doi: 10.17221/103/2016-VETMED

Evaluation of diagnostic coelioscopy including liver and kidney biopsies in cinereous vultures (*Aegypius monachus*)

S.H. Seok[1,3], D.H. Jeong[2], H.C. Lee[1], I.H. Hong[1], S.C. Yeon[1,3]

[1]College of Veterinary Medicine, Gyeongsang National University, Jinju, Republic of Korea
[2]Species Restoration Technology Institute of Korea National Park Service, Gurye, Republic of Korea
[3]Gyeongnam Wildlife Center, Jinju, Republic of Korea

ABSTRACT: Diagnostic coelioscopy, including liver and kidney biopsies, was performed in seven cinereous vultures (*Aegypius monachus*). A 5-mm endoscopy system was used for examination of coelomic viscera. The endoscopist rated the ease of entry into the coelom and visualisation. Coelioscopic biopsy was performed using a 5-mm biopsy forceps following the diagnostic coelioscopy, and the diagnostic quality of the samples was evaluated. The endoscopic entry and visualisation scores ranged from satisfactory to excellent for all coelomic structures, except for the oesophagus, spleen, epididymis/oviduct and pancreas in all vultures. The coelioscopic examinations of coelomic structures and biopsy samples were carried out safely and easily. The biopsy samples were suitable for histopathological examination. Thus, minimally invasive coelioscopy using a 5-mm endoscopy system can be considered a useful technique suitable for visceral examination of large raptors such as cinereous vultures.

Keywords: biopsy; avian endoscopy; minimally invasive endosurgery; large raptor

The cinereous vulture (*Aegypius monachus*) is one of the largest birds of prey and is one of the rarest species of raptors in the world. The worldwide population of the cinereous vulture is assumed to be about between 7 200 and 10 000 pairs, with 1 700–1 900 pairs in Europe, and 5 500–8 000 pairs in Asia (BirdLife International 2008). As a result of increased concern for wildlife ecology and preservation, many injured or sick animals are sheltered and rescued in wildlife centres that rehabilitate sick animals and reintroduce healthy animals back into their natural environment.

Endoscopy has been shown to be an effective diagnostic tool in veterinary medicine, and it also has been widely used in various species such as birds, reptiles and fish (Taylor 1994; Stetter 2002; Monnet and Twedt 2003; Hernandez-Divers and Hernandez-Divers 2004; Hernandez-Divers et al. 2004; Jekl and Knotek 2006; Boone et al. 2008; Tams and Rawlings 2011). Endoscopy is specially designed to visualise, examine, and perform biopsies of internal organs and tissues via a small surgical incision. Due to these features, minimally invasive endoscopic examination enables safe biopsy procedures without lengthy, invasive surgery (Richter 2001; Hernandez-Divers and Hernandez-Divers 2004; Tams and Rawlings 2011). Avian endoscopy systems commonly incorporate a small rigid telescope such as a 2.7-mm system, housed within an operating sheath, through which basic instruments can be inserted. However, this system may be restricted for use with large raptors such as cinereous vultures due to the limited operating radius.

Endoscopy offers many advantages including the capability of performing biopsies immediately under direct visual control when an abnormal structure or pathological lesion is noted. Some of the different biopsy techniques, such as percutaneous, endoscopic or open surgical procedures have been tried and tested for the collection of liver and kid-

doi: 10.17221/103/2016-VETMED

ney samples in mammals, birds and reptiles for diagnostic purposes (Kerwin 1995; Richter 2001; Rawlings et al. 2003; Muller et al. 2004; Hernandez-Divers et al. 2007). Although studies of endoscopic procedures in avian practice have been reported, including studies of some species of psittacine birds, falcons, pigeons and raptors, published information on endoscopic procedures for certain avian species including the large raptor species such as cinereous vultures is very limited (Crosta et al. 2002; Muller et al. 2004; Clayton and Ritzman 2005; Hernandez-Divers et al. 2006; Jekl et al. 2006).

Therefore, using the cinereous vulture as a large raptor model, this study aimed to investigate a technique for diagnostic coelioscopy, evaluate its ability to visualise visceral structures, and to assess the quality of the liver and kidney through the biopsy samples obtained.

MATERIAL AND METHODS

Animals. This study was approved by the Institutional Animal Care and Use Committee at Gyeongsang National University (approval number: GNU-140128-E0005). Seven cinereous vultures were used in this study. They were hospitalised in the wildlife centre in the province of Gyeongsangnam-do, Republic of Korea. They had received appropriate treatment to correct their health problems but were not healthy enough to reintroduce back into the wild. The health status of vultures was assessed by physical examination, diagnostic imaging, complete blood count (CBC) and serum biochemical analyses. The birds were acclimated to the rehabilitation programme for at least seven days prior to the study. The birds were then transferred to temporary individual housing units, and food was withheld for 12 h prior to anaesthesia, but access to water was provided *ad libitum*. Although two of these cinereous vultures recovered normal health status, euthanasia was carried out due to permanent disabilities.

Anaesthesia and monitoring. Anaesthesia was induced with 5% isoflurane (Ifran®, Hana Pharm, Korea) in 100% oxygen (3 l/min) delivered via a mask in a circle system. A non-rebreathing anaesthetic circuit (Modified Jackson Rees anaesthesia circuit) was used in this study. After induction, the trachea was intubated with an uncuffed endotracheal tube. After endotracheal intubation, anaesthesia was maintained by spontaneous ventilation with isoflurane in 100% oxygen (3 l/min) to produce a surgical depth of anaesthesia. The end tidal CO_2 partial pressure ($PetCO_2$), end tidal isoflurane concentration (ETiso) and respiratory rate were monitored with a calibrated multigas monitor (AS3®, Datex-Ohmeda Division Instrumentarium Corp., Finland). Oxyhaemoglobin saturation was also monitored continuously with a pulse oximeter. Heart rate and electrocardiogram (ECG) were monitored with the monitor noted above during the procedure. Body temperature was also recorded with an oral probe linked to the patient monitor. Throughout the surgery, the body temperature was maintained at 39–40 °C with a circulating water blanket (Medi-Therm®, Gaymar Industries Inc., USA), and 0.9% normal saline was administered intravenously during the surgery at a rate of 10 ml/kg/h.

