EXOTIC ANIMAL ENDOSCOPY
in association with
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Coelomic Endoscopy of the Green Iguana, *Iguana iguana*

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**ABSTRACT:** This is the first study evaluating coelomic endoscopy in any reptile species, and describes a bilateral coeloscopic technique that was performed on 32 juvenile green iguanas, *Iguana iguana*, under general anesthesia using a 2.7 mm telescope system. The levels of difficulty to enter the coelom, insufflate the coelomic cavity, and visualize the viscera were objectively scored by the endoscopists. In addition, the level of difficulty to locate and examine the coelomic organs and structures were objectively scored. Endoscope entry, insufflation, and general coelomic visualization were straightforward. Results indicated that there was no significant difference between left and right visualization scores for lung, liver, pancreas, small intestine, large intestine, ovary, oviduct, testis, epididymis, vas deferens, bladder, fat body, or kidney. However, a left lateral approach to the heart, stomach, and spleen, and a right lateral approach to the gall bladder and adrenal gland were significantly superior (P < 0.05). In conclusion, endoscopy is advocated as a safe and effective method for the detailed examination of coelomic viscera in the green iguana.

**KEY WORDS:** green iguana, *Iguana iguana*, endoscopy, coelom, viscera.

**INTRODUCTION**

The green iguana, *Iguana iguana*, is a large saurian originating from Central and South America. This species is popular as a zoological exhibit and exotic pet, and is farmed in large numbers for the pet trades of Europe and the United States. The simple coelom of the green iguana, lacking post-hepatic and post-pulmonary membranes, makes it an ideal candidate for endoscopic research. In addition, their frequent presentation to veterinarians further advocates the need for detailed objective data regarding coeloscopy of this particular species.

Endoscopy has become an important diagnostic and surgical tool in zoological medicine and has been used in a variety of nondomestic species (Burrows and Heard, 1999). Although reported as early as the 1970s, reptile coeloscopy has only recently been exploited thanks to improvements in equipment design and procedural development (Divers, 1998, Divers, 1999, Schildger, et al, 1999). The preferred equipment for rigid reptile endoscopy includes a 2.7 mm telescope within a sheath, light guide cable, xenon light source, endovideo camera, monitor and some means of coelomic insufflation (Divers, 1998, Channess, 1999).

There have been several reports detailing coeloscopy of lizards, and the clinical application for disease diagnosis has been documented (Divers, 1999, Schildger, et al, 1999). In general a left paralumbar approach to the iguanid coelum is described for the evaluation of most visceral structures (Divers, 1998, Divers, 1999, Schildger et al, 1999). Despite these recent advances, there have been no studies to objectively assess coeloscopy in any reptile. The aims of this study were to objectively evaluate the ability of the rigid endoscope to safely evaluate the iguanid coelom, and to determine whether there were any advantages to a left or right paralumbar approach. The results are reported with statistical significance, and the established methodology is advocated as the basis for coeloscopy in other saurian species.

**MATERIALS AND METHODS**

Thirty-two green iguanas, 21 females and 11 males, maintained by a private breeder were utilized for this study. All animals were 18-m old and captive-bred siblings from the same clutch. The methodology described was reviewed and accepted by the University of Georgia’s Institutional Animal Care & Use Committee (IACUC #A2003-10045-0). The breeder housed the iguanas in groups of two to seven in plastic-coated wire enclosures measuring 2m x 1.4m x 1m or 2m x 2.7m x 2.7m. Heating was provided by ceramic heaters (ReptiCare Ceramic Heat Emitters, 60 and 100 w, Zoo Med Laboratories, San Luis Obispo, CA), and broad-spectrum lighting was provided by mercury halide lamps (PowerSun UV, 160 w, Zoo Med Lab). Enclosure temperature gradients
varied between 21 - 35°C (70 - 95°F) during the day and 18 - 29°C (65 - 85°F) at night. General humidity was maintained at 80% and the enclosures were sprayed daily using tap water. Diet consisted of collared greens, kale, squash, and carrot, supplemented with calcium powder (Rep-Cal Calcium with Vitamin D₃, Rep-Cal Research Labs, Los Gatos, CA) and multivitamins (Reptivite Reptile Vitamins, Zoo Med Lab). Fresh water was available at all times. The iguanas were transported to the University of Georgia's College of Veterinary Medicine the morning of the procedure. The iguanas were maintained in heated incubators (Animal Intensive Care Unit, Animal Care Products, Lyon Electric Company, Chula Vista, CA) at 29.5°C (85°F), for at least one hour pre-operatively. The examination and surgery areas were maintained at 24°C (75°F). Each iguana was physically examined following standard protocols at admission (Divers, 1999). Body weights, resting heart rate, and respiratory rates were recorded. Each animal was identified by means of a numbered tape label secured around a forelimb.

Premedication using 1 mg/kg butorphanol (Torbugesic, 10 mg/ml, Fort Dodge Animal Health, Overland Park, KS) IM was given 20 - 30 min prior to anesthetic induction. Anesthesia was induced using 10 mg/kg propofol (Propofol, 10 mg/ml, Abbott Lab, North Chicago, IL) IV into the caudal (ventral tail) vein. Iguanas were intubated with 14 g intravenous catheters (Abbocath-T radio-opaque IV cannula, 51 mm, Abbott Ireland, Sligo, Republic of Ireland). Anesthesia was maintained using 1 - 4% isoflurane and 1 - 2 L/min oxygen by intermittent positive pressure ventilation (Small Animal Ventilator VT-9093, BAS Vetronics, West Lafayette, IN), adjusted to individual patient requirements. Typical settings for respiratory rate and maximum inspiratory airway pressure were 8 - 12 per min and 0.4 - 0.5 KPa (ventilator reading 4 - 5 cm H₂O) respectively. Patient monitoring included palpation, corneal, and withdrawal reflexes, end-tidal capnography (ETCO₂/SPO₂ Monitor, CO₂ SMO Novametrix Medical Systems, Wallingford, Wallingford, CT), V9004 End Tidal CO₂ Monitor with Digital Pulse Oximeter, SurgiVet, Waukesha, WI), cardiac doppler ultrasound (Ultrasone Doppler, Parks Electric Laboratory, Aloha, OR), pulse oximetry (V3301 Pulse Oximeter, SurgiVet), and esophageal temperature (Precision Thermometer, Tandy, Fort Worth, TX). Patient temperatures were maintained using circulating warm water pads (Temperature Therapy Pad TP22G and Temperature Therapy Pump TP500T, Gaymar, 10 Centre Drive, Orchard Park, NY 14127) set to 40°C (105°F). Snout to vent lengths were recorded in the anesthetized animals to ensure accurate measurement. All iguanas were implanted between the shoulders with subcutaneous microchips (Avid Friendship Identification System, Avid, Norco, CA).

The following sterile endoscopy procedure was performed in all iguanas. Each lizard was initially placed in right lateral recumbency with the fore- and hindlimbs taped to the operating table. The left paralumbar region was aseptically prepared using chlorhexidine scrub solution (Hibiclens, AstraZeneca Pharmaceuticals, Wilmington, DE) and 70% alcohol. A 3 - 4 mm cranio caudal skin incision was made in the centre of the left paralumbar region (Figure 1). To avoid damage to visceral structures, the skin and underlying musculature were pinched and elevated using thumb and forefinger before small hemostats were inserted through the incision and bluntly forced through the coelomic musculature into the coelom. The hemostats were removed and replaced with a 14.5 Fr operating sheath and obturator (67065 CC, Karl Storz Veterinary Endoscopy-America [KSVEA], Goleta, CA). One of the sheath ports was connected to a CO₂ insufflator. The obturator was removed from the sheath and replaced with a 2.7 mm telescope (Hopkins Telescope, 2.7 mm x 18 cm, 30°, KSVEA). The telescope was attached to a 175 watt xenon light source (201320-20, KSVEA) via a light guide cable (495 NA, KSVEA) and connected to a veterinary endovideo camera (69235106, KSVEA) and monitor (Sony Monitor, 19”, 9219-B, KSVEA). Coelomic insufflation was achieved using a dedicated CO₂ insufflator (26012 C and 264305-20, KSVEA) set to 1.0 - 1.5 L/min CO₂ and 0.4 - 0.7 KPa (insufflator reading 3 - 5 mmHg). Endoscopic images were digitally captured and recorded (Sony Image Capture Device and Digital Photo Printer, 9525-P, KSVEA).

The endoscopist scored the ease of entry into the coelom (including skin incision, hemostat penetration, sheath placement, endoscopy entry and camera connection) and the ability to visualize the coelomic visceria using a scale from 1 to 5 (1 = impossible, taking more than 15 min, 2 = very difficult, taking between 11 - 15 min, 3 = difficult, taking between 6 - 10 min, 4 = straightforward, taking between 2 - 5 min, 5 = easy, taking less than 2 min) (Table 1). A standard coeloscopic evaluation was performed including location and examination of all major organs and structures in the coelomic cavity (Figure 2). For each structure, the score was based on the ease of examination and visualization from 1 - 5 (1 = impossible, 2 = difficult, requiring an extensive search and significant movement of viscera; 3 = straightforward, but requiring some searching and manipulation of viscera; 4 = simple to locate, but requiring minor manipulation to see clearly; 5 = obvious, and clear visualization with no manipulation required) (Table 1). Upon completion of the procedure, the endoscopy equipment was removed, and the CO₂ aspirated from the coelom. The skin incision was closed in a routine manner using a single horizontal mattress suture of absorbable monofilament polydioxanone (PDS II, 2 metric, Ethicon, Somerville, NJ). The animal was then returned to right lateral recumbency, and the endoscopy procedure and scoring technique were repeated on the right side. Upon completion of bilateral endoscopy, 25 ml/kg half strength lactated ringsers solution and 2.5% dextrose (Braun Medical, Irvine, CA) warmed to 29.5°C (85°F) was administered by intracoelomic injection. Iguanas were returned to heated incubators set to 29.5°C (85°F), and closely monitored until righting reflexes returned. They were maintained in the incubators until no further signs of sedation were noted. Iguanas were discharged back into the care of their owner one to eight hours after surgery and were housed as previously described. Any sutures that had not been shed by six weeks were manually removed.

RESULTS

Body weights and snout-vent lengths of the iguanas ranged from 358.6 - 1310.9 g and 21.0 - 33.0 cm respectively. Most animals appeared to be clinically healthy and in good body condition pre-operatively, although several exhibited evidence of previous minor trauma including rostral abrasions, missing nails, and chronic skin or tail wounds. In addition, animal #26
Table 1. Endoscopy score results for left and right coelioscopy in 32 green iguanas, *Iguana iguana*. + 1 = impossible, taking > 15 mins; 2 = very difficult, taking between 11 – 15 mins; 3 = difficult, taking between 6 – 10 mins; 4 = straightforward, taking between 2 – 5 mins; 5 = easy, taking less than 2 mins. Visceral structure score 1 = impossible; 2 = difficult, requiring an extensive search and significant movement of viscera; 3 = straightforward, but requiring some searching and manipulation of viscera; 4 = simple to locate, but requiring minor manipulation to see clearly; 5 = obvious, and clear visualization with no manipulation required.

