Evaluation of an endoscopic liver biopsy technique in green iguanas

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Objective—To establish a safe and effective endoscopic technique for collection of liver biopsy specimens from lizards by use of a 2.7-mm rigid endoscope system that is commonly available in zoologic veterinary practice.

Design—Prospective study.

Animals—11 subadult male green iguanas (*Iguana iguana*).

Procedures—Each lizard was anesthetized, and right-sided coelioscopic examination of the right liver lobe and gallbladder was performed. Three liver biopsy specimens were collected from each lizard by use of a 2.7-mm rigid endoscope and 1.7-mm (5-F) biopsy forceps. Biopsy samples were evaluated histologically for quality and crush artifact. Ten days following surgery, all iguanas were euthanatized and underwent full necropsy examination.

Results—For all 11 iguanas, the right liver lobe and gallbladder were successfully examined endoscopically, and 3 biopsy specimens of the liver were collected without complications. Mean ± SD durations of anesthesia and surgery were 24 ± 7 minutes and 6.8 ± 1.0 minutes, respectively. At necropsy, there was no evidence of trauma or disease associated with the skin or muscle entry sites, liver, or any visceral structures in any iguana. All 33 biopsy specimens were considered acceptable for histologic interpretation; in most samples, the extent of crush artifact was considered minimal.

Conclusions and Clinical Relevance—By use of a 2.7-mm rigid endoscope, liver biopsy procedures can be performed safely, swiftly, and easily in green iguanas. Biopsy specimens obtained by this technique are suitable for histologic examination. For evaluation of the liver and biopsy specimen collection in lizards, endoscopy is recommended. (*J Am Vet Med Assoc* 2007;230:1849–1853)

In small animal medicine, various antemortem techniques for the collection of liver tissue samples for diagnostic purposes have been tried and tested. Currently, percutaneous, endoscopic, or open surgical procedures are most commonly used to collect samples for cytologic or histologic examination.[1-3] Percutaneous biopsy procedures allow liver tissue samples to be safely collected by use of biopsy needles in dogs that weigh > 10 kg (22 lb); fine-needle aspiration by use of smaller gauge hypodermic needles is preferred in smaller animals.[1,2] In most instances, collection of such samples is performed with ultrasound guidance. Comparisons of liver biopsy specimens and fine-needle aspiration samples obtained from dogs and cats have revealed that findings of cytologic examination of aspirates is only diagnostic in 30% to 61% of samples, compared with findings of histologic examination of tissue specimens.[4,5] Compared with wedge tissue samples collected surgically, large-gauge–needle biopsy specimens yielded a diagnosis in only 48% of cases, and findings from examination of the latter should be interpreted with caution.[6] Although the diagnostic value of surgical biopsy procedures is greater than that of needle biopsy and fine-needle aspiration techniques, the invasiveness of those procedures is also greater; however, that disadvantage has been largely overcome since the development of minimally invasive endoscopic techniques.[3,7,8] Retrospective studies[9,10] in humans have revealed comparable diagnosis rates for laparoscopic and laparotomy-associated liver biopsy procedures, but application of endoscopy has the advantage of decreased duration of hospitalization.

In reptile medicine, the diagnostic approach to liver disease is similar to that of domesticated animals, and typically, results of an examination of liver tissue samples are required for a definitive diagnosis.[11] Large-gauge–needle biopsy is seldom performed in small reptiles because of the dangers of iatrogenic trauma, and ultrasound-guided fine-needle aspiration frequently yields specimens from which a diagnosis cannot be made because of the difficulties of microscopic interpretation without tissue architecture. In snakes that weigh > 1 kg (2.2 lb), percutaneous ultrasound-guided needle biopsy has provided diagnostic samples; however, 2 to 4 attempts were required to obtain liver tissue, and penetration of the gastrointestinal tract was a

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complication.[12] The failure of the procedure was associated with movement of the snake, and anesthesia was therefore deemed essential. Endoscopic liver biopsy has been advocated for a variety of reptile species, even in animals that weigh ≤ 100 g (0.22 lb).[13-16] Anesthesia is required, but to our knowledge, no complications have been reported with use of appropriate anesthetic techniques. The purpose of the study reported here was to establish a safe and effective endoscopic technique for collection of liver biopsy specimens from lizards by use of a 2.7-mm rigid endoscope system that is commonly available in zoologic veterinary practice.