Diagnostic coelioscopy. The cinereous vultures were positioned in 45° right-oblique dorsal recumbency on a surgical positioning table (Tippy table®, Biovision Veterinary Endoscopy, USA) designed to facilitate the rotation of the animal from dorsal recumbency to right or left lateral recumbency while maintaining an aseptic surgical field (Figure 1A). The left wing and pelvic limb were secured caudo-dorsally over the back at the edge of the Tippy table with a 7.5-cm self-adherent bandage (3-in Coban™, 3M, USA). Then, the vulture was turned about 80–90° to the right as a tilt of 45° to the right of the Tippy table. After removing feathers from the left flank, and taping around it, the flank was aseptically prepared with a chlorhexidine scrub solution and a single final wipe of 70% alcohol (Figure 1B). Using a No. 11 scalpel blade, a 4-mm skin incision was made behind the last rib as it crosses the centre of the left flank region. Then, lifting the skin by Allis tissue forceps and dissecting bluntly through the body wall by small haemostats, the first port was made by inserting a trocar into the caudal thoracic air sac. Through this port, a 30° forward-oblique, 5 mm × 30 cm telescope (Panoview Plus®, Richard Wolf GmbH, Germany) with a 1 CCD video camera (Single Chip Camera 5512®, Richard Wolf GmbH, Germany) and a light source (Auto LP 4251®, Richard Wolf GmbH, Germany) was inserted and any damages and haemorrhage upon insertion of the trocar were evaluated. Under laparoscopic observation, the second 5-mm port was made about 2–3 cm caudal from that point in the same way as

doi: 10.17221/103/2016-VETMED

Figure 1. Diagnostic coelioscopy in cinereous vulture (*Aegypius monachus*). (**A**) A cinereous vulture was positioned in 45° right-oblique dorsal recumbency on the Tippy table; (**B**) after removing feathers and taping around it, the left flank was aseptically prepared; (**C** and **D**) diagnostic coelioscopy was performed with the double entry technique

previously described. This port served as the instrument port during the surgical procedure. The 5-mm telescope was inserted through the first port into the caudal thoracic air sac. Then, exploration of adjacent cranial thoracic and abdominal air sacs was performed by pressing the tip of the telescope against the air sac membrane and advancing the telescope in a gentle sweeping motion until the air sac membranes were breached. If necessary, 5-mm grasping forceps (Atraumatic grasping forceps®, Richard Wolf GmbH, Germany) were used through the second port during the exploration of the coelom (Figures 1C and 1D). The endoscopist rated the ease of entry into the coelom using a previously published scale from 1 to 5 (1 = impossible, taking more than 15 min; 2 = difficult, taking between 11 and 15 min; 3 = satisfactory, taking between 6 and 10 min; 4 = good, taking between 2 and 5 min; 5 = excellent, taking less than 2 min; Hernandez-Divers and Hernandez-Divers 2004; Hernandez-Divers et al. 2007). In addition, the eases of location and visualisation of the heart, lung, oesophagus, proventriculus, ventriculus, small intestine, large intestine, spleen, liver, pancreas, adrenal, reproductive tract, and kidney were also scored using a previously published scale of 1 to 5 (1 = impossible; 2 = difficult, requiring an extensive search and significant movement of viscera; 3 = satisfactory, requiring some searching and minor manipulation of viscera; 4 = good, easy to locate but requiring minor manipulation to see clearly; 5 = excellent, obvious and clear visualisation with minimal to no manipulation required; Hernandez-Divers and Hernandez-Divers 2004; Hernandez-Divers et al. 2007).

Coelioscopic biopsy procedures. Coelioscopic biopsy was performed following the diagnostic coelioscopy. The telescope was inserted through the first port into the caudal thoracic air sac. A small incision was made through the cranio-ventral coelomic membrane using 5-mm endoscopic scissors (Metzenbaum scissors®, Richard Wolf GmbH, Germany) to approach the liver for biopsy. By using 5-mm (15 French) biopsy forceps (Biopsy forceps®, Richard Wolf GmbH, Germany) inserted through the second port, a liver biopsy specimen was collected from the caudal edge of the left liver lobe. To approach the kidney for biopsy, the telescope was advanced to the abdominal air sac. After the cranial division of the left kidney was located, a kidney biopsy specimen was collected by use of 15 French biopsy forceps. After coelioscopic biopsy, the telescope and instrument and portal cannulas were removed. Open surgical biopsy performed on two cinereous vultures led to subsequent euthanasia because of permanent injuries and disability. The incision site used as a first portal was extended dorso-ventrally to expose the liver and kidney for biopsy. Then, open surgical wedge biopsy was performed. All tissue samples were transferred to a biopsy cassette using a sterile cotton-tipped applicator moistened with sterile saline (0.9% NaCl). Then, the cassette was immediately placed in 10% neutral buffered formalin. The incision length of the abdominal wall was measured using a digital

doi: 10.17221/103/2016-VETMED

vernier calliper and closure of the skin was achieved by means of a single absorbable monofilament polydioxanone suture (2-0 PSD II®, Ethicon, USA). All vultures were monitored closely on recovery and 0.5 mg/kg/day of meloxicam was administered orally for five days as a postoperative analgesic.

Histopathology. Liver and kidney biopsy samples were routinely processed through graded alcohols to xylene, embedded in paraffin, sectioned at 4 µm, stained with haematoxylin and eosin, covered with a coverslip, and examined microscopically. For each biopsy specimen, the degree of crush artifact resulting in an inability to recognise cell types or evaluate tissue parenchyma was graded, using a previously demonstrated scoring system as follows: minimal = less than or equal to 10% affected; mild = 11–20% affected; moderate = 21–50% affected; and severe = greater than or equal to 51% affected (Hernandez-Divers et al. 2007). Through a comprehensive assessment of tissue architecture and cellular preservation, the diagnostic quality of the biopsy samples was scored as poor, good, or excellent.

