the advantages and disadvantages of a smaller versus a larger biopsy specimen. In the present study, the number of portal triads and central veins for both sizes of biopsy specimens varied widely and was zero in some cases. However, as expected, the numbers of both architectural landmarks were greater for the larger biopsy specimens. The size of the specimens obtained with the 1.7-mm biopsy forceps following formalin fixation was relatively consistent (generally between 1.5 and 2.0 mm), compared with the size of the specimens obtained with the 3.0-mm biopsy forceps, which were at least 4.0 mm (and up to 8.0 mm) in length. However, the maximum calculated volume of a specimen obtained with 3-mm biopsy forceps is approximately 20 mm³ (ie, 10 times as large as that obtained with 1.7-mm biopsy forceps).

A retrospective study of 66 dogs with suspected liver disease that underwent laparoscopic liver biopsy, which was compatible with the recommendations in human medicine, showed that all liver biopsy specimens had from 8 to 13 portal triads. However, these specimens were taken with 5.0-mm biopsy forceps, instead of the 1.7- or 3.0-mm biopsy forceps as used in the present study. The sizes of the actual biopsy specimens collected in that study were not provided.

A prospective study of 12 dogs comparing 5 liver biopsy methods showed that use of a 4-mm biopsy punch and 16-gauge biopsy needle produced <6 portal triads/specimen, compared with >20 portal triads/specimen obtained with the ligature method, >14 portal triads/specimen when clamshell laparoscopic biopsy forceps (size of the specimen not provided) was used, and >29 portal triads/specimen when an ultrasonic-activated scalpel was used. The median number of portal triads found in a prospective study of 98 dogs and 26 cats when an 18-gauge biopsy needle was used was 4 portal triads/specimen (range, 0 to 8 portal triads/specimen for dogs and 0 to 11 portal triads/specimen for cats), suggesting that needle liver biopsy specimens of dogs and cats must be interpreted with caution.

For the 2 cited studies involving dogs, the mean weights of the animals (25 and 17.9 kg [55 and 39.38 lb]) were substantially greater than the weight of the rabbits (2.45 kg) in the present study. The size of the animal directly impacts the size of endoscopic equipment that can be used. Although larger laparoscopic equipment could be potentially used in rabbits (ie, 5.0-mm telescope), the choice of the equipment used in the present study (2.7-mm telescope) was made on the basis of the most common telescope system used in exotic animal medicine. The authors considered use of this equipment of great importance when evaluating laparoscopic liver biopsy technique in rabbits, considering that this will most likely be the instrument available to clinicians who work with exotic animal species.

Most 3.0-mm biopsy specimens had greater variation in size but provided more continuous tissue for evaluation than two 1.7-mm biopsy specimens. Further, all but one 3.0-mm biopsy had ≥1 portal triad suitable for evaluation, and all had ≥1 central veins. In contrast, half of the 1.7-mm biopsy specimens had no discernible portal triads, and many also had no discernible central veins for evaluation. Subjectively, 3.0-mm biopsy specimens had superior hepatocellular detail on histologic evaluation than the smaller biopsy specimens, most likely as the result of the greater effects of tissue crush artifact on the smaller specimens.

 obscuring of hepatocellular detail was likely the result of prefixation collection-induced crush artifact. The larger size of the 3.0-mm biopsy specimens allows for tissue evaluation even with some crush artifact. However, even these specimens were susceptible to tissue crush artifact in some cases and were more likely to undergo postfixation artifact (eg, fragmentation) dur
ing routine histologic processing of paraffin-embedded specimens. Therefore, gentle tissue collection and handling of biopsy specimens is crucial for the most accurate assessment by the pathologist.

Similar to cytologic specimens, results from the present study suggested that 1.7-mm biopsy specimens are suitable when the suspected disease processes would most likely involve diffuse distribution throughout the liver, such as in cases of hepatic lipidosis, diffuse neoplasia, toxic insult (eg, aflatoxicosis, lead, or NSAIDs), or diffuse cirrhosis. The smaller biopsy specimens may also be useful for evaluation of focal, potentially large lesions such as abscesses or primary liver tumors when specimen collection is aided by additional diagnostic modalities such as imaging. For some infectious diseases (eg, lymphoma, bile duct carcinoma or adenocarcinoma, and metastases), evaluation of numerous portal triads and central veins is important; therefore, larger biopsy specimens are indicated. In some cases, neoplasms and other diseases may be associated with a specific distribution pattern, such as perportal lymphocytic infiltrates with lymphoma, which could be missed with 1.7-mm biopsy specimens, given their frequent lack of portal triads. Detection of a neoplastic focus, infectious process, or parasitic migratory tract or life stage can be easily missed during ante-mortem specimen collection by fine-needle aspiration for cytologic evaluation or biopsy for histologic evaluation. A distinct advantage of biopsy over fine-needle aspiration is long-term archiving of the tissue-embedded paraffin blocks and the ability to cut additional slides for additional diagnostic testing, including special histochemical stains to view bacteria, fungi, or protozoa; immunohistochemical staining with neoplastic or infectious organism markers; or PCR assay.

In conclusion, cytologic evaluation of liver specimens is a good overall diagnostic modality when looking for evidence of diffuse disease that does not require evaluation of architecture, for the reason that cytologic interpretation depends largely on individual cell morphology. It is a rapid, less expensive, minimally invasive technique that works well if the aspirated tissue exfoliates well and the associated slides are well prepared, with good cell distribution, minimal breakage, and limited blood contamination. Alternatively, for diseases more likely to have a multifocal distribution or when evaluation of tissue architecture is important for a diagnosis, biopsy is indicated. Although larger biopsy specimen sizes provide more tissue and therefore an increased potential for detection of disease processes, the risk to the patient must be considered. Alternatively, multiple small biopsy specimens (eg, 1.7 mm) could provide a larger amount of tissue or multiple sites for evaluation. Future studies in rabbits with liver disease are necessary to better evaluate the best method of liver specimen collection as well as associated advantages and disadvantages.

References