<table>
<thead>
<tr>
<th>Procedure/Structure</th>
<th>Left</th>
<th>Right</th>
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<tbody>
<tr>
<td></td>
<td>Min</td>
<td>Max</td>
</tr>
<tr>
<td>Ease of entry*</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Coelomic visualization*</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Heart</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Lung</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Stomach</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Small intestine</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Large intestine</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Spleen</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Liver</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pancreas</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Adrenal</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Ovary</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Oviduct</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Testis</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Epididymis</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Vas deferens</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Kidney</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Bladder</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Fat body</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

Table 2. Comparison between left and right endoscopy approaches and the ease of coelomic viscera visualization in the green iguana, *Iguana iguana*.

<table>
<thead>
<tr>
<th>Coelomic structure</th>
<th>Left Paralumbar Approach</th>
<th>Right Paralumbar Approach</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>Yes – left side</td>
<td>Yes – right side, vena cava visible</td>
</tr>
<tr>
<td>Lung</td>
<td>Yes – left lung</td>
<td>Yes – right lung</td>
</tr>
<tr>
<td>Stomach</td>
<td>Yes</td>
<td>Difficult to impossible</td>
</tr>
<tr>
<td>Small intestine</td>
<td>Yes – mainly ileum</td>
<td>Yes – mainly duodenum</td>
</tr>
<tr>
<td>Large intestine</td>
<td>Yes – terminal colon</td>
<td>Yes – sacculated colon</td>
</tr>
<tr>
<td>Gall bladder</td>
<td>Difficult to impossible</td>
<td>Yes</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Yes – below spleen, close to stomach</td>
<td>Yes – caudal to gall bladder between loops of duodenum</td>
</tr>
<tr>
<td>Adrenal glands</td>
<td>Yes – left adrenal, occasionally both</td>
<td>Yes – right adrenal, occasionally both</td>
</tr>
<tr>
<td>Reproductive tract</td>
<td>Yes – often both gonads</td>
<td>Yes – often both gonads</td>
</tr>
<tr>
<td>Kidney</td>
<td>Yes – often both kidneys</td>
<td>Yes – often both kidneys</td>
</tr>
<tr>
<td>Bladder</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fat body</td>
<td>Yes</td>
<td>Yes</td>
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exhibited evidence of healed coccygeal vertebral fracture, kyphosis, and possibly stunted growth, as it was the smallest animal in the group.

Butorphanol premedication, propofol induction and maintenance with isoflurane/oxygen by intermittent pressure ventilation proved to be an effective anesthetic regime. Variations in anesthetic depth were evident during some endoscopy procedures and appeared to be related to coelomic CO₂ insufflation reducing lung ventilation. Increasing the maximum inspired pressure to 0.8 - 1.3 KPa (6 - 10 mmHg) and rarely up to 2 KPa (15 mmHg) proved effective in maintaining adequate lung ventilation and a surgical plane of anesthesia despite coelomic insufflation.

Endoscopy score data (Table 1) were analyzed for normalcy. Based on the non-normalized data distribution, a Wilcoxon ranked sum test was used to compare ease of visualization of each organ from the left versus the right side. Statistical significance was considered if $P < 0.05$. The ease of endoscope entry, insufflation and visualization, were consistently scored as 5 (easy, taking less than 2 min to accomplish). On the left side, all the structures of interest, except the gall bladder, received a mean score of > 4 (simple to locate or obvious, requiring little or no manipulation). On the right side, all structures of interest, except stomach and spleen, also received mean scores > 4. Left scores were significantly greater for heart ($P < 0.0001$), stomach ($P < 0.0001$), and spleen ($P < 0.0001$), and right scores were significantly greater for gall bladder ($P < 0.0001$) and adrenal gland ($P = 0.0099$). There were no significant differences between left and right scores for any other organs and structures examined (Table 1). In all cases, the optics of the telescope provided excellent, clear images (Figures 3 - 38). Animal #9 had urinated immediately prior to induction and consequently the empty bladder was only visible from the left side. The only anatomical variations noted were the apparent absence of a gall bladder of one animal (Figure 37) and a ves-tigial yolk sac attached to the small intestine of another (Figure 38).

No technical difficulties were appreciated during the endoscopy procedures and all animals recovered uneventfully. The owner reported that all animals were behaving and feeding normally by 24 hr post surgery. A single animal (#26) died three weeks after surgery. A gross postmortem examination failed to indicate any internal trauma or infection that may have been caused by the endoscopic procedure. The carcas had been frozen and histologic evaluation was not performed.

**DISCUSSION**

The green iguana proved to be an appropriate model for evaluating coelioscopy in lizards. The size and anatomical configuration of the coelomic cavity were ideal for coelioscopy, as all the major visceral organs are visible on left or right, or both sides. Physical examination findings were largely unremarkable as minor scratches and damaged claws are common in multi-iguana enclosures. One iguana (#26) appeared to be particularly affected, and as the smallest animal in the group, was considered to be the runt of the clutch. The owners had been aware of the reduced growth of this individual. Although there was no direct evidence of endoscopic trauma or infection, it is possible that the added stress and demands of anesthesia and surgery hastened the demise of this animal. No adverse signs were reported in any of the other iguanas, and the coelioscopy procedure described is recommended as safe and effective for the detailed examination of coelomic viscera.

Propofol and isoflurane provided effective and controllable anesthesia; however, eight animals became light during the procedure. We believe that the mechanical collapse of the lungs due to the positive coelomic pressure of insufflation reduced ventilation in these animals to a point where uptake of anesthetic gas was inadequate to maintain their anesthesia. Increasing the maximum inspired ventilation pressure resulted in re-inflation of the lungs and return to adequate minute ventilation. Direct endoscopic visualization of the lungs confirmed the improvement in lung ventilation following an increase in maximum inspiratory airway pressure. The ability to accurately control and maintain a constant inspiratory pressure using the electrical ventilator was considered essential in maintaining a steady anesthetic state. Accurate monitoring can be demanding and although pulse oximetry in reptiles is controversial, initial research suggests it is reliable in the green iguana (Diehl, 2001). These observations have been further supported by research at the University of Georgia, which has investigated blood gas measurements, pulse oximetry, and end-tidal capnography for monitoring anesthesia in green iguanas (Sonja Hernandez-Divers, Juergen Schumacher and Stephen Hernandez-Divers, unpublished observations).

The endoscopy scores indicated no significant differences between the cumulative mean scores of left and right approaches to the lung, liver, pancreas, small intestine, large intestine, ovary, ovotestis, testis, epididymis, vas deferens, bladder, fat body, and kidney. However, it should be noted that usually only the left or right organ, or aspect of an organ, was visible from either side. It was generally not possible to visualize lungs, fat bodies, or the entire liver from a unilateral approach. Nevertheless, it was occasionally possible to visualize both gonads, adrenal glands, and kidneys from either side. A ventral midline approach may provide better visualization of ventral viscera but examination of the urogenital tract would be very difficult.

Different parts of an organ or organ system were often visualized from left and right sides. For example, there was no significant difference between left and right scores for the large intestine because parts were equally visible from either side; however, the sacculated colon was much more visible from the right side, and the terminal colon from the left side. Therefore, although the results indicate that either a left or right approach is appropriate for the large intestine, a right approach is more appropriate for the sacculated colon, and a left for the terminal colon. Despite the lack of statistical significance in left and right scores, differences in visualization of the pancreas were obvious and attributed to anatomical asymmetry within the coelom. The iguand pancreas is typically tri-lobed, with one portion running along the bile duct towards the gall bladder, another closely associated with the duodenum, and a third limb with a distinct distal lobe that runs to the spleen. The gall bladder, duodenum, and their associated pancreatic lobes are located on the right side of the coelom making them more amenable to a right-sided approach. The most consistent way to locate the pancreas on the right side involved identification of the gall bladder and
careful examination caudal to the gall bladder, adjacent to the duodenum. On the left side, the splenic lobe of the pancreas was usually located just ventromedial to the spleen, close to the stomach.

There were statistical differences between left and right approaches for the heart, stomach, spleen, gall bladder, and adrenal glands. The heart was partially obscured by the caudal vena cava on the right side, making the score for the left side greater. The higher scores for the stomach and spleen on the left were attributed to their asymmetrical anatomical location on the left side of the coelom. Although it was possible to locate these structures from the right side, a left approach is recommended, as visualization from the right side was difficult. Similarly, the right coelomic position of the gall bladder made a right score higher, as this structure was impossible to locate from the left side. The right adrenal was easier to locate on the right side, than the left adrenal on the left side. This may be related to anatomical asymmetry in the green iguana. The left adrenal gland lies between the left gonad and the left renal vein, and may be obscured by the gonad, whereas the right adrenal gland is separated from the gonad by the renal vein (Bennett and Mader, 1996). Recommendations for the endoscopic evaluation of coelomic structures in the green iguana have been summarized in Table 2. Anatomical variations, which occasionally impeded visualization, included large fat bodies, fluid-distended bladders, and gas-distended large intestines. Therefore, fasting for two days is recommended for elective coelioscopy as distension of the intestinal tract could hinder visualization of other structures. Digital manipulation of the cloaca prior to anesthesia will often stimulate urination and decrease problems associated with a large bladder. The inability to visualize the gall bladder of animal #5 was scored as a one because a complete absence of the structure could not be confirmed. The other anatomical variation, a vestigial yolk sac attached to the small intestine of animal #13, (Figure 38) was considered an incidental finding, and did not appear to be associated with pathology.

The coelioscopic examination of reptiles has been advocated as a safe and important diagnostic procedure (Divers, 1999, Schildger, et al., 1999). This study confirms the safe and effective use of endoscopy to examine the coelomic structures in the green iguana, summarizes the best approach for most coelomic structures, and should serve as a basis for coelioscopy of other lizard species.

Figure 1. Right lateral positioning of a green iguana, Iguana iguana, for left coelioscopy. “X” marks the incision and telescope entry site in the left paralumbar area.

Figure 2. Diagram illustrating lizard position and correct camera-telescope handling for performing right-sided coelioscopy in the green iguana, Iguana iguana.
Figure 3. Left coelioscopic view of the green iguana, Iguana iguana: heart in ventricular diastole (H) and deflated left lung (L).

Figure 4. Left coelioscopic view of the green iguana, Iguana iguana: heart in ventricular systole (H) and deflated left lung (L). Dorsal aorta also visible (arrow).

Figure 5. Left coelioscopic view of the green iguana, Iguana iguana: deflated left lung.

Figure 6. Left coelioscopic view of the green iguana, Iguana iguana: ventral surface of the left liver lobe.

Figure 7. Left coelioscopic view of the green iguana, Iguana iguana: dorsal surface of the left liver lobe.