Materials and Methods

Animals—The study protocol was approved by the University of Georgia's Institutional Animal Care and Use Committee (IACUC No. A2003-10074-m2). Eleven healthy subadult male green iguanas (*Iguana iguana*) were maintained in conditions approved by the Association for Assessment and Accreditation of Laboratory Animal Care. The iguanas were housed individually and maintained in a room with an ambient temperature of 23.8°C (75°F) at night and 27.2°C (81°F) during the day. A mercury-halide incandescent lamp suspended above each enclosure provided a daytime basking area (35°C [95°F]) and broad-spectrum lighting. The iguanas were exposed to cycles of 12 hours of light followed by 12 hours of dark. The diet consisted of commercial iguana pellets[a] soaked in water and supplements of several varieties of lettuce, collard greens, cabbage, and kale; water was available at all times. General environmental humidity was maintained at 80% through daily spraying. The iguanas were physically examined on arrival and acclimatized to the research facilities for 7 days prior to the start of the study. Food was withheld from all 11 iguanas for 48 hours prior to anesthesia, although access to water was maintained.

Anesthesia—Iguanas were accurately weighed and received butorphanol (1 mg/kg [0.45 mg/lb], IM) 20 minutes prior to induction of anesthesia with propofol (10 mg/kg [4.5 mg/lb], IV). Following intubation, anesthesia was maintained by use of isoflurane in oxygen, adjusted to the requirements of each iguana, and delivered via a pressure-cycle ventilator.[b] The risk of development of hypothermia was reduced by maintaining the anesthesia and surgery areas at 23.8°C and placing the iguanas on recirculating warm water blankets. Anesthetic depth was monitored by evaluating reflexes, heart rate, end-tidal CO_2 concentration, peripheral pulse, and oxygen saturation (as measured by pulse oximetry).

Surgery—Iguanas were positioned in left lateral recumbency (dorsum facing the surgeon) on a level surgery table (**Figure 1**). Following aseptic preparation of the right flank, a 3-mm vertical skin incision was made in the center of the paralumbar region. Then, with the surgeon pinching the skin and underlying external oblique musculature, a 4.8-mm (14.5-F) operating sheath with obturator[c] was inserted through the skin incision, directed craniad through the external abdominal oblique musculature, and placed into the coelomic cavity. The obturator was removed and replaced with a 30° telescope[d] that was connected to an endoscopic video system[e] (including camera, monitor, xenon light source, and CO_2 insufflator); CO_2 insufflation flow and pressures were set to 0.5 L/min and 3 to 5 mm Hg, respectively. Once the coelom was inflated, the liver was identified and the entire right liver lobe and gallbladder were examined. By use of 1.7-mm (5-F) biopsy forceps[f] inserted down the instrument channel of the operating sheath, 3 liver biopsy specimens were collected from the caudal edge of the right liver lobe. Tissue samples were transferred from the forceps to a histology cassette by use of a sterile cotton-tipped applicator moistened with sterile saline (0.9% NaCl) solution and then immediately placed in neutral-buffered 10% formalin. Following the manual expression of coelomic CO_2, the telescope and sheath were removed and the skin incision was closed by use of 3-0 polydioxanone suture in a horizontal mattress pattern. Certain intervals were recorded to the nearest second as follows: time from initial skin incision to insufflation and first clear observation of the liver, time from first clear observation of the liver to completion of the examination of the right lobe and gallbladder, time from start to completion of collection of all 3 liver biopsy specimens, and time from start of CO_2 expression from the coelom to completion of skin incision closure. Duration of anesthesia was defined as the time from propofol injection to return to spontaneous respiration following cessation of isoflurane administration and was measured to the nearest minute. In addition, any complications associated with the anesthesia or surgical procedures were recorded. After recovery from anesthesia, the iguanas were returned to their enclosures. General behavior and food intake were monitored for 10 days.