Statistical analysis. All the statistical tests were performed using the IBM SPSS Statistics 21® statistical software (IBM Corp., USA). The endoscopy scores were expressed as mean and standard deviation. A Mann-Whitney *U*-test was used to compare the heart rate, respiratory rate, $PetCO_2$, ETiso, oxyhaemoglobin saturation and body temperature before and after entry into the coelomic cavity. A level of $P < 0.05$ was considered statistically significant.

RESULTS

Diagnostic coelioscopy

Body weights of the cinereous vultures ranged from 7.9 to 10.2 kg (mean ± SD of body weight, 8.71 ± 0.96 kg) before surgery. Endoscopy scores are summarised in Table 1. Entry into the caudal thoracic air sac, cranial thoracic air sac and abdominal air sac was easy and uncomplicated in all vultures. The endoscopic location and visualisation scores ranged from satisfactory to excellent (> 3) for all coelomic structures except the oesophagus, spleen, epididymis/oviduct, and pancreas in all vultures (Figures 2–5). The oesophagus was impossible to locate from within the cranial thoracic air sac. The spleen was impossible to locate from within the abdominal air sac. An extensive search and significant movement of the intestines were required to locate and visualise the pancreas and epididymis/oviduct.

Table 1. Endoscopy scores associated with coelioscopy in seven cinereous vultures (*Aegypius monachus*), data are presented as mean ± SD

Items	Endoscopy score[a]
Ease of entry into coelom	4.6 ± 0.5
Visualisation scores	
Lung	5.0 ± 0.0
Ventriculus	5.0 ± 0.0
Liver	5.0 ± 0.0
Heart	5.0 ± 0.0
Proventriculus	5.0 ± 0.0
Oesophagus	1.0 ± 0.0
Kidney	4.3 ± 0.5
Adrenal	3.1 ± 0.4
Testis/ovary	3.3 ± 0.5
Epididymis/oviduct	1.8 ± 0.7
Spleen	1.0 ± 0.0
Pancreas	1.7 ± 0.5
Small intestine	5.0 ± 0.0
Large intestine	4.6 ± 0.5
Cloaca	4.1 ± 0.7

[a]The ease of entry into the coelom: 1 = impossible, taking more than 15 min; 2 = difficult, taking between 11–15 min; 3 = satisfactory, taking between 6–10 min; 4 = good, taking between 2–5 min; 5 = excellent, taking less than 2 min. The ease of location and visualisation: 1 = impossible; 2 = difficult, requiring an extensive search and significant movement of viscera; 3 = satisfactory, requiring some searching and minor manipulation of viscera; 4 = good, easy to locate but requiring minor manipulation to see clearly; 5 = excellent, obvious and clear visualisation with minimal to no manipulation required

Coelioscopic biopsy

The coelioscopic biopsies of the liver and kidney were completed successfully without any complications in four vultures (Figures 6 and 7). The open surgical wedge biopsy was also completed in two of the four vultures.

Histopathology

Representative samples harvested in open surgical and coelioscopic biopsies for liver and kidney were

doi: 10.17221/103/2016-VETMED

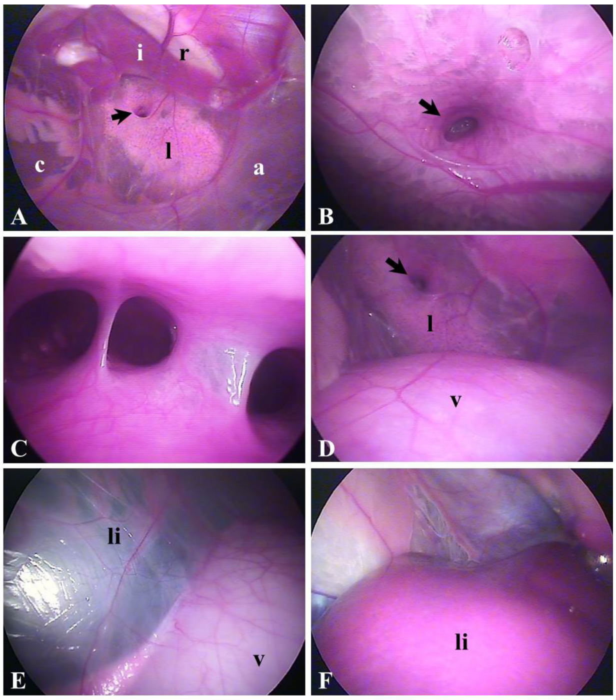

Figure 2. Coelioscopic views within the caudal thoracic air sac using a 5-mm telescope in cinereous vultures (*Aegypius monachus*). (**A**) The primary ostium (arrow) and the ventrolateral aspect of the lung (l) are located directly ahead with the ribs (r) and intercostal muscles (i) above; the cranial air sac (c) and abdominal air sac (a) located on the left and right, respectively; (**B**) the primary ostium (arrow) is visible; (**C**) the normal appearance of parabronchi and lung tissue are demonstrated through the view within the primary ostium of the caudal thoracic air sac; (**D**) the ventriculus (v) located below the primary ostium (arrow) and lung (l) is demonstrated; (**E**) the normal appearance of the liver (li) and ventriculus (v); (**F**) the normal appearance of the liver (li)