Figure 8. Left coelioscopic view of the green iguana, Iguana iguana: ventrolateral view of the stomach.

Figure 9. Left coelioscopic view of the green iguana, Iguana iguana: caudal view of the stomach.

Figure 10. Left coelioscopic view of the male green iguana, Iguana iguana: stomach (St), spleen (S), and left testis (T).

Figure 11. Left coelioscopic view of the female green iguana, Iguana iguana: stomach (St), spleen (S), left ovary (O), and fat body (F).

Figure 12. Left coelioscopic view of the male green iguana, Iguana iguana: left testis (T), left epididymis (E), and the left adrenal gland (arrow).

Figure 13. Left coelioscopic view of the male green iguana, Iguana iguana: both left and right testes are visible.

Figure 14. Left coelioscopic view of the male green iguana, Iguana iguana: close-up of the dorsal left testis (T), left epididymis (E), and left adrenal gland (arrow).
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REFERENCES


A Review of Reptile Diagnostic Coelioscopy

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ABSTRACT: Diagnostic endoscopy has proven to be an important diagnostic tool for minimally-invasive visualization and biopsy of internal structures in a variety of species. In reptile medicine, the lack of pathognomonic clinical signs, variable hematology and inconsistent plasma biochemistry results, make disease diagnosis challenging. In many cases reaching a definitive diagnosis relies upon biopsies for histology and microbiology. The ability to explore the coelom and collect biologic samples with targeted precision and minimal trauma using a small diameter rigid telescope with intergrated sheath and operating channel offers a significant diagnostic advantage over surgical coeliotomy or ultrasound-guided techniques. This review describes available equipment, approaches and techniques for examination of coelomic viscera for members of the Squamata, Crocodilia and Chelonia. Emphasis is placed upon the 2.7 mm telescope system as this size is suitable for the majority of reptile species; however, 5 and 10 mm equipment for larger species is also described.

KEY WORDS: reptile, surgery, endoscopy, coelioscopy, diagnosis, biopsy.

INTRODUCTION


Clinical, research and teaching experience suggests that endoscopy offers unparalleled opportunities for visualization and biopsy in reptiles, and has been advocated as a standard diagnostic technique (Schildger and Wicker, 1992, Schildger, 1994, Divers, 1999, Schildger, et al, 1999, Hernandez-Divers, 2003, 2004, Hernandez-Divers, et al, 2004a, Hernandez-Divers, et al, 2004b). A standard single-entry system incorporates a rigid telescope housed within a sheath, through which basic instruments can be inserted into the field of view. For reptiles over 10 kg, larger sheathed telescopes, separate cannulae, and instruments are used by triangulation (Magne and Tams, 1999, McCarthy, 2005). This paper reviews diagnostic coelioscopy in reptiles, and describes techniques that have been efficacious in a variety of squamates, crocodilians, and chelonians.

Rigid endoscopy equipment — Older rigid endoscopes incorporated a convex glass lens system, in which small glass lenses were separated by large air spaces. By contrast, modern telescopes incorporate a rod lens design that utilizes comparatively longer rods of glass and smaller air spaces. The advantages of the rod lens system are greater light transmission, better image resolution, wider field of view and image magnification. The general consensus among endoscopists is that rod lens telescopes are superior. The authors use the system designed by Professor Harold Hopkins and manufactured by Karl Storz (Karl Storz Veterinary Endoscopy America Inc, Goleta, CA) (Table 1). Human cystoscopy equipment (2.7 mm) was adopted for avian coelioscopy and is equally applicable to
most reptiles under 10 kg. In contrast to a 0° telescope, the 30° Hopkins telescope not only enables a straight-ahead view but, by rotating the scope around its longitudinal axis, a greater area can be surveyed. The 2.7 mm telescope can be housed within an examination and protection sheath or an operating sheath with an intergrated instrument channel. The operating sheath provides two stop-cocks for uses such as insufflation, aspiration and irrigation and an operating channel that accommodates various instruments, including scissors, grasping forceps, biopsy forceps, fine aspiration/injection needle, wire-basket snare, laser, and radiosurgical probes (Figure 1). The biopsy forceps are used to harvest tissue samples for histopathology and microbiology. The small sample size permits the collection of biopsies for various laboratory tests, and for sequential biopsies over time to monitor disease progress. Grasping forceps (5 Fr) are useful for manipulating tissues, debridement, and foreign body removal. The fine aspiration/injection needle is used for aspiration, irrigation and drug administration. A smaller 1.9 mm telescope version is available with 3 Fr grasping and biopsy instruments, and is ideally suited to animals less than 100g (Table 1).

For larger reptiles, over 5 – 10 kg, cannulae are used to create multiple ports for the insertion of telescopes and instruments (Magne and Tams, 1999; McCarthy, 2005) (Figures 2 and 3). The endotip cannula is a recent improvement that has an external screw-thread to enable gradual advancement by rotation (Figures 2 and 3) (Termanim and Deit tel, 1999) The cannula does not require trocar or axial penetration force during insertion. A telescope within the cannula provides a magnified view during entry into the coelom. As the cannula is advanced through a small skin incision, the fascia and then the muscle fibers spread radially and are transposed onto the cannula’s outer thread. The thin pleuroperitoneal membrane is transilluminated so that viscera, vessels and/or adhesions are visualized before entry into the coelom. The risks of iatrogenic visceral damage are therefore greatly reduced. A 5 mm endotip cannula can be used with a 2.7 mm telescope sheathed within a 3.5 mm protection sheath, while 5 and 10 mm telescopes and instruments can be inserted through 6 and 11 mm endotip cannulae respectively. Telescopes and instruments of the same size can be used interchangeable between multiple endotip ports.

For reptiles between 5 and 100 kg, 5 mm telescopes and instruments are used, but for animals over 100 kg, 10 mm equipment may be preferred. Various 5 and 10 mm instruments that accompany the different diameters of cannulae are available for performing endoscopic surgery. However, for the purposes of this review, diagnostic instruments will be discussed (Figure 3).

There are two types of light source available, tungsten-halogen and rare-earth xenon, and either is connected to the telescope via a flexible, fiber-optic cable. Halogen is sufficient for rigid endoscopy using the eye-piece in small animals. However, xenon is generally preferred because the greater intensity and quality of light provides better real-life and recorded images. Xenon becomes more beneficial as the telescope diameter decreases or the patient size increases above 1 kg. A xenon light source with a dedicated endoscopy camera and a recording device (e.g. analogue video, digital video, digital still image capture, still image print-out) is recommended for wide species application, case records, and client education (Figure 4). Cameras that relay the endoscopic image from the eye-piece to a monitor (endovideo cameras) were once considered optional but clinical, research and training experience has indicated that surgeon ergonomics and ability are greatly improved by their routine use (Figures 1A and 2C).

Insufflation is essential for reptile coelioscopy in order to create the necessary working space. Several gases have been used, but medical grade CO2 is inert, non-toxic, readily absorbed, quickly excreted, and is preferred. Dedicated CO2 endoflators accurately control gas flow to maintain the desired insufflation pressure; however, it is possible to

### Table 1. Rigid Endoscopy Equipment Used for Reptile Diagnostic Coelioscopy

<table>
<thead>
<tr>
<th>Essential equipment for rigid endoscopy</th>
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<tbody>
<tr>
<td>Nova xenon light source, 175 watts</td>
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<tr>
<td>Light guide cable, 3.5 mm x 230 cm</td>
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<tr>
<td>Veterinary video camera ii</td>
</tr>
<tr>
<td>Medical grade monitor</td>
</tr>
<tr>
<td>CO2 endoflator (or aquarium air pump) and insufflation line</td>
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<table>
<thead>
<tr>
<th>Basic equipment for reptiles &lt; 0.1 kg</th>
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<tbody>
<tr>
<td>Hopkins rigid telescope, 1.9 mm x 10 cm, 30°</td>
</tr>
<tr>
<td>Arthroscope sheath for 1.9 mm telescope, 2.8 mm outer diameter</td>
</tr>
<tr>
<td>Obturator, blunt for arthroscope sheath</td>
</tr>
<tr>
<td>Biopsy forceps, flexible, elliptical cup 3 Fr x 34 cm</td>
</tr>
<tr>
<td>Grasping forceps, flexible, 3 Fr x 34 cm</td>
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<table>
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<tr>
<th>Basic equipment for reptiles 0.1 – 10 kg</th>
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<tbody>
<tr>
<td>Hopkins telescope, 2.7 mm x 18 cm, 30°</td>
</tr>
<tr>
<td>Operating sheath, 14.5 Fr, 5 Fr instrument channel</td>
</tr>
<tr>
<td>Examination and protection sheath, 3.5 mm outside diameter</td>
</tr>
<tr>
<td>Biopsy forceps, 5 Fr x 34 cm</td>
</tr>
<tr>
<td>Grasping forceps, 5 Fr x 34 cm</td>
</tr>
<tr>
<td>Scissors, single action, 4 Fr x 34 cm</td>
</tr>
<tr>
<td>Injection/aspiration needle, flexible with Teflon guide</td>
</tr>
<tr>
<td>Stone (wire) basket, flexible, 5 Fr x 60 cm, consisting of 3-ring-handle, wire basket, and coil</td>
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<table>
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<tr>
<th>Basic equipment preferred for reptiles &gt; 10 kg</th>
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<tbody>
<tr>
<td>Hopkins telescope, 5 mm x 29 cm, 0°</td>
</tr>
<tr>
<td>Hopkins telescope, 5 mm x 29 cm, 30°</td>
</tr>
<tr>
<td>Hopkins telescope, 10 mm x 57 cm, 0° (suitable for giant reptiles &gt; 100 kg)</td>
</tr>
<tr>
<td>Termanim endotip cannula, with insufflation stopcock and multifunctional valve, 6 mm x 10.5 cm (2)</td>
</tr>
<tr>
<td>Termanim endotip cannula, with insufflation stopcock and multifunctional valve, 11 mm x 10.5 cm (suitable for giant reptiles &gt; 100 kg)</td>
</tr>
<tr>
<td>Blakesley biopsy forceps, 5 mm x 43 cm, plastic handle without ratchet</td>
</tr>
<tr>
<td>Metzenbaum scissors, serrated, conical, curved 12 mm jaws, 5 mm x 43 cm, plastic handle without ratchet</td>
</tr>
<tr>
<td>Babcock grasping forceps,atraumatic fenestrated jaws with multiple teeth, 5 mm x 43 cm, plastic handle with hemostat style ratchet</td>
</tr>
<tr>
<td>A greater variety of instruments are required for endoscopic surgery</td>
</tr>
</tbody>
</table>
use a simple aquarium air pump to provide room air for insufflation.