Necropsy and tissue sample processing and examination—Ten days following surgery, all iguanas were weighed and then euthanized via IV administration of pentobarbital sodium. Each iguana underwent a full necropsy examination. The liver was evaluated for any evidence of trauma or disease; liver tissue samples (along with any other tissues of abnormal appearance) were collected for histologic examination. Biopsy specimens and tissue samples obtained during necropsy were processed routinely, embedded in paraffin, sectioned at 5 µm, stained with H&E, and examined microscopically. For each biopsy specimen, the degree of crush artifact (ie, damage resulting in an inability to recognize

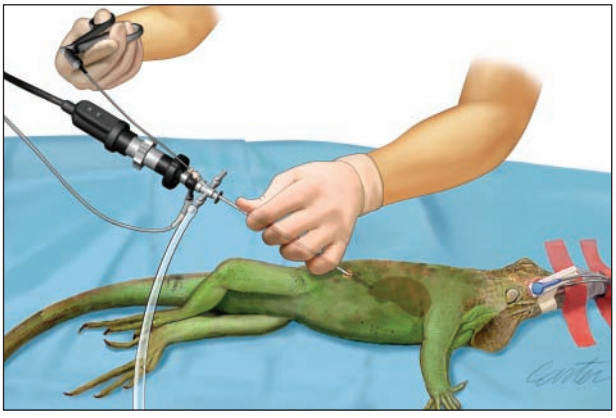

Figure 1—Illustration of an endoscopic liver biopsy procedure in a green iguana (Prepared by Kip Carter; printed with permission from Educational Resources, College of Veterinary Medicine, University of Georgia, Athens, Ga).

cell types or evaluate the hepatic parenchyma) in each section was graded as follows: minimal, ≤ 10% affected; mild, 11% to 20% affected; moderate, 21% to 50% affected; and severe, ≥ 51% affected. The number of portal triads included in each section was also counted.

Results

Before surgery, the mean ± SD iguana body weight was 441 ± 42 g (0.97 ± 0.092 lb). Complete endoscopic examination of the right liver lobe and gallbladder and liver biopsy procedures were completed successfully and without complication in all iguanas (**Figure 2**). The mean time from initial skin incision to insufflation and first clear observation of the liver was 95 ± 31 seconds. Time from first clear observation of the liver to completion of the examination of the right lobe and gallbladder was 44 ± 9 seconds. Time from start to completion of collection of all 3 liver biopsy specimens was 209 ± 57 seconds, and time from start of CO_2 expression from the coelom to completion of skin incision closure was 57 ± 17 seconds. Duration of anesthesia and surgery was 24 ± 7 minutes and 6.8 ± 1.0 minutes, respectively. Recovery from anesthesia was complete and uneventful in all iguanas. All iguanas returned to apparently normal behavior and feeding patterns by the day following surgery; behavior and feeding remained unchanged until the time of euthanasia 10 days after surgery. At that time, the mean body weight was 449 ± 54 g (0.988 ± 0.119 lb).

At necropsy, there was no clinically important evidence of trauma or disease associated with the skin or muscle entry sites, liver, or any other visceral structures in any iguana. In 4 iguanas, 1 or more small (1- to 2-mm-long) fibrin tags were detected at the endoscopic biopsy sites. In the remaining 7 iguanas, there was no gross evidence of the endoscopic biopsy sites.

Three liver biopsy specimens from each iguana were examined histologically (33 evaluations overall). Crush artifact did not affect > 50% of any tissue section. Among the 33 biopsy specimens, sections of 8 (24.2%) had minimal crush artifact (**Figure 3**), sections of 11 (33.3%) had mild crush artifact, and sections of 14 (42.4%) had moderate crush artifact. In each instance, the crush artifact was confined to the periphery of the section and there was a central area of intact and undamaged parenchyma that could be evaluated histologically. The mean number of portal triads per section was 1.96. Triads were more frequently observed in sections with minimal crush artifact (2.9 triads/section) and mild crush artifact (1.9 triads/section) than in sections with moderate crush artifact (1.5 triads/section). No triads were apparent in 3 sections with moderate crush artifact; triads may have been present but unrecognizable in these sections.