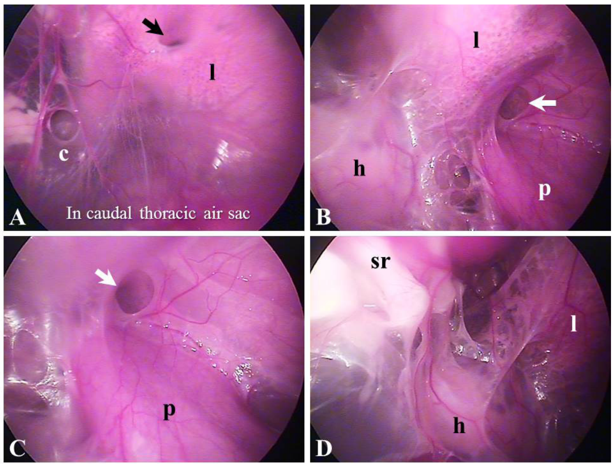

Figure 3. Coelioscopic views within the cranial thoracic air sac using a 5-mm telescope in cinereous vultures (*Aegypius monachus*). (**A**) Entry into the cranial thoracic air sac (c) is achieved by a gentle sweeping motion of the telescope through the interface between the caudal thoracic and cranial thoracic air sacs; the lung (l) and the primary ostium connected to the caudal thoracic air sac; (**B**) the normal appearance of the heart (h), lung (l), proventriculus (p) and primary ostium (arrow) connected to the cranial thoracic air sac; (**C**) the normal appearance of the primary ostium (arrow) and proventriculus (p); (**D**) the left lateral aspect of the heart (h) ,lung (l) and sternal ribs (sr) are visible

compared (Figures 8 and 9). All liver biopsy samples had minimal to mild crush artifact. There were a number of hemosiderin granules in all liver biopsy samples. There were no difficulties in evaluating the coelioscopic liver biopsy samples, although boundaries of the cytoplasm were observed to be somewhat unclear. All kidney biopsy samples had moderate to severe crush artifact. There were no difficulties in evaluating structures of renal tubules and glomeruli in the coelioscopic kidney biopsy samples, although boundaries of the cytoplasm were somewhat unclear and nuclear morphologies varied to a certain degree. In all samples, crushing was confined to the periphery of the section, and central areas of intact parenchyma could be histologically evaluated. All liver and kidney biopsy samples were determined to be of good to excellent cellular preservation and tissue orientation. While conducting diagnostic coelioscopy, a small lesion was noted in the liver of one of the tested vultures, and a biopsy was performed. Histopathology confirmed the lesion to be a fatty liver. It was difficult to distinguish the normal liver tissues because numerous portions of the cytoplasm were replaced by cytoplasmic lipid droplets (Figure 10).

Incision length in the abdominal wall

The mean ± SD incision length in the abdominal wall was 1.36 ± 0.11 cm in the coelioscopic biopsy group, and 8.05 ± 0.35 cm in the open surgical wedge biopsy group.

Anaesthesia and monitoring

There were no differences between before and after entry into the coelomic cavity with regard to

doi: 10.17221/103/2016-VETMED

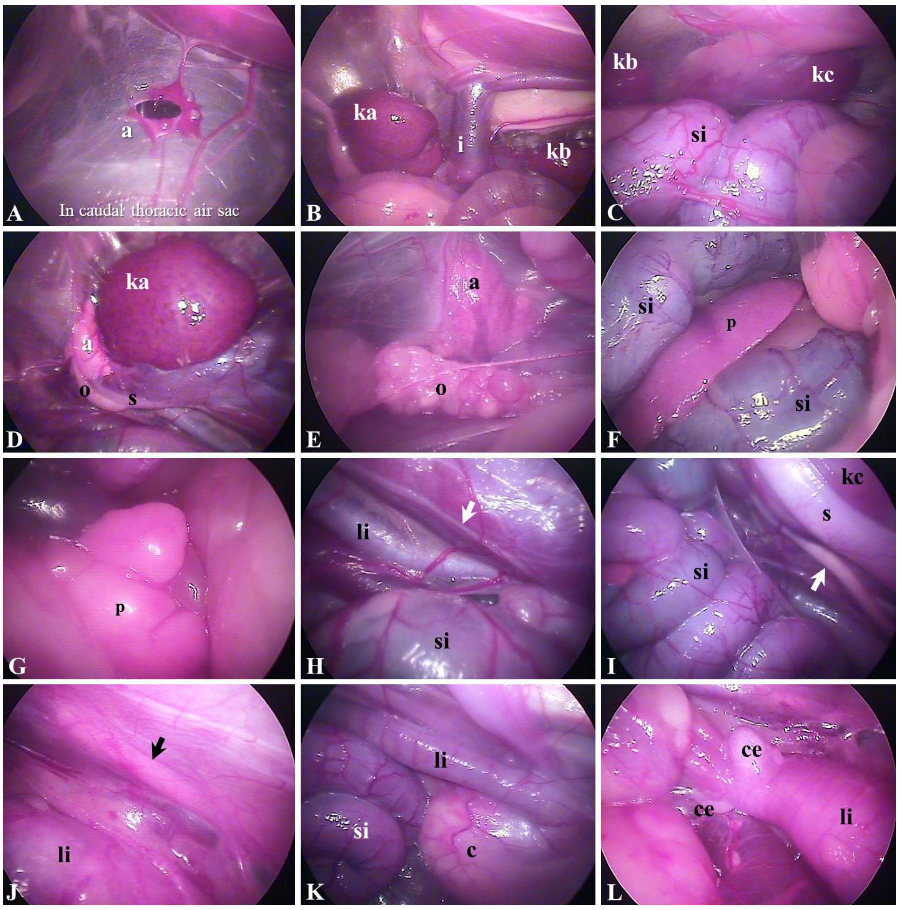

Figure 4. Coelioscopic views within the abdominal air sac using a 5-mm telescope in cinereous vultures (*Aegypius monachus*). (**A**) Entry into the abdominal air sac (a) is achieved by a gentle sweeping motion of the telescope through the interface between the caudal thoracic and abdominal air sacs; (**B**) external iliac vein (i) running between the cranial (ka) and middle (kb) divisions of the left kidney; (**C**) the middle (kb) and caudal (kc) divisions of the left kidney, and the small intestine (si) are visible; (**D**) the adrenal gland (a) closed to cranial division (ka) of the left kidney lies dorsally with the ovary (o) and suspensory ligament (s) below; (**E**) the ovary (o) at maturity close to the adrenal gland (a); (**F**) the pancreas (p) close to the small intestine (si) is visible; (**G**) the normal appearance of the pancreas (p); (**H**) the large (li) and small (si) intestines are visible with the immature oviduct (arrow) above; (**I**) the suspensory ligament (s) closely running along the caudal division of the left kidney (kc), with the ureter (arrow) ventromedially. The small intestine (si) is also visible; (**J**) the normal appearance of the large intestine (li) and ureter (arrow); (**K**) the large (li) and small (si) intestines, and the cloaca (c) are visible; (**L**) large intestine (li) and two small ceca (ce) are visible

heart rate, respiratory rate, $PetCO_2$, and oxyhaemoglobin saturation (Table 2). The mean ETiso and body temperature were significantly changed after entry (Table 2).