Operating room design and layout are important. The light source, camera unit, endoflator, and documentation device are best stored on a mobile cart that can be easily moved and positioned in the operating room (Figure 5A). An endovideo camera coupled to a monitor facing the surgeon at eye-level will greatly improve the ability of the endoscopist and reduce fatigue. Standard surgical and endoscopic equipment and supplies should also be arranged within easy reach (Figure 5B). After the equipment is cleaned using a neutral pH enzymatic cleaner, it can be sterilized using hydrogen peroxide vapor or ethylene oxide gas. Cold sterilization using glutaraldehyde according to the recommendations of the manufacturer is acceptable. Autoclaving has not been routinely advised because of fears of reduced equipment longevity; however, most modern telescopes are autoclavable.

**Patient preparation** — For elective procedures, reptiles should be fasted to reduce the volume of the gastrointestinal tract. The precise duration of fasting will depend upon species, age, and dietary preferences. In those reptiles that possess a urinary bladder, digital stimulation of the cloaca prior to anesthetic induction may promote urination and reduce the size of the bladder, thereby reducing the chances of iatrogenic trauma during telescope entry into the coelom. Alternatively, urinary catheterization and emptying of the bladder may be possible.

Laparoscopy and CO$_2$ insufflation are known to be painful procedures that necessitate general anesthesia in humans (Golditch, 1971, Kehlet, 1999). Therefore general anesthesia and analgesia are considered equally essential for reptile coelioscopy. Insufflation causes visceral displacement and places tension on visceral suspensory ligaments, making sedation and/or local anesthesia of the entry site inadequate for clinical practice. Following the induction of general anesthesia, tracheal intubation and artificial ventilation are essential to overcome apnea and the adverse effects of insufflation on lung inflation. Insufflation gas must be evacuated from the coelom before final closure to reduce post-operative discomfort.

**Telescope, instrument and biopsy handling** — A sheath, although increasing overall diameter, is recommended to avoid damage to the smaller 1.9 and 2.7 mm telescopes, and in most cases an operating sheath is preferred to allow...
When moving around the coelom, the surgeon should sit facing the monitor at eye-level. The surgeon’s inferior hand is used to hold the shaft of the sheath, close to where it enters the coelom, while the dominant hand is used to support and control the main body of the telescope-sheath-camera unit (Figure 6a). This handling technique enables the endoscopist to maneuver the telescope around the coelom with maximum control and minimal tremor. Before using an instrument, this two-handed grip must be modified. The inferior hand is used to form a fist around the shaft of the sheath with the thumb slid proximally to prevent rotation. This enables the inferior hand to take the weight and maintain the position of the telescope-sheath-camera, while freeing the dominant hand to manipulate an instrument into the operating channel of the sheath (Figure 6b). Consider the collection of a kidney biopsy from a lizard (Figure 6). Once the instrument has been inserted into the endoscopic field of view, it is important to move the instrument-telescope-sheath-camera as a single unit when approaching the structure of interest. Independent movement of the instrument is not only more difficult but often results in poor control. The biopsy instruments are sharp and delicate, and it is not necessary to forcibly close the forceps with great pressure. The spring action of the handle is often sufficient, but light assistance to close the jaws is all that is ever required. Excessive force will increase crush artifact, and risk instrument damage. In some situations, the membranes covering an organ may be tough and incision using scissors may improve access for tissue collection (Figures 7 and 11).

Upon withdrawal of the biopsy instrument from the operating sheath, the endoscopist opens the biopsy jaws and an assistant, using a moistened sterile cotton-tipped applicator, gently rolls the biopsy onto the applicator. The biopsy is then transferred to a foam-sandwiched histology cassette, which is closed and placed into neutral buffered formalin for histology. For microbiologic culture, the tissue biopsy can be placed either into transport medium or, if submitted immediately, a sterile blood tube containing a small volume of sterile saline (not bacteriostatic water) to prevent tissue desiccation.

**COELIOSCOPY - SAURIA AND CROCODILIA**

**Patient positioning** — Coelioscopy of the green iguana has been objectively assessed and serves as a useful model for most saurians (Hernandez-Divers, et al, 2004a). Given the small size of most lizard species, entry in a paramedian or paralumbar area will permit examination of most, if not all, coelomic structures (Figure 8). For a left paralumbar approach, the lizard is positioned in right lateral recumbency with the left hindlimb taped caudad against the tail base. The surgical area is bordered by the ribs, spine and hindlimb, and a central paralumbar entry is standard. Small crocodilians are placed in dorsal recumbency for a ventral paramedian approach because osteoderms make the paralumbar approach more difficult.

**Endoscopic procedure** — The precise entry point will be dictated by diagnostic imaging, anatomic asymmetry of the coelomic visceras, and the preferences of the endoscopist. Following aseptic preparation, a 2 – 4 mm skin...
incision is made in the middle of the paralumbar region. To avoid damage to visceral structures, the skin and underlying musculature are pinched and elevated using thumb and forefinger before the operating sheath and obturator are forced through the thin coelomic wall (composed of the internal and external oblique muscles and pleuroperitoneum) and into the coelom (Figure 9). Blunt penetration tends to ensure an adequate seal and prevent insufflation gas leakage around the sheath. Alternatively, a surgical cutdown procedure and dissection through the muscle layers can be adopted as long as a pursestring suture is placed around the sheath. A sheath stop-cock is connected to the CO2 endoflator and set to 0.4 – 0.7 KPa (3 – 5 mmHg). The obturator is removed and replaced with the telescope. When using an aquarium air pump for insufflation, the second sheath stop-cock is left open to avoid over-inflation. Air is permitted to continuously escape from the system because, unlike a dedicated endoflator, an aquarium air pump cannot be set to control the gas flow to maintain a precise insufflation pressure. Occluding this second stop-cock with a finger increases insufflation pressure, while lifting the finger off the stop-cock opening decreases insufflation. By careful finger control, insufflation can be crudely controlled. Alternatively, the second stop-cock can be partially closed to balance the inflow and outflow of gas.

Once the endoscope has been inserted, it is often necessary to gently touch the tip of the telescope against a pleuroperitoneal membrane to clean the terminal lens of condensation or fluid. If there is fat or blood on the lens it is usually more effective to remove the telescope from the sheath, clean with sterile damp gauze, and then replace. It is important not to continue with a dirty lens as poor visualization will reduce the endoscopist’s ability and increase procedure time.

Upon entry, the first organ to find for orientation is the large, brown liver lying in the mid-ventral coelom (Figures 10 and 11). Advancing the telescope cranid against the heart, and dorsad the lungs (Figure 12). There are no diaphragmatic, post-pulmonary, or longitudinal membranes in most saurians. However, these membranes do exist to a greater or lesser extent in tegus, monitors, and crocodilians (Perry, 1998). Minor perforation of these membranes with the telescope will not cause any significant harm as long as the lung, intestinal tract and bladder are not perforated. Dorsal to the heart and extending from the cranial coelomic inlet to mid-coelom are the paired lungs. In most species the caudal lung becomes thin and sac-like, and in the Chamaeleonidae finger-like projections are observed. Although reptiles will tolerate hypoxia, a mechanical ventilator is recommended to maintain lung ventilation during coelioscopy. Lung ventilation will be substantially reduced by insufflation and careful communication with the anesthetist is required to balance inspiration and insufflation pressures. Inspiration pressure must exceed coelomic insufflation pressure for lung expansion, and decrease below insufflation pressure for expiration.

Caudal to the lungs, the stomach resides in the mid-coelom (Figure 13). The duodenum is biased towards the right side, in close association with the majority of the pancreas, while the ileum is more easily located on the left, often caudoventral to the stomach. The large intestine can often be appreciated from both sides but in hindgut
fermenting species like the green iguana (Iguana iguana) it is often displaced to the right.

The gonads are located in mid-coelom, on each side of the dorsal midline (Figure 14). Sex identification can be determined endoscopically at an early age, even in monomorphic species of the genera Tiliqua, Corucia, Varanus, and Heloderma. Endoscopy also provides feedback on gonadal activity and disease. The testes are ovoid and smooth and the immature or inactive ovaries appear as small clusters of clear, fluid-filled, follicles. The gonads may enlarge tremendously during seasonal reproductive activity. The vasa deferentia of males and oviducts of females can be followed caudad to the kidneys and urodeum, respectively. Depending upon species, kidneys may be examined on each side of the dorsal midline in the caudal coelom (Figure 15).

The spleen is closely associated with the greater curvature of the stomach, and in some species, careful examination ventromedial to the stomach and spleen reveals the splenic limb of the pancreas (Figure 16A and 16B). The adrenal glands are dorsal to the gonads and lie along the renal veins, and the bladder, if present, is found within the most dependent aspect of the caudal coelom, close to the caudoventral fat-body (Figure 16C and D).

Figure 7. Instrument handling and biopsy technique. (A) Endoscopic view of an iguanid kidney; (B) endoscopic scissors inserted down the instrument channel of the sheath into the field of view, and used to incise the renal capsule; (C) the scissors are withdrawn and the incised capsule reveals the renal parenchyma below; (D) biopsy forceps inserted into view and through the capsular incision to collect a tissue sample.

Figure 8. Lizard positioning for coelioscopy. (A) Green iguana (Iguana iguana) in lateral recumbency with paralumbar region delineated and the preferred entry site marked (X); (B) paramedian entry (arrow) in a leopard gecko (Eublepharis macularius) with the position of the paired pelvic veins draining into the midline abdominal vein indicated.
Figure 9. Lizard coelisocopic technique with drapes removed for photography. (A) Following aseptic preparation and using aseptic techniques, the skin and coelomic musculature is pinched and elevated using thumb and forefinger; (B) a 2-4 mm incision is made through the skin; (C) while holding the skin and muscle, the sheath and obturator are inserted through the skin incision and gently forced into the coelom; (D) the CO2 line is connected (arrow) and, following insufflation, coelioscopy can commence using the two-handed technique. The endoscopy light visible through the body wall can be helpful for orientation.

Figure 10. Lizard coelisocopy. (A) Right liver lobe (1) and gall bladder (2) in a green iguana (Iguana iguana); (B) left liver lobe in green iguana, note the dark pigmented areas of melanomacrophage aggregation; (C) numerous pale foci within the liver of a veiled chameleon (Chameleo calyptratus) with multifocal bacterial hepatitis; (D) hepatomegaly due to amyloidosis in a green iguana.

Figure 11. Endoscopic liver biopsy in lizards and chelonians. (A) Caudal edge liver biopsy using 5 Fr biopsy forceps in a green iguana (Iguana iguana); (B) note the minimal hemorrhage from the biopsy site (arrow); (C) incision through the pleuroperitoneal and hepatic membranes of an Egyptian tortoise (Testudo kleinmanni) in preparation for liver biopsy; (D) insertion of the biopsy forceps into the liver of the same Egyptian tortoise to collect a deeper parenchymal biopsy.