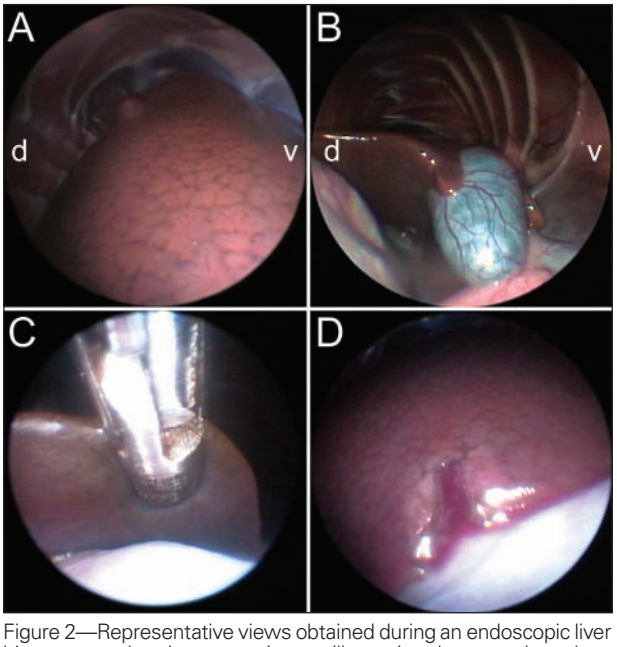

Figure 2—Representative views obtained during an endoscopic liver biopsy procedure in a green iguana illustrating the ventrolateral aspect of the right liver lobe (A), gallbladder and caudal edge of the right liver lobe (B), the caudal edge of the right liver lobe during biopsy specimen collection by use of 1.7-mm biopsy forceps (C), and the liver after completion of the biopsy procedure (D). For orientation, dorsal and ventral aspects of views A and B have been identified (d and v, respectively).

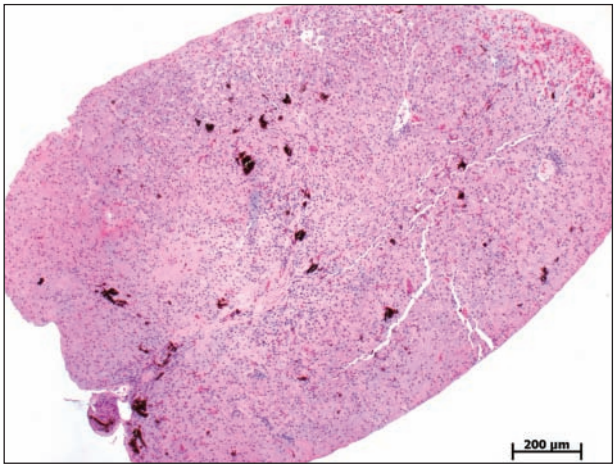

Figure 3—Photomicrograph of a section of a liver biopsy specimen that was obtained endoscopically from an iguana. The specimen has minimal crush artifact, which is confined to the periphery of the section. H&E stain; bar = 200 µm.

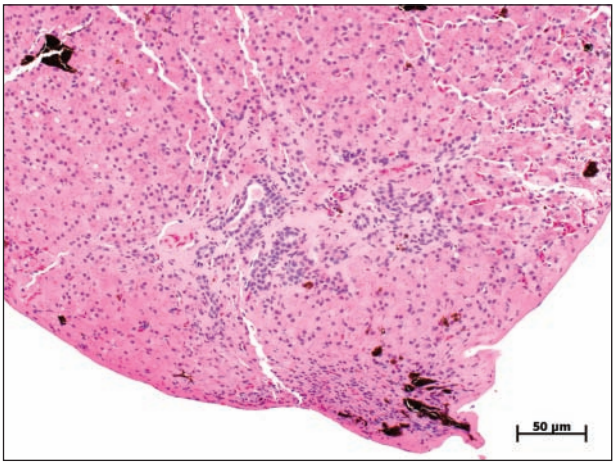

Figure 4—Photomicrograph of a section of a liver biopsy specimen obtained endoscopically from an iguana. Mild hyperplasia of the biliary ductules is apparent. H&E stain; bar = 50 µm.