Recovery and complications

After the procedure, all of the vultures recovered from the anaesthesia uneventfully, and no arrhythmia or respiratory instabilities were observed during the study. All of the vultures tested in this experiment were treated and put through the rehabilitation program. Although mild haemorrhage occurred from all biopsy sites, excessive uncontrolled haemorrhage was not observed, and haemostasis was accomplished naturally within 1 min.

DISCUSSION

Although there have been some reports of avian endoscopy, the information limited to only a few avian species, especially in wild birds. Thus, this

doi: 10.17221/103/2016-VETMED

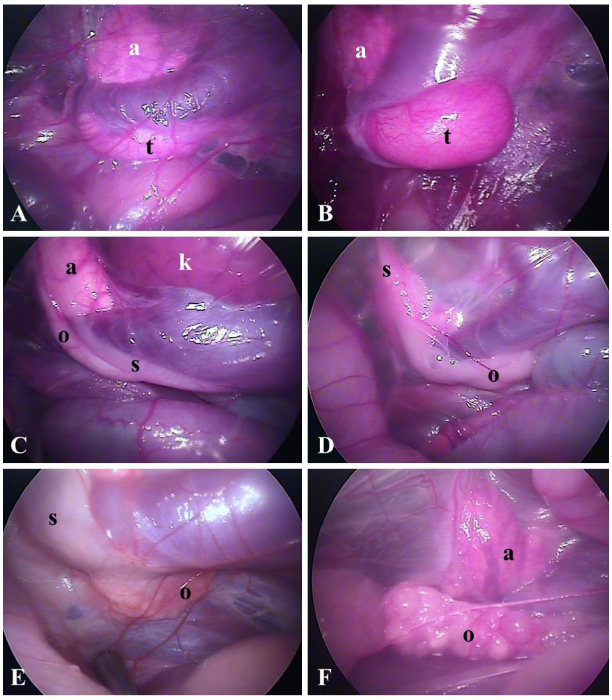

Figure 5. Coelioscopic gonadal examination from within the left abdominal air sac in cinereous vultures (*Aegypius monachus*). (**A**) The immature testis (t) and adrenal gland (a); (**B**) the testis (t) at maturity and adrenal gland (a); (**C**) the immature ovary (o), suspensory ligament (s), adrenal gland (a), and cranial division of the kidney (k); (**D**) the immature ovary (o) and suspensory ligament (s); (**E**) the ovary (o) at maturity and suspensory ligament (s); (**F**) the ovary (o) at maturity and adrenal gland (a)

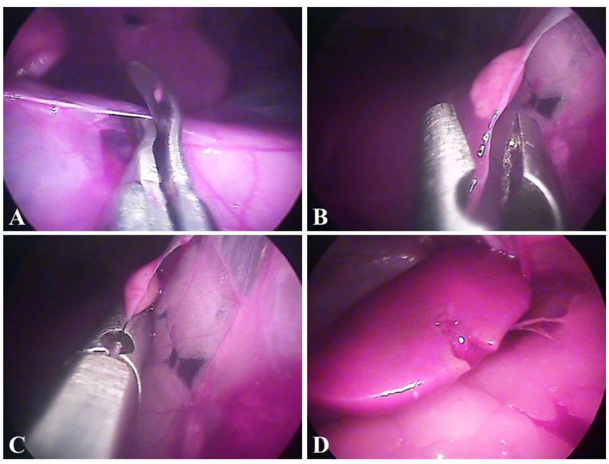

Figure 6. Coelioscopic biopsy technique in the liver of cinereous vultures (*Aegypius monachus*). (**A**) 5-mm (15 French) scissors inserted down the instrument port used to incise the coelomic membrane; (**B**) 5-mm (15 French) biopsy forceps advanced to collect a liver sample; (**C**) the biopsy sample from the caudal edge of the left liver lobe with fat infiltration is harvested; (**D**) view of the liver immediately after biopsy with the minimal haemorrhage

Table 2. Data of heart rate (HR), respiratory rate (RR), end tidal CO_2 partial pressure ($PetCO_2$), oxyhaemoglobin saturation (SpO_2), end-tidal isoflurane concentration (ETiso), and body temperature (BT) in seven cinereous vultures (*Aegypius monachus*) anaesthetised with isoflurane that were breathing spontaneously before and after entry into the coelomic cavity

Variables	Before entry	After entry
HR (beats/min)	108 ± 26	116 ± 22
RR (breaths/min)	9 ± 2	10 ± 3
$PetCO_2$ (mmHg)	44 ± 4	44 ± 3
SpO_2 (%)	99 ± 2	99 ± 1
ETiso (%)	1.5 ± 0.0	1.5 ± 0.1[a]
BT (°C)	39.6 ± 0.2	39.4 ± 0.3[a]

[a]$P < 0.05$, statistical difference between value before and after entry

study investigated the effectiveness of diagnostic coelioscopy and coelioscopic biopsy in the cinereous vulture as a model of a large raptor.