Figure 12. Lizard coelisocopy. (A) Heart (1) and deflated lung (2) in the cranial coelom in a green iguana (Iguana iguana); (B) Deflated lung (1) and spine (2) in a green iguana; (C) post-pulmonary membrane in an Iranian monitor (Varanus bengalensis); (D) multiple urate tophi on the lung surface of a green iguana suffering from chronic renal disease.
The right paralumbar approach will provide greater access to the gall bladder at the caudal edge of the right liver lobe, while the pancreas is located adjacent to the duodenum (Figure 10A and 13B). In addition, the iguanid sacculated colon is more readily appreciated from the right side (Figure 13D).

Abnormal structures should be documented and samples collected using biopsy forceps (Figure 17). When dealing with potentially cystic structures, the fine aspiration needle reduces the risk of post-sampling leakage and contamination of the coelom compared to biopsy forceps. Care should be taken when collecting samples from the surface of the gastrointestinal or urogenital tracts as perforation may result in leakage and coelomitis. In addition, blood vessels and nerves should be avoided unless lesions are large and can be sampled without damaging the integrity of structure. Following coelioscopy, insufflation gas is aspirated followed by routine skin closure using a single suture and/or tissue adhesive (VetBond, 3M, St. Paul, MN). There is no need to repair the small puncture wound in the coelomic musculature.

COELIOSCOPY – SERPENTES

Patient positioning — Coelioscopy in the snake is not as rewarding or straightforward as it is in the lizard. The elongated body design of the snake makes it impossible to examine all organs from a single entry point. In addition, the more diffuse fat bodies, and numerous fascial planes make insufflation and navigation more difficult. A targeted
coelioscopic approach can be used to examine and biopsy from a specific area, e.g. liver or kidneys. The precise entry point is governed by species-specific anatomy (McCracken, 1999).

Endoscopic procedure — Snakes are placed in lateral recumbency. In small snakes the telescope may be inserted between the first and second row of lateral scales to enter the coelom ventromedial to the ribs. In larger specimens, the telescope can enter through the intercostal muscles, between two ribs. The difficulty of telescope introduction in snakes can be reduced with a Veress needle to induce pneumocoelom prior to sheath-obturator insertion, or by an optical or endotip cannula that can be inserted under direct visual control (Ternamian and Deitel, 1999). The less distendable coelom of snakes may also warrant increased insufflation pressures of up to 0.8 – 1.4 KPa (6 – 10 mmHg) to create an adequate pneumocoelom. Targeted examination and biopsy of liver, kidney, and splenopancreas are possible (Figure 18). The rigid telescope can be used for lung examination via a temporary pneumotomy. A small coeliotomy approach is performed to identify the lung. While maintaining maximum inspiration a stab incision is made into the lung and the telescope is inserted. A purse-string suture is used to ensure an adequate seal and prevent the escape of anesthetic gas.

COELIOSCOPY – CHELONIA

Patient positioning — The most useful endoscopic approach to the chelonian coelom is through the prefemoral fossa. Unless, diagnostic imaging or anatomic considerations dictate otherwise, the decision about a left or right approach can be determined by the preference of the endoscopist. The conformation of the shell and prefemoral fossa makes a left fossa approach easier for right-handed surgeons, and a right fossa approach easier for left-handed surgeons.

The chelonian is positioned in lateral recumbency using a vacuum positioning cushion (Vac-Pacs, Olympic Medical, Seattle, WA) or sand-bags. The pelvic limb is retracted and secured caudad to expose the prefemoral fossa (Figure 19). In chelonians with a pronounced caudal plastron hinge it is usually necessary to place a wedge between the caudal plastron and carapace to maintain adequate exposure of the prefemoral fossa.

Endoscopic procedure — Following aseptic preparation of the prefemoral area and surrounding shell, a 2 – 4 mm cranial to caudal skin incision is made in the center of the fossa. The subcutaneous fat and connective tissues are bluntly dissected using hemostats. This dissection is continued to the level of the coelomic aponeurosis, which is formed by the broad tendinous portions of the transverse and oblique abdominal muscles. Muscle damage and hem-

Figure 16. Lizard coelioscopy. (A) Elongated spleen (1) and closely associated stomach (2), and testis (3) in a green iguana (Iguana iguana); (B) spherical spleen (1) and stomach (2) in a Yemen chameleon (Chameleo calyptratus); (C) adrenal gland (arrow) dorsal to the epididymis (1) and testis (2) in a male iguana; (D) urinary bladder in an iguana.

Figure 17. Lizard coelioscopy. (A) Vestigial yolk sac (arrow) attached via a short stalk to the intestinal tract (1) of a sub-adult green iguana (Iguana iguana); (B) two fungal granulomas attached to the pleuroperitoneal membrane of the coelomic wall in a green iguana (Iguana iguana); (C) hepatic cyst (arrow) attached to the liver (1) and close to the small intestine (2) in a bearded dragon (Pogona vitticeps); (D) multifocal pale streaks within the internal abdominal oblique muscle of an iguana with chronic renal disease, biopsy confirmed metastatic soft tissue mineralization.
orrhage can be avoided by remaining cranial and ventral to the sartorius and iliacus muscles, respectively. Entry into the coelom is accomplished by penetrating the coelomic aponeurosis with the sheath and obturator, aiming towards the mid-point of the cranial rim of the carapace (Figure 20). Insufflation is essential and is provided using the same techniques at the same pressures as previously described for lizards.

Identification of the prominent liver is used to orientate the endoscopist (Figure 21). The stomach lies in a craniodorsal location, often partially obscured by the liver. The intestinal tract may be viewed from either side, although the duodenum is easier to locate from the right (Figure 22). The inactive ovary and oviduct are situated in the caudodorsal coelom but once mature may occupy much of the central region (Figure 23a–c). The male testis, often cream, yellow or brown in color, epididymis, and vas deferens are readily visible (Figure 23d). The adrenal glands lie craniomedial to the gonads and the retrocoelomic kidneys are located in the caudodorsal coelom. The retrocoelomic kidneys may be obscured behind a pigmented coelomic membrane making it impossible to identify or biopsy unless this covering is incised and reflected (Figure 24). The pancreas and spleen can be challenging to find, but are more commonly located on the right side (Figure 25a,b). The lungs are situated dorsad and typically only the ipsilateral lung is visualized from a lateral approach. In

![Figure 18. Coelioscopic view of the kidney (1), fat body (2), ribs and intercostal muscles (3) in a boa constrictor (Boa constrictor). Note the relatively poor degree of coelomic expansion despite insufflation, which is common in snakes.](image)

**Figure 19.** Chelonian positioning for coelioscopy. (A) Loggerhead sea turtle (*Caretta caretta*) placed in lateral position with the pelvic limb secured to expose the prefemoral fossa and telescope entry site (arrow); (B) diagram to illustrate the regional anatomy of the prefemoral area, and in particular the location of the coelomic aponeurosis, sartorius and iliacus muscles. Adapted from Bojanus (1819).
some species, particularly aquatic chelonia (e.g., Loggerhead sea turtle, *Caretta caretta*), the post-pulmonary membrane is very thin and the lungs can be easily visualized. However, in many terrestrial chelonia (e.g., Greek tortoise, *Testudo graeca*) the post-pulmonary membrane, or *septum horizontale*, is more prominent making it impossible to observe the lungs directly (Figure 25c,d). The heart lies outside the visceral coelomic cavity, within a distinct cranioventral pericardial sac, while the urinary bladder is variable in size and can occupy much of the dependent coelom (Figure 26a,b). Fluids and tissues can be sampled using previously described techniques (Figure 26c). Following insufflation gas removal, it is not necessary to repair the coelomic membrane or aponeurosis during closure (Figure 26d). The skin is closed as previously described. Water-proofing the surgical site with tissue glue is recommended for aquatic species.

An extra-coelomic approach to the chelonic kidney has also been described (Hernandez-Divers, 2004). This technique involves advancement of the sheathed telescope in a caudodorsal direction between the coelomic aponeurosis and the broad iliacus muscle. A combination of gentle lateral movements of the telescope tip coupled with intermittent insufflation is required to separate the coelomic aponeurosis from musculature, and reveal the retrocoelomic kidney(s).

**Postoperative care** — As long as insufflation gas is evacuated, most reptiles recover quickly from minimally-invasive endoscopy, but as with any surgical procedure continued provision of an appropriate thermal environment, analgesia, assisted ventilation, fluid therapy, and nutritional support should be considered. Antimicrobials are not routinely used following coelioscopy unless infection or contamination is identified at the time of surgery.

**DISCUSSION**

Endoscopy is a surgical procedure and, as such, is limited by any contraindication for general anesthesia, and the abilities of the surgeon. Debilitated animals should be medically stabilized prior to coelioscopy. It is important to
Figure 21. Chelonian coelioscopy. (A) Normal liver in a Greek tortoise (*Testudo graeca*) demonstrating multifocal pigmented areas of melanoma-macrophage aggregation; (B) diffusely pale liver in a male Hermann’s tortoise (*Testudo hermanni*) due to hepatic lipidosis; (C) pale area in the liver of a leopard tortoise (*Geochelone pardalis*) due to focal bacterial hepatitis; (D) liver biopsy from a juvenile loggerhead sea turtle (*Caretta caretta*).

Figure 22. Chelonian coelioscopy. (A) Stomach (1), liver (2) and cranial oviduct (3) in a Greek tortoise (*Testudo graeca*); (B) stomach (1), ileum (2), large intestine (3) and lung (4) in a loggerhead sea turtle (*Caretta caretta*); (C) large intestine in a Greek tortoise (*Testudo graeca*); (D) distended large intestine (arrow) due to impaction in a leopard tortoise (*Geochelone pardalis*).

Figure 23. Chelonian coelioscopy. (A) Immature ovary (arrow) in a loggerhead sea turtle (*Caretta caretta*); (B) ovary (1) largely obscured by the closely associated infundibulum (2) and pale liver (3) in an adult female Greek tortoise (*Testudo graeca*), note that increased hepatic fat is physiologic and normal during vitellogenesis; (C) involuted oviduct in a juvenile leopard tortoise (*Geochelone pardalis*); (D) testis (1), vas deferens (arrow), epididymis (2), and closely associated retrocoelomic kidney (3) in a red-eared slider (*Trachemys scripta elegans*).

Figure 24. Chelonian coelioscopy. (A) Retrocoelomic kidney (arrow) in a female Hermann’s tortoise (*Testudo hermanni*); (B) testis (1), retrocoelomic kidney (2) and vas deferens (3) in an adult male Greek tortoise (*Testudo graeca*); (C) incision through the coelomic membrane (1) using endoscopic scissors (2), to reveal the retrocoelomic kidney (3) of a box turtle (*Terrapene carolina*); (D) biopsy from the retrocoelomic kidney of the same box turtle.
remember that intracoelomic administration of fluids may subsequently impede coelioscopy unless they are aspirated at the beginning of the procedure. Stabilization is not always possible and many procedures have been successfully accomplished in moderate to high risk patients (Hernandez-Divers, 2004). Fluid therapy, assisted ventilation, thermal control, anesthetic monitoring, minimal surgical trauma and species-specific anatomic knowledge, and reduced operating times compared to standard coeliotomy are critically important for minimizing surgical risks.