Mild hyperplasia of the biliary ductules was detected in 1 biopsy specimen obtained from 1 iguana (**Figure 4**), and this finding was confirmed via histologic examination of the liver tissue specimen obtained at necropsy. Histologic abnormalities were not detected in any other biopsy specimen sections or sections of liver tissue collected at necropsy.

Discussion

The minimally invasive liver biopsy procedure described in this report was based on accepted endoscopic methods for reptiles, and for iguanas in particular.[13,14,16] Endoscopic liver biopsy was safe, simple to perform with appropriate equipment, and yielded tissue samples suitable for histologic interpretation in the present study. Coelioscopic examination (performed from the right side) provided excellent views not only of the right liver lobe and gallbladder, but also of portions of the gastrointestinal, urogenital, and cardiorespiratory systems. However, if the liver was the sole organ of interest in a particular iguana, a ventral approach (performed with care to avoid the midline abdominal vein) would permit evaluation of both the left and right liver lobes.

In small reptiles, ultrasound-guided fine-needle aspiration of the liver is possible but seldom recommended because practitioners are typically less familiar with their anatomic features; thus, the risk of iatrogenic damage resulting from the procedure is increased. In addition, cytologic interpretation of aspirates from reptiles is often difficult; therefore, there is a preference for histologic samples that preserve tissue architecture. Endoscopy and needle biopsy both require that the patient is anesthetized and yield biopsy specimens of comparable histologic quality; however, endoscopy may be preferable because direct observation of the organ of interest during sample collection reduces the risk of iatrogenic trauma.[12,13,16]

The size of the biopsy specimen is dictated by the size of the endoscopic forceps used. The expected tissue volumes from 1-mm (3-F), 1.7-mm (5-F), 3-mm (9-F), and 5-mm (15-F) biopsy forceps would be 0.5, 2.4, 14.1, and 65.4 mm^3, respectively. Whereas the biopsy specimens collected from iguanas in the present study were small (2.4-mm^3 volume), they were considered appropriate for small-sized iguanas (mean weight, 441 g) and did yield tissue that was suitable for histologic examination and histopathologic interpretation. In larger reptiles, the use of 3- or 5-mm forceps may be preferable, and a 1-mm instrument may be better suited for use in reptiles that weigh < 100 g.

Although retrospective human studies[9,10,17] have revealed that evaluation of liver tissue collected during endoscopy is associated with a diagnosis rate for liver disease of nearly 98%, such data are currently lacking for reptiles. In the present study, biliary hyperplasia was correctly diagnosed in 1 iguana via examination of biopsy specimens and confirmed via examination of tissue specimens obtain during necropsy. However, with regard to liver disease among iguanas or other small reptiles, further research involving comparisons between biopsy specimens obtained endoscopically and tissue samples obtained during surgery or necropsy is needed before the diagnostic capability of coelioscopic techniques can be definitively determined. Our clinical experience with a wide variety of reptile species has indicated that endoscopic collection of visceral biopsy specimens can be of considerable benefit; in the face of equivocal clinicopathologic results, examination of those tissue samples often enables a diagnosis to be made.[18-20] Overall, it appears that endoscopic liver biopsy in iguanid lizards can be recommended for the collection of tissue samples that are suitable for histologic interpretation.

a. Adult iguana diet, Ziegler Bros Inc, Gardners, Pa.
b. VT-5000, small animal ventilator, BAS Vetronics, Bioanalytical Systems Inc, West Lafayette, Ind.
c. 67065C, operating sheath for 64018BSA telescope, 14.5-F outer diameter, Karl Storz Veterinary Endoscopy America Inc, Goleta, Calif.
d. 64018BSA, autoclavable Hopkins rigid telescope, 2.7 mm × 18 cm working length, 30°, Karl Storz Veterinary Endoscopy America Inc, Goleta, Calif.
e. 69235106, veterinary video camera II, 9219-B Sony medical grade monitor, 201320-20 xenon light source, 26012C CO_2 insufflator, Karl Storz Veterinary Endoscopy America Inc, Goleta, Calif.
f. 67161Z, flexible biopsy forceps, 5-F × 34-cm, Karl Storz Veterinary Endoscopy America Inc, Goleta, Calif.