The coelioscopic examination of coelomic viscera and biopsies of the liver and kidney were safe, simple to accomplish with appropriate equipment, and harvested tissue samples that were suitable for histological and histopathological tests in our study. Some previous studies have reported that coelioscopy seems to be a safe and useful diagnostic

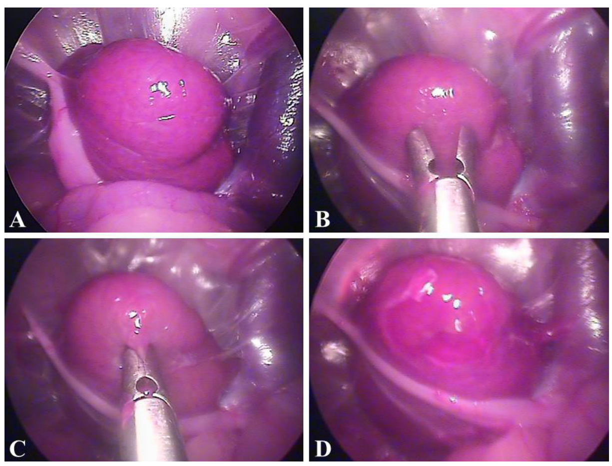

Figure 7. Coelioscopic biopsy technique in the kidney of cinereous vultures (*Aegypius monachus*). (**A**) View of the cranial division of the left kidney; (**B**) 5-mm (15 French) biopsy forceps advanced to the renal tissue; (**C**) the biopsy sample of the cranial division of the left kidney is harvested; (**D**) view of the liver immediately after biopsy with minimal haemorrhage

doi: 10.17221/103/2016-VETMED

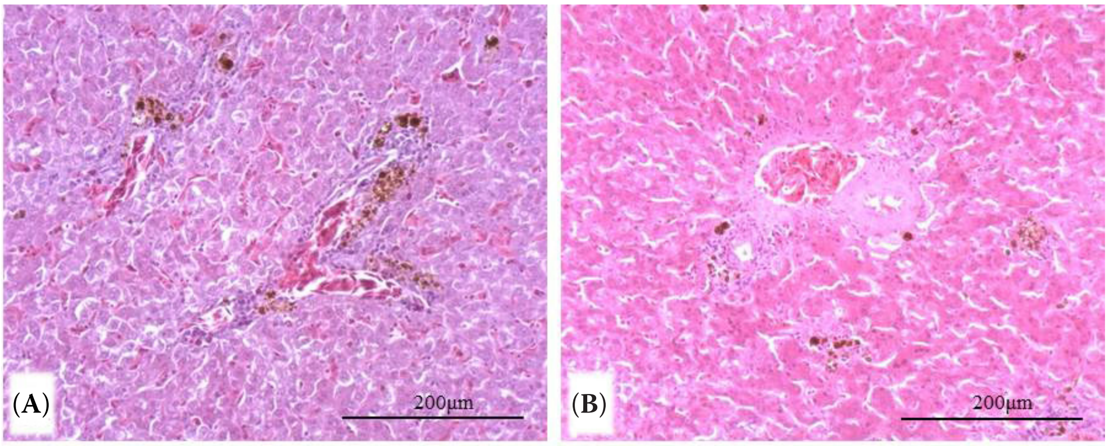

Figure 8. Histological sections of liver samples collected by open surgical (**A**) and coelioscopic biopsy (**B**). Both methods of tissue collection typically produced sections with good to excellent preservation of hepatocellular architecture. A number of hemosiderin granules were identified in all liver biopsy samples, haematoxylin and eosin stain, × 200, scale bar = 200 µm

tool for the examination and visualisation of coelomic viscera in some avian species (Taylor 1994; Hernandez-Divers and Hernandez-Divers 2004; Divers 2010).

Endoscopic equipment commonly used for a basic avian endoscopy system and biopsy procedures include a 2.7 mm × 18 cm (30° forward oblique) rigid telescope; a 4.8-mm (14.5 French) operating sheath; and 1.7-mm (5 French) instruments such as grasping forceps, biopsy forceps, aspiration/injection needles and single action scissors (Taylor 1994; Hernandez-Divers and Hernandez-Divers 2004; Hernandez-Divers et al. 2006; Divers 2010). Typically, a 2.7-mm rigid telescope housed within an operating sheath into which basic instruments can be inserted, has been used in procedures. However, a 5 mm × 30 cm (30° forward-oblique) rigid telescope and 5-mm (15 French) instruments, specifically a traumatic grasping forceps, Metzenbaum scissors and biopsy forceps were used in this study. Large raptors have a wide coelom and are large in length and depth of body, so a 2.7-mm endoscopy system may be limiting for endoscopic procedures in cinereous vultures because of a restricted operating radius. On the other hand, it is possible to explore the coelom deeply using the 5-mm endoscopy system with a wide operating range. In addition, no significant difference is

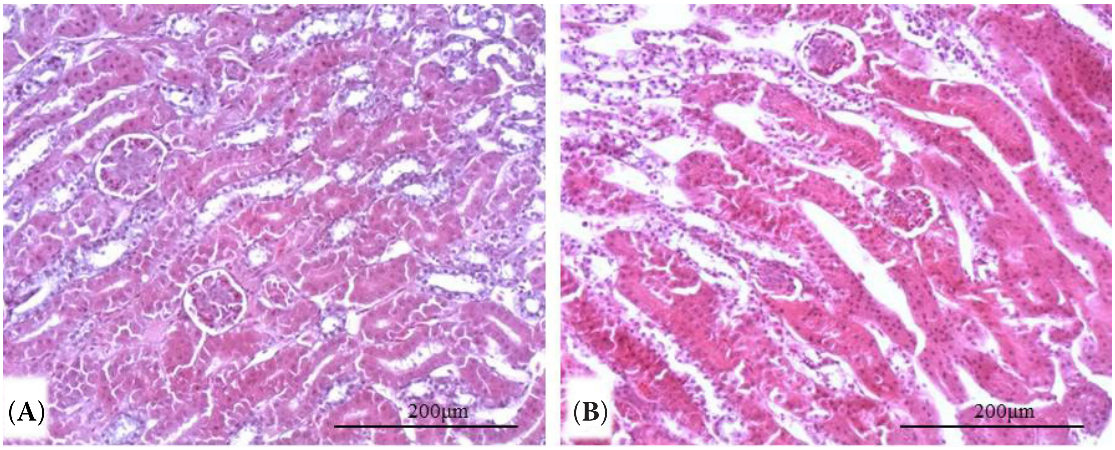

Figure 9. Histological sections of kidney samples collected by open surgical (**A**) and coelioscopic biopsy (**B**). In the open surgical biopsy specimen, glomerular and tubular morphologies were excellently preserved. In the coelioscopic biopsy specimen, there were no been difficulties in evaluating structures of renal tubules and glomeruli, although boundaries of the cytoplasm were somewhat unclear and nuclear morphology varied to a certain degree, haematoxylin and eosin stain, × 200, scale bar = 200 µm

doi: 10.17221/103/2016-VETMED

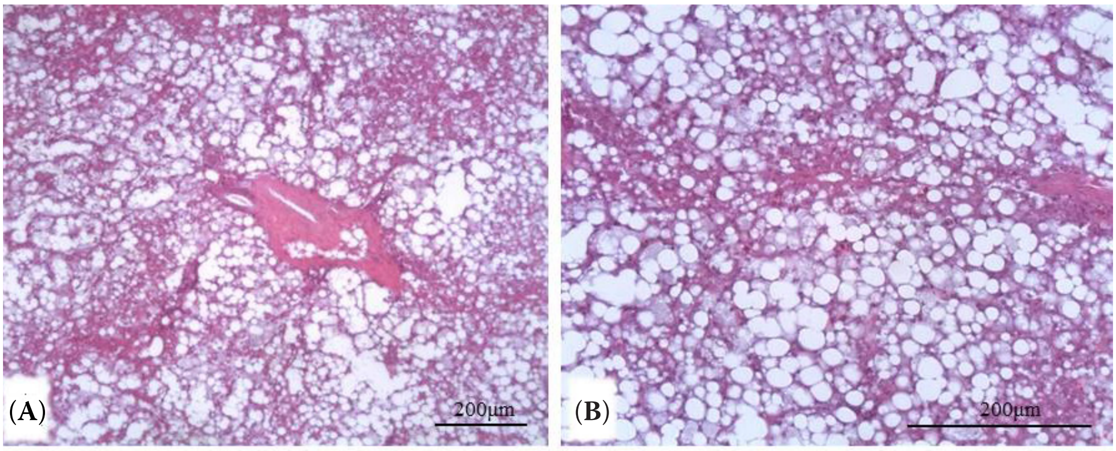

Figure 10. Histological sections of the liver lesion collected by coelioscopic biopsy in a cinereous vulture (*Aegypius monachus*). The normal hepatocellular architecture is not well defined. Numerous portions of the cytoplasm are replaced by cytoplasmic lipid droplets, haematoxylin and eosin stain, × 100 (**A**), × 200 (**B**), scale bar = 200 μm

expected in incision length in the abdominal wall because the 2.7-mm telescope housed within the 4.8 mm operating sheath is similar to the 5-mm telescope in diameter.

When using the endoscopy system to examine the coelomic viscera, the effects of creating a direct connection between the air sacs and the external environment could complicate anaesthesia. The leakage of anaesthetic gas and respiration through the telescope entry site can cause anaesthetic instability. According to previous studies (Touzot-Jourde et al. 2005; Hernandez-Divers et al. 2006), the use of mechanical ventilation for coelioscopy offered some advantages over the use of spontaneous ventilation and is not associated with clinically important adverse cardiopulmonary changes. However, in our study, spontaneous ventilation was used during anaesthesia. The leakage at the level of the air sac perforation and anaesthetic instability could be prevented by use of portal cannulas with rubber stoppers during coelioscopy. In addition, cannulas offer the advantage of allowing the operator to introduce and withdraw the telescope and endoscopic instruments repeatedly for cleaning. Although statistically significant differences in ETiso were observed in our study when comparing values before and after entry into the coelom, these were momentary changes due to changes in ventilation when the coelom was opened, and therefore clinically unremarkable. The body temperature was maintained within normal ranges, although the temperature slightly decreased over the time of anaesthesia.

There are four basic approaches to the coelom: left, right, ventral and inter-clavicular. In our study, we used the left approach, which is the most commonly employed procedure, because it permits visualisation of the overall internal organs (Divers 2010). In addition, in the case of male birds, the testes and other reproductive structures are bilaterally paired, whereas in the female birds, only the left-side structures usually develop and become functional, and only a few species have bilateral ovaries (Proctor and Lynch 1993).

It has been well documented in human medicine that minimally invasive laparoscopy offers several advantages over traditional open techniques, such as rapid and accurate disease diagnosis, decreased need for extensive laparotomy, decreased surgical stress and pain, enhanced postoperative pulmonary function, decreased surgical time, rapid recovery and shorter hospital stays (Golditch 1971; Hasson et al. 1993; Vandervelpen et al. 1994; Yu et al. 1997; Kehlet 1999). Endoscopic surgery is a minimally invasive technique and has been accepted as a viable alternative to traditional open procedures in veterinary medicine. Several studies (Davidson et al. 2004; Devitt et al. 2005; Culp et al. 2009) investigated the advantages of endoscopic surgery over the traditional open techniques in dogs. These studies (Davidson et al. 2004; Devitt et al. 2005; Culp et al. 2009) indicated that reduced postoperative pain and stress as well as rapid postsurgical activity could be observed following endoscopic procedures compared to traditional open techniques. In wildlife medicine, particularly in raptors, evaluating the intensity of postoperative pain and surgical stress responses is difficult because of the tendency of wild animals to hide clinical signs of

doi: 10.17221/103/2016-VETMED

disease until their condition is severe. Wild birds can undergo a lot of stress when being restrained and handled for postoperative treatment, which often leads to debilitating health problems or even death.

In dogs weighing more than 10 kg, safe collection of percutaneous liver samples using biopsy needles can be achieved. On the other hand, in the case of smaller animals, it is preferable to use smaller gauge hypodermic needles for fine-needle aspiration (Kerwin 1995; Cholongitas et al. 2006). In most cases, such samples are obtained under ultrasound guidance. In avian medicine, biopsies can be commonly made from the kidneys, gonads, liver, spleen, pancreas, lungs, fat, air sac, coelomic musculature and, in general, any abnormal soft tissue structure. It is important to examine as much of the target structure as possible to determine whether pathology is focal, multifocal or diffuse (Hernandez-Divers and Hernandez-Divers 2004; Divers 2010). In cases of diffuse renal or hepatic disease such as tubulonephrosis, nephrocalcinosis, hepatic lipidosis and hepatitis, two or three biopsies performed from the most convenient sites are generally of diagnostic value. At this moment, ultrasound-guided and blind percutaneous biopsy techniques may be useful in diagnosing diffuse disease; however, they are seldom recommended because of the increased risk of iatrogenic trauma associated with poorer visualisation of closely surrounded structure (Divers 2010). In our study, we verified that the three-lobed kidneys are lying at the dorsal wall of the abdominal cavity, tucked into a concave spaced formed by the ilium and synsacrum of the pelvis, and several major vessels and nerves lie across the around the surface of this organ. Endoscopic kidney biopsy techniques have proven to be superior over ultrasound-guided techniques in dogs, and their safety and effectiveness have been shown in birds of prey (Grauer et al. 1983; Rawlings et al. 2003; Muller et al. 2004).

Aspiration cytology was found to be of diagnostic value in only 30–61% of cases compared with histology in studies comparing liver biopsy and fine-needle aspiration in dogs and cats (Roth 2001; Wang et al. 2004). In comparison to surgical wedge samples, large-gauge needle-biopsies were of diagnostic value in only 48% of cases and they should be interpreted with a certain degree of caution (Cole et al. 2002). Since the rapid development of minimally invasive endoscopic techniques, the disadvantages of surgical intervention have been slowly yet largely overcome, although the improved diagnostic value of surgical biopsy over needle biopsy and fine-needle aspiration has been well documented in multiple studies (Richter 2001; Monnet and Twedt 2003; Twedt and Monnet 2005). Although traditional open surgery biopsy promises a higher rate of diagnosis, it has the disadvantages of being more invasive and requiring a longer period of recovery. Especially with a large raptor like cinereous vultures, due to a larger abdominal muscle mass with deeper coelom, open surgery biopsy can cause a moderate amount of haemorrhage along with tissue damage. If haemorrhage is not controlled in a timely fashion, blood can be exposed to the air sac, and the haemorrhage can be fatal.

Although retrospective human studies (Orlando et al. 1990; Falcone et al. 1993; Esposito et al. 1997) have shown that evaluation of liver tissue collected with endoscopy has led to a diagnosis rate for liver disease of nearly 98%, this finding may not be applicable in avian species due to lack of data. In this study, a lesion in the liver was detected in one of the testing vultures and coelioscopic biopsy was performed to arrive at a diagnosis. However, further research comparing endoscopic collections of tissue samples obtained during surgery or necropsy is needed before the diagnostic capability of coelioscopic techniques in liver disease can be definitively determined in cinereous vultures or other birds.

In conclusion, minimally invasive coelioscopy using the 5-mm telescope system is a safe and effective procedure for visceral examination of large raptors such as cinereous vultures, and endoscopic biopsy is recommended for the collection of tissue samples that are suitable for histological and histopathological interpretation.

REFERENCES

Birdlife International (2008): Threatened Birds of the World. Birdlife international CD-ROM. Cambridge, UK.

Boone SS, Hernandez-Divers SJ, Radlinsky MG, Latimer KS, Shelton JL (2008): Comparison between coelioscopy and coeliotomy for liver biopsy in channel catfish. Journal of the American Veterinary Medical Association 233, 960–967.

Cholongitas E, Senzolo M, Standish R, Marelli L, Quaglia A, Patch D, Dhillon AP, Burroughs AK (2006): A systematic review of the quality of liver biopsy specimens. American Journal of Clinical Pathology 125, 710–721.

doi: 10.17221/103/2016-VETMED

Clayton LA, Ritzman TK (2005): Endoscopic-assisted removal of a tracheal seed foreign body in a cockatiel (Nymphicus hollandicus). Journal of Avian Medicine and Surgery 19, 14–18.

Cole TL, Center SA, Flood SN, Rowland PH, Valentine BA, Warner KL, Erb HN (2002): Diagnostic comparison of needle and wedge biopsy specimens of the liver in dogs and cats. Journal of the American Veterinary Medical Association 220, 1483–1490.

Crosta L, Gerlach H, Marcellus B, Timossi L (2002): Endoscopic testicular biopsy technique in psittaciformes. Journal of Avian Medicine and Surgery 16, 106–110.

Culp WTN, Mayhew PD, Brown DC (2009): The effect of laparoscopic versus open ovariectomy on postsurgical activity in small dogs. Veterinary Surgery 38, 811–817.

Davidson EB, Moll HD, Payton ME (2004): Comparison of laparoscopic ovariohysterectomy and ovariohysterectomy in dogs. Veterinary Surgery 33, 62–69.

Devitt CM, Cox RE, Hailey JJ (2005): Duration, complications, stress, and pain of open ovariohysterectomy versus a simple method of laparoscopic-assisted ovariohysterectomy in dogs. Journal of the American Veterinary Medical Association 227, 921–927.

Divers SJ (2010): Avian diagnostic endoscopy. Veterinary Clinics of North America – Exotic Animal Practice 13, 187–202.

Esposito C, Garipoli V, Vecchione R, Raia V, Vajro P (1997): Laparoscopy-guided biopsy in diagnosis of liver disorders in children. Liver 17, 288–292.

Falcone RE, Wanamaker SR, Barnes F, Baxter CG, Santanello SA (1993): Laparoscopic vs. Open wedge biopsy of the liver. Journal of Laparoendoscopic Surgery 3, 325–329.

Golditch IM (1971): Laparoscopy: Advances and advantages. Fertility and Sterility 22, 306–310.

Grauer GF, Twedt DC, Mero KN