Human endoscopists benefit from artificial teaching devices and prolonged supervised instruction by experienced surgeons. Human laparoscopy trainers are expensive and do not relate to the 2.7 mm system commonly used in reptile practice. In addition, there are limited opportunities to learn reptile endoscopy during traditional surgery or exotic animal residencies. Therefore, within the veterinary field initial instruction is best achieved through participation in continuing education courses and practical laboratories. While every opportunity should be taken to practice these techniques on cadavers, reptile carcasses represent a useful but imperfect model due to rapid deterioration after death. In those countries that permit and regulate the use of live animals for training veterinarians, non-recovery endoscopy laboratories using anesthetized reptiles offer an unparalleled opportunity for establishing competence before embarking on clinical cases.

Endoscopy should ideally be performed after baseline clinicopathology and diagnostic imaging. The information such diagnostic procedures provide assist in determining structures of primary interest, and the best endoscopic approach. Entry of the 2.7 mm telescope, which is suitable for the majority of reptiles presented to clinicians, only requires a 2 – 4 mm skin incision and minimal blunt dissection. The approaches described have not resulted in any significant morbidity. Insufflation is considered essential for reptile coelioscopy to provide sufficient telescope-tissue distance for examination and sample collection. Required insufflation pressures may varied from 0.4 – 0.7 KPa (3 – 5 mmHg) for lizards, crocodilians, and chelonians; however, pressures up to 1.4 KPa (10 mmHg) were occasionally required in large snakes due to their reduced coelomic space. Nevertheless, pressures were consistently lower than those used in mammalian laparoscopy, typically 1.6 – 2.0 KPa (12 – 15 mmHg) (Magne and Tams, 1999). Use of higher insufflation pressures in reptiles are likely to result in blood vessel compression and reduced venous return because of the lower diastolic blood pressures of reptiles (Hicks, 1998).

Most reptiles lack any form of muscular diaphragm, although testudines, teiids, varanids, and crocodilians may possess post-pulmonary and/or post-hepatic membranes that may be substantial (Perry, 1998). The lack of a true, muscular diaphragm does result in lung compression during coelomic insufflation even at the relatively low
pressures recommended. Tidal volume is maintained when using a volume-cycle ventilator but with pressure-cycle ventilators the inspiration pressure should be increased to counteract the effects of insufflation on pulmonary function. Most procedures are performed with the animal in lateral or dorsal recumbency and although no adverse effects have been noted as a consequence, it is possible that the dependent lung may be collapsed by the weight of overlying viscera. Postural factors should be considered as potential causes of ventilation-perfusion mismatches, particularly in large reptiles (Wang, et al. 1998).

Carbon dioxide is recommended for insufflation in human and veterinary laparoscopy because it is readily absorbed, rapidly eliminated, and has been associated with fewer complications (Golditch, 1971, Magne and Tams, 1999). However, on the rare occasions when air was used, no deleterious effects were observed ((Divers, 1998, 2000). In all cases pneumocoeolom should be resolved prior to closure to reduce post-operative discomfort.

Magnification provided by the telescope assists with the identification and biopsy of lesions, with minimal collateral damage to adjacent structures. Instruments used through the operating channel of the sheath enables visualization and biopsy through a single-entry technique in most reptiles under 10 kg. In larger animals, two or three ports (telescope and one or two instruments) can be triangulated, but again a surgeon can usually accomplish diagnostic procedures unassisted.

There are very few pathognomonic signs or consistent clinicopathologic changes associated with known disease states of reptiles. In many cases a definitive diagnosis of disease relies upon the demonstration of a host pathologic response, and if infectious, culture and identification of the causative pathogen. Tissue biopsies are, therefore, frequently essential, and endoscopic biopsy provides a minimally-invasive technique for their collection. Correct handling of biopsy forceps and collected tissue reduces crush artifact, improves biopsy quality, and enhances histopathologic interpretation. For example, the results of a renal biopsy study in 23 green iguanas indicated that endoscopic biopsy collection produced excellent samples with negligible trauma to the patient (Hernandez-Divers, et al, 2004b). Common alternatives to endoscopic biopsy include conventional surgical and ultrasound-guided techniques. Reports from human surgeons indicate that considerable benefits may be gained from minimally-invasive endoscopic surgery, compared to other techniques (Golditch, 1971, Corson and Grochmal, 1990, Vander Velpen, et al, 1994, Yu, et al, 1997, Kehlet, 1999, Lagares-Garcia, et al, 2003). Human laparoscopy has been credited with more rapid and accurate diagnosis, reduced need for extensive laparotomy, reduced surgical stress, improved postoperative pulmonary function, reduced hypoxemia, reduced surgical time, and faster recovery (Yu, et al, 1997, Kehlet, 1999). The disadvantage of human laparoscopy appears minimal and restricted to misdiagnosis in less than 1% of cases. No significant morbidity has been demonstrated with appropriate laparoscopic technique (Vander Velpen, et al, 1994). In the few comparative studies have been published in veterinary medicine, endoscopic techniques provided superior sample quality with reduced complication rates compared to ultrasound-guided procedures (Kovak, et al, 2002, Rawlings, et al, 2003).

The most substantial limitation to successful ante-mortem diagnosis is the relative small size and delicate nature of most reptiles. Both of these limitations can be largely overcome using diagnostic endoscopy which provides focal magnification, illumination, and minimally-invasive surgical access to the coelom. Obesity is a frequent hindrance in mammals, but the lack of extensive fat deposition around the visceral organs of most reptiles (except for the diffuse fat bodies of snakes) makes this less of a concern. However, inappropriate patient positioning or telescope entry into a fat body will certainly hinder endoscopic evaluation. Large bladders, voluminous intestinal tracts and active female reproductive systems can present more serious obstacles that should be appreciated and avoided. In addition, order, suborder and family differences in anatomy necessitate the application of general principles rather than rigidly adhered to techniques. For example, the ability to perform prefemoral coelioscopy in a chelonian is affected by the shape and conformation of the prefemoral fossa and shell.

No significant morbidity has been demonstrated with appropriate laparoscopic technique in humans (Vander Velpen, et al, 1994). The efficacy, complications, and long term effects of coelioscopy have not been extensively documented in reptiles, although previous and on-going studies at the University of Georgia continue to critically evaluate these procedures (Hernandez-Divers, 2004, Hernandez-Divers, et al, 2004a, Hernandez-Divers, et al, 2004b).

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The authors thanks Karl Storz Veterinary Endoscopy for providing photographs for figures 1B and 1C, and for their continued support of the endoscopy training, research, and development at the College of Veterinary Medicine, University of Georgia. We also thank our colleagues that assisted with endoscopic procedures used in writing this review, including Drs. Clarence Rawlings, Heather Wilson, Anneliese Strunk, Christopher Hanley, and Michael McBride. Thanks also to Kip Carter of Educational Resources for preparing the illustrations used in figures 5B and 6.
REFERENCES

The ball python (*Python regius*) is a medium-sized snake of the family **Boidae** and is native to West Africa. Because of its gentle nature, moderate size, and variably attractive skin patterns, this snake is a popular species maintained in captivity. The respiratory system of snakes has been extensively reviewed.¹ The trachea is long and narrow and composed of incomplete cartilaginous rings that are supported by a dorsal ligament. The trachea terminates into 2 short primary bronchi because ball pythons, like other boids, have both left and right lungs. Each bronchus continues a short distance as an intrapulmonary bronchus before terminating in the cranial portion of the lung. Each lung is composed of 3 areas: a highly vascular faveolar region in which gaseous exchange occurs; a short semisaccular (transitional) zone; and a larger saccular area, which is thin, semitransparent, and poorly vascularized.

As in other species of captive snakes, bacterial and fungal respiratory diseases are common in ball pythons and are often related to suboptimal temperature, humidity, or ventilation.² In addition, paramyxovirus-associated respiratory tract disease in boids and tracheal chondromas in ball pythons have been reported.³ Given that specific treatment requires accurate diagnosis, the collection of exudates and tissue samples from the respiratory tract is important.⁴ Although various sampling techniques have been described, endoscopy provides the least invasive means of direct lung examination and biopsy and has been described for snakes and other reptiles.⁵⁻⁷ The long narrow trachea of snakes makes it difficult to impossible to use most rigid and flexible endoscopes to evaluate the distal portion of the trachea and lung via an endotracheal approach. Consequently, transcutaneous insertion of a rigid endoscope directly into the lung has been advocated.⁸⁻¹² The purpose of the evaluation of transcutaneous pulmonoscopy for examination and biopsy of the lungs of ball pythons and determination of preferred biopsy specimen handling and fixation procedures

**Evaluation of transcutaneous pulmonoscopy for examination and biopsy of the lungs of ball pythons and determination of preferred biopsy specimen handling and fixation procedures**

Scott J. Stahl, DVM, DABVP; Stephen J. Hernandez-Divers, BVetMed, DZooMed, DACZM; Tanya L. Cooper; Uriel Blas-Machado, DVM, PhD, DACVP

**Objective**—To establish a safe and effective technique for the endoscopic examination and biopsy of snake lungs by use of a 2.7-mm rigid endoscope system.

**Design**—Prospective study.

**Animals**—17 subadult and adult ball pythons (*Python regius*).

**Procedures**—The right lung of each anesthetized snake was transcutaneously penetrated at a predetermined site. Endoscopic lung examination was objectively scored, and 3 lung biopsies were performed. Tissue samples were evaluated histologically for diagnostic quality. One year later, 11 of the 17 snakes again underwent pulmonoscopy and biopsy; specimens were placed in various fixatives to compare preservation quality. All 17 snakes were euthanatized and necropsied.

**Results**—No major anesthetic, surgical, or biopsy-associated complications were detected in any snake. In 16 of 17 pythons, ease of right lung entry was satisfactory to excellent, and views of the distal portion of the trachea; primary bronchus; intrapulmonary bronchus; cranial lung lobe; and faveolar, semisaccular, and saccular lung regions were considered excellent. In 1 snake, mild hemorrhage caused minor procedural difficulties. After 1 year, pulmonoscopy revealed healing of the previous transcutaneous lung entry and biopsy sites. Important procedure-induced abnormalities were not detected at necropsy. Diagnostic quality of specimens that were shaken from biopsy forceps into physiologic saline (0.9% NaCl) solution before fixation in 2% glutaraldehyde or neutral-buffered 10% formalin was considered good to excellent.

**Conclusions and Clinical Relevance**—By use of a 2.7-mm rigid endoscope, lung examination and biopsy can be performed safely, swiftly, and with ease in ball pythons. Biopsy specimens obtained during this procedure are suitable for histologic examination. (J Am Vet Med Assoc 2008;233:xxx–xxx)
study reported here was to establish a safe and effective technique for transcutaneous endoscopic examination and biopsy of the lungs of snakes by use of a 2.7-mm rigid endoscope.

**Materials and Methods**

**Animals**—Seventeen recently imported adult ball pythons (15 females and 2 males) were obtained from a reptile wholesaler for use in the study. All procedures and methods were reviewed and accepted by the University of Georgia’s Institutional Animal Care and Use Committee (IACUC No. A2006-10076-0). The pythons were maintained in conditions approved by the Association for Assessment and Accreditation of Laboratory Animal Care. Snakes were housed in groups of 3 or 4 in large plastic containers maintained in a room at an ambient temperature of 24°C (75°F) during the night and 27°C (81°F) during the day. Mercury halide incandescent lamps that were suspended above each enclosure provided a daytime basking area at 35°C (95°F). Pythons were exposed to a repeating cycle of 12 hours of light followed by 12 hours of darkness and a general humidity level of 50%. The snakes were physically examined on arrival and found to be clinically normal adults. The snakes were acclimatized to the research facilities for 7 days prior to the start of the study. They were not offered food during this acclimatization period, but were permitted to recover from anesthetic or surgical procedures. The examination, anesthesia, and surgery areas were maintained at 24°C (75°F) during the night and 27°C (81°F) during the day. Mercury halide incandescent lamps that were maintained in a room at an ambient temperature of 24°C (75°F) for at least 1 hour prior to commencement of experimental procedures. The examination, anesthesia, and surgery areas were maintained at 24°C. Body weight and resting respiratory and heart rates were recorded for each snake. Each snake was identified by use of a unique number written with a permanent marker pen on the dorsal aspect of the cranium.

**Anesthesia**—Each python was premedicated with butorphanol tartrate (1 mg/kg [0.45 mg/lb]) administered via injection into the epaxial muscles 20 minutes prior to induction of anesthesia via intracardiac injection with propofol (5 mg/kg [2.27 mg/lb]). Following intubation, anesthesia was maintained by use of 1% to 3% isoflurane in 100% oxygen (flow rate, 1 L/min) and adjusted to the individual’s requirements. Throughout the anesthetic period, assisted ventilation was provided by use of a pressure-cycle ventilator; adjustments were made to maintain end-tidal CO₂ readings > 10 mm Hg. Hypothermia was minimized by placing the snake on recirculating warm water blankets that were set to 40°C (105°F). Monitoring included assessments of tongue and tail withdrawal reflexes and ventral muscle tone, end-tidal capnography, cardiac Doppler ultrasonography, pulse oximetry, and esophageal temperature measurement.

**Endoscopy**—Each python was positioned in left lateral recumbency (with the dorsum facing the surgeon) on a horizontally level surgery table. The surgical entry site was identified at 90 ventral scales caudal to the head and 9 scales lateral on the right side. Following aseptic preparation, a vertical 8- to 10-mm incision was made through the interscalar skin. The subcutis was bluntly dissected until the underlying ribs and intercostal space were identified. Small straight mosquito hemostats were used to penetrate the intercostal muscles and separate the 2 adjacent ribs. The serosal surface of the right lung was identified as a thin semitransparent membrane containing a latticework of small blood vessels, which inflated in association with ventilation. The lung was penetrated by use of small hemostats to create a 3- to 4-mm pneumotomy and facilitate insertion of the 30° telescope (2.7 mm X 18 cm) that was housed within a 14.5-F operating sheath and connected to a xenon light source, endovideo camera, monitor, and digital recorder.

Endoscopic examinations were performed by 2 experienced reptile endoscopists (SJS and SJHD). Each endoscopist scored the ease of entry into the lung (including skin incision, hemostat penetration, and entry of the endoscope) on a scale from 1 to 5 (1 = impossible [interval to insertion of endoscope, > 15 minutes]; 2 = difficult [interval to insertion of endoscope, 11 to 15 minutes]; 3 = satisfactory [interval to insertion of endoscope, 6 to 10 minutes]; 4 = good [interval to insertion of endoscope, 2 to 5 minutes]; and 5 = excellent [interval to insertion of endoscope, < 2 minutes]). Additionally, the endoscopist scored the ease of location and observation of various structures associated with the right side of the lower respiratory tract, including the distal portion of the trachea; primary bronchus; intrapulmonary bronchus; and regions of faveolar (cranial, vascular) lung, semisaccular (transitional zone) lung, and saccular (avascular air sac) lung on a scale of 1 to 5 (1 = impossible, 2 = difficult, 3 = satisfactory, 4 = good, and 5 = excellent).

**Biopsy specimen collection**—Once the evaluation was completed, 3 biopsies were performed endoscopically; samples were collected from the right faveolar region by use of 5-F biopsy forceps through the instrument channel of the endoscope sheath. Each biopsy specimen was gently transferred from the forceps to a biopsy cassette by use of a moistened cotton-tipped applicator; the cassette was then closed and placed in neutral-buffered 10% formalin. Hemorrhage from the biopsy sites was recorded on a scale of 1 to 3 (1 = no hemorrhage, 2 = minor hemorrhage, and 3 = major hemorrhage).

**Completion of procedure**—Only the skin was closed by use of a single 4-0 polydioxanone horizontal mattress suture. Any complications associated with the anesthetic or surgical procedures were recorded. Eleven snakes were permitted to recover from anesthesia and were provided with postoperative analgesia (0.2 mg of meloxicam/kg [0.09 mg/lb], IM). Six pythons were not permitted to recover but were euthanized via IV injection of pentobarbital for immediate necropsy.

**Repeat pulmonoscopy and biopsy specimen collection**—The 11 remaining snakes were maintained...
for 12 months before undergoing repeat anesthesia and transcutaneous pulmonoscopy, as described. This second procedure was not scored, and the entry site was located at 95 ventral scales caudal to the head and 9 scales lateral on the right side to facilitate examination of the previous surgical approach. The right lung was evaluated for signs of disease or trauma that could be associated with the previous surgery. In particular, the surgical entry site into the lung and biopsy sites were evaluated.

Three endoscopic biopsy specimens were collected from the faveolar region of each snake (33 in total), but to avoid any physical damage to the harvested tissue, each biopsy was gently shaken from the forceps into a sterile red-top blood collection tube containing 1 mL of physiologic sterile saline (0.9% NaCl) solution. The sterile saline solution was then decanted and replaced with 1 of 3 fixatives; neutral-buffered 10% formalin solution, 2% glutaraldehyde, or Davidson’s medium. Biopsy specimens were processed routinely for histologic evaluation.

Necropsy and histologic examination of tissue—Six pythons were euthanatized via IV administration of pentobarbital immediately following the original endoscopic procedure, and each snake underwent a full gross necropsy examination. The remaining 11 snakes were similarly euthanatized and examined 12 months later, immediately following the second endoscopy procedure. In all instances, the right lung was evaluated for any evidence of trauma or disease, and samples of lung (and any other abnormal tissues) were collected into neutral-buffered 10% formalin for routine histologic evaluation. Biopsy and necropsy tissues were processed routinely, embedded in paraffin, sectioned at approximately 5 µm, stained with H&E stain, and examined microscopically. Histologically, biopsy and necropsy tissues were subjectively compared to determine whether biopsy specimens collected during endoscopy were representative of tissue collected during necropsy. In addition, the diagnostic quality of each biopsy specimen was scored on a scale of 1 to 4 (1 = nondiagnostic, 2 = poor, 3 = good, and 4 = excellent); the criteria used included relative size of the biopsy sample in relation to the area biopsied, presence of crushing artifacts, quality of architectural detail preservation achieved via fixation, and tinctorial quality of the stained tissue.

Results

Among the 17 ball pythons, mean ± SD body weight and snout-to-vent length were 1,348 ± 327 g (2.972 ± 0.721 lb) and 109.9 ± 9.4 cm, respectively. All snakes appeared to be clinically normal adults and were in acceptable body condition. There were no significant changes in body weights during the course of the study. Premedication with butorphanol, induction of anesthesia with propofol, and maintenance of anesthesia with isofluorane in oxygen via intermittent pressure ventilation resulted in a surgical plane of anesthesia without complications in all snakes. Pre- and intraoperative variables were recorded (Table 1).

### Table 1—Pre- and perioperative findings in 17 ball pythons that underwent transcutaneous rigid pulmonoscopy of the right lung.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative respiratory rate (breaths/min)</td>
<td>8.3 ± 4.9</td>
</tr>
<tr>
<td>Preoperative heart rate (beats/min)</td>
<td>44 ± 8</td>
</tr>
<tr>
<td>Butorphanol premedication dose* (mg/kg)</td>
<td>1.0 ± 0.03</td>
</tr>
<tr>
<td>Propofol dose† for induction of anesthesia (mg/kg)</td>
<td>5.2 ± 1.4</td>
</tr>
</tbody>
</table>

Intraoperative ventilation rate‡ (breaths/min) 6.4 ± 0.9
Intraoperative maximum inspiratory pressure (mm Hg) 4.0 ± 0.9
Intraoperative end-tidal CO2 pressure (mm Hg) 12.4 ± 1.7
Intraoperative heart rate (beats/min) 32 ± 7
Intraoperative esophageal temperature (°C[°F]) 27.4 ± 0.8 (81.3 ± 1.4)

*Administered via injection into the epaxial muscles. †Administered via intracardiac injection. ‡Assisted ventilation was provided throughout the anesthetic period by use of a pressure-cycle ventilator.

### Table 2—Assessments (mean ± SD scores) of ease of entry into the right lung, ease of observation of anatomic structures, and hemorrhage from biopsy sites in 17 ball pythons that were anesthetized and underwent transcutaneous rigid pulmonoscopy of the right lung.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ease of initial entry*</td>
<td>4.2 ± 1.0</td>
</tr>
<tr>
<td>Ease of location and observation of various structures†</td>
<td>4.9 ± 0.2</td>
</tr>
<tr>
<td>Distal portion of the trachea</td>
<td>4.3 ± 0.5</td>
</tr>
<tr>
<td>Primary bronchus</td>
<td>5.0 ± 0.0</td>
</tr>
<tr>
<td>Intrapulmonary bronchus</td>
<td>5.0 ± 0.0</td>
</tr>
<tr>
<td>Faveolar lung region</td>
<td>5.0 ± 0.0</td>
</tr>
<tr>
<td>Semisaccular lung region</td>
<td>5.0 ± 0.0</td>
</tr>
<tr>
<td>Saccular lung region</td>
<td>5.0 ± 0.0</td>
</tr>
<tr>
<td>Postbiopsy hemorrhage†</td>
<td>1.1 ± 0.3</td>
</tr>
</tbody>
</table>

*Ease of entry into the lung was assessed on a scale from 1 to 5 (1 = impossible [interval to insertion of endoscope, > 15 min]; 2 = difficult [interval to insertion of endoscope, 11 to 15 min]; 3 = satisfactory [interval to insertion of endoscope, 6 to 10 min]; 4 = good [interval to insertion of endoscope, 2 to 5 min]; and 5 = excellent [interval to insertion of endoscope, ≤ 2 min]). †Ease of location and observation of various structures was assessed on a scale from 1 to 5 (1 = impossible, 2 = difficult, 3 = satisfactory, 4 = good, and 5 = excellent). Hemorrhage from the biopsy sites was assessed on a scale of 1 to 3 (1 = no hemorrhage, 2 = minor hemorrhage, and 3 = major hemorrhage).

Endoscopy score data were not normally distributed (Table 2). All mean endoscopy scores were > 4 (good), and the mean hemorrhage score was only 1.1 (Figure 1). In 16 of 17 snakes, the ease of entry score was considered satisfactory to excellent; however, in 1 snake, entry was considered difficult (score of 2) because of mild hemorrhage following hemostat penetration into the lung. Observation of the structures associated with the lower respiratory tract (accessed via the right lung), including the distal portion of the trachea; primary bronchus; intrapulmonary bronchus; cranial lung lobe; and faveolar, semisaccular, and saccular lung regions, was considered excellent (score of 5) in 16 of 17 pythons. In the snake with mild hemorrhage, observations of the trachea and primary bronchus were considered good (score of 4) and satisfactory (score of 3), respectively. However, endoscopic examination and biopsy procedures were still performed without complication in that snake.
Among the 17 snakes, minor hemorrhage was rarely associated with entry into the lung but more commonly developed following lung biopsy. In the initial experiment, biopsy procedures were associated with minor and clinically unimportant bleeding in 15 of 17 snakes. In 2 snakes, intraoperative bleeding was considered severe in the endoscopic views, but in one of those snakes, no major hemorrhage was identified during necropsy performed immediately after completion of the experiment. The other affected python recovered completely and uneventfully, without any evidence of mucous membrane pallor associated with severe hemorrhage.

All 11 snakes that were allowed to recover from anesthesia did so uneventfully, without any evidence of morbidity and with no deaths associated with the procedure. Repeat pulmonoscopy 1 year later revealed healing of the previous biopsy sites, which appeared as small defects within the faveolar lung; typically, the original pneumotomy site was barely visible as a small scar.

Necropsy examinations did not reveal any notable damage to the skin, subcutis, and pulmonary or other visceral tissues. Diagnostic quality scores of the biopsy specimens obtained via pulmonoscopy were assessed; criteria for score allocation included the presence of crushing artifacts, preservation of architectural detail, and tinctorial quality of H&E-stained tissues. Specimens collected from 17 snakes (3 specimens/snake; 51 biopsy specimens in total) during the initial endoscopic procedure were transferred to a biopsy cassette by use of a cotton-tipped applicator, and the cassette was placed in neutral-buffered 10% formalin; for these samples, mean ± SD quality score was 2.5 ± 0.8. Specimens were also collected from 11 of those snakes during a repeat procedure 1 year later (3 specimens/snake; 33 biopsy specimens in total). Samples were each shaken from forceps into saline solution and transferred to a biopsy cassette prior to placement in neutral-buffered 10% formalin, glutaraldehyde, or Davidson’s medium; for these samples, mean ± SD quality score was 3.0 ± 0.0, 3.3 ± 0.5, and 2.0 ± 0.0, respectively.

Regardless of the fixative solution and method used for tissue handling, biopsy specimens were well-fixed and representative of the luminal half of the faveolar lung (Figure 2). Although most of the specimens were of adequate size, some were too small or were crushed during collection or transfer to the fixatives. In other specimens, the faveolar septae were clumped or collapsed; such artifact was detected most frequently in specimens that were transferred from forceps directly to fixative solution by use of a cotton-tipped applicator, and the cassette was placed in neutral-buffered 10% formalin, glutaraldehyde, or Davidson’s medium; for these samples, mean ± SD quality score was 3.0 ± 0.0, 3.3 ± 0.5, and 2.0 ± 0.0, respectively.

Figure 1—Representative endoscopic views obtained from 17 ball pythons that were anesthetized and underwent transcutaneous rigid endoscopy of the right lung. A—Cranial view of the faveolar lung region. B—Close-up view of the faveolar lung region to illustrate the primary (p), secondary (s), and tertiary (t) septae of the vascular portion of the lung. C—View of the semisaccular (or transitional zone) region of the lung. D—Caudal view of the saccular lung region illustrating its thin nature and poor vascularity. E—View of the cranial aspect of the faveolar lung region illustrating the intrapulmonary bronchus (l), short primary bronchus (b), trachea (t), and anterior lung lobe (a). F—Close-up view illustrating the intrapulmonary bronchus (l), short primary bronchus (b), trachea (t), and anterior lung lobe (a). G—View into the anterior lung lobe (a) in which the primary bronchus (b) is also visible. H—View inside the distal portion of the trachea (t). Notice the incomplete cartilaginous rings and dorsal ligament (d). I—View of a biopsy procedure in the faveolar lung region involving use of B-F biopsy forceps. J—View to illustrate the minimal bleeding that was typically observed immediately following a biopsy procedure. K—View of a typical healed biopsy site after an interval of 1 year. Notice the healed defect within the faveolar tissue. b = Primary bronchus. L—View of an original pneumotomy entry site after an interval of 1 year. Notice the complete healing and minimal scarring (arrow) of the tissues.
Extensive morphometric data for many species of snakes has been summarized\(^1\) and can be used to accurately determine entry into the semisaccular lung. However, in the authors’ experience, entry into the right lung of most snake species can be approximated by identifying the location of the heart and selecting a point halfway between the heart and the vent (ie, at approx 40% to 45% of the total snout-to-vent length).

Propofol and isoflurane provided effective and controllable anesthesia in the ball pythons of the present study. End-tidal CO\(_2\) values have been poorly investigated in reptiles. Observations in green iguanas have indicated that there may be poor correlation between end-tidal CO\(_2\) and arterial PCO\(_2\) values because of intracardiac or intrapulmonary shunting.\(^9\) However, clinical observations by the authors have suggested that maintaining the end-tidal CO\(_2\) value at > 10 mm Hg in snakes reduces the time to return to unassisted respiration following anesthesia. Insertion and movement of the endoscope within the right lungs of the study snakes did not appear to interfere with the maintenance of surgical anesthesia and did not alter the measured physiologic variables; however, the ability to accurately control and maintain respiration by use of the electrical ventilator was likely essential. A tight seal between the endoscope and the snake’s skin, combined with closure of all the sheath ports, was important for preventing gas exchange across the surgical site. If a port were accidentally left open, the pressure cycle ventilator would not trigger and end-tidal CO\(_2\) values would decrease to zero.

The surgical approach used for endoscopic lung examination in snakes in the present study was more lateral and less extensive but otherwise similar to that described previously.\(^1\) In 16 of the 17 snakes, the ease of entry score was considered satisfactory to excellent; however, in 1 snake, entry was considered somewhat difficult because of mild hemorrhage following hemothorax penetration into the lung. Endoscopic evaluation of the structures associated with the right lower respiratory tract was considered excellent in 16 of 17 pythons; in the snake with hemorrhage, observations of the trachea and primary bronchus were considered good and satisfactory, respectively, but this did not impede completion of the examination and biopsy procedures.

Minor hemorrhage was occasionally associated with entry into the lung but most commonly evident following lung biopsy. Biopsy procedures were associated with minor bleeding in 15 of the 17 snakes. In 2 snakes, intraoperative bleeding appeared severe endoscopically; in 1 snake, no major hemorrhage was detected at necropsy immediately after the endoscopic examination, and the other recovered without clinical signs of severe hemorrhage. Although pre- and postoperative Hct values were not determined, on the basis of the clinical and necropsy findings, we concluded

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**Discussion**

The use of ball pythons for evaluation of the safety and effectiveness of rigid endoscopy to examine the lower respiratory tract of snakes was highly successful. The right lung was chosen for this procedure because the left lung is either absent or reduced in most snakes.\(^1\) However, in booids with disease that affects the left lung, a left (or bilateral) approach could be undertaken. The endoscope entry site into the right lung was specifically determined to coincide with the reduced vascularity of the semisaccular (transitional) portion of the lung. In addition to minimizing hemorrhage, entry at this level permitted an excellent view as far cranial as the distal portion of the trachea and as far caudal as the caudal extent of the saccular lung (air sac). In ball pythons, the entry site was located at 90 ventral scales caudal to the head and 9 scales lateral on the right side. This equates to 44% of the total snout-to-vent length. This landmark was determined by the anatomic evaluation and scale counts of several dissected specimens and published morphometric data.\(^1\) Jekl and Knotek\(^2\) suggest a similar entry point (35% to 45% of the total snout-to-vent length) for ball pythons, boa constrictors (Boa constrictors), and Burmese pythons (Python molurus bivittatus).
that hemorrhage was minor and clinically unimportant but was magnified by the optics of the endoscope. All snakes permitted to recover from anesthesia after the initial evaluation did so uneventfully and without any evidence of morbidity and with no deaths during the following 12-month period.

Lung biopsy tissues are delicate. In the present study, lung tissue architecture was damaged during transfer of specimens by use of cotton-tipped applicators, resulting in poor to satisfactory diagnostic quality (mean quality score, 2.5). Diagnostic quality of tissues was improved by gently shaking specimens free from the forceps into sterile saline solution, before proceeding with fixation in neutral-buffered 10% formalin or glutaraldehyde (mean quality score, 3.0 and 3.3, respectively). Compared with those fixation techniques, use of Davidson's medium resulted in poorer staining (mean quality score, 2.0) and is therefore not recommended for processing of snake lung biopsy specimens.

Transcutaneous pulmonoscopy appears to be safe and effective for examination of the lower respiratory tract of snakes and is recommended when fine-diameter flexible or rigid endoscopes cannot reach the lungs via an endotracheal approach. Furthermore, biopsy procedures performed during lung endoscopy appear to be tolerated well and yield tissue samples of diagnostic quality.

References

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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)
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 (35ºC [95ºF]) 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 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. 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 CO2 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 was inserted through the skin incision, directed cranial through the external abdominal oblique musculature and placed into the coelomic cavity. The obturator was removed and replaced with a 30º telescope that was connected to an endoscopic video system (including camera, monitor, xenon light source, and CO2 insufflator); CO2 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 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 CO2, 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 CO2 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 euthanatized 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...
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 CO₂ 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](image1.png)  
**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](image2.png)  
**Figure 3—Photomicrograph of a section of a liver biopsy specimen 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](image3.png)  
**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 = 80 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.\textsuperscript{13,14,16} Endoscopic liver biopsy was 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.\textsuperscript{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\textsuperscript{3}, respectively. Whereas the biopsy specimens collected from iguanas in the present study were small (2.4 mm\textsuperscript{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-mm forceps may be better suited for use in reptiles that are suitable for histologic interpretation.

Involving comparisons between biopsy specimens obtained endoscopically and tissue samples obtained during surgery or necropsy is needed before the diagnostic capability of endoscopic 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.\textsuperscript{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.

References