References

1. Cholongitas E, Senzolo M, Standish R, et al. A systematic review of the quality of liver biopsy specimens. *Am J Clin Pathol* 2006;125:710–721.
2. Kerwin SC. Hepatic aspiration and biopsy techniques. *Vet Clin North Am Small Anim Pract* 1995;25:275–291.
3. Richter KP. Laparoscopy in dogs and cats. *Vet Clin North Am Small Anim Pract* 2001;31:707–727.
4. Roth L. Comparison of liver cytology and biopsy diagnoses in dogs and cats: 56 cases. *Vet Clin Pathol* 2001;30:35–38.
5. Wang KY, Panciera DL, Al-Rukibat RK, et al. Accuracy of ultrasound-guided fine-needle aspiration of the liver and cytologic findings in dogs and cats: 97 cases (1990–2000). *J Am Vet Med Assoc* 2004;224:75–78.
6. Cole TL, Center SA, Flood SN, et al. Diagnostic comparison of needle and wedge biopsy specimens of the liver in dogs and cats. *J Am Vet Med Assoc* 2002;220:1483–1490.
7. Twedt DC, Monnet E. Laparoscopy: technique and clinical experience. In: McCarthy TC, ed. *Veterinary endoscopy for the small animal practitioner*. St Louis: Elsevier, 2005;357–385.
8. Monnet E, Twedt DC. Laparoscopy. *Vet Clin North Am Small Anim Pract* 2003;33:1147–1163.
9. Falcone RE, Wanamaker SR, Barnes F, et al. Laparoscopic vs open wedge biopsy of the liver. *J Laparoendosc Surg* 1993;3:325–329.
10. Orlando R, Lirussi F, Okolicsanyi L. Laparoscopy and liver biopsy: further evidence that the two procedures improve the diagnosis of liver cirrhosis. A retrospective study of 1,003 consecutive examinations. *J Clin Gastroenterol* 1990;12:47–52.
11. Hernandez-Divers SJ. Diagnostic techniques. In: Mader DR, ed. *Reptile medicine and surgery*. 2nd ed. St Louis: Elsevier, 2005;490–532.
12. Ramiro I, Ackerman N, Schumacher J. Ultrasound-guided percutaneous liver biopsy in snakes. *Vet Radiol Ultrasound* 1993;34:452–454.
13. Hernandez-Divers SJ. Diagnostic and surgical endoscopy. In: Raiti P, Girling S, eds. *Manual of reptiles*. 2nd ed. Cheltenham, England: British Small Animal Veterinary Association, 2004;103–114.
14. Taylor WM. Endoscopy. In: Mader DR, ed. *Reptile medicine and surgery*. 2nd ed. St Louis: Elsevier, 2006;549–563.
15. West G. Endoscopic hepatic biopsy in Coahuilan box turtles, *Terrapene coahuila*. *J Herpetol Med Surg* 2001;11:28–29.
16. Hernandez-Divers SJ, Hernandez-Divers SM, Wilson GH, et al.

A review of reptile diagnostic coelioscopy. *J Herpetol Med Surg* 2005;15:16–31.
17. Esposito C, Garipoli V, Vecchione R, et al. Laparoscopy-guided biopsy in diagnosis of liver disorders in children. *Liver* 1997;17:288–292.
18. Hernandez-Divers SJ, Stahl S, Stedman NL, et al. Renal evaluation in the green iguana (*Iguana iguana*): assessment of plasma biochemistry, glomerular filtration rate, and endoscopic biopsy. *J Zoo Wildl Med* 2005;36:155–168.
19. Hernandez-Divers SJ. Endoscopic renal evaluation and biopsy in chelonia. *Vet Rec* 2004;154:73–80.
20. Hernandez-Divers SJ, Shearer D. Pulmonary mycobacteriosis caused by *Mycobacterium haemophilum* and *M marinum* in a royal python. *J Am Vet Med Assoc* 2002;220:1661–1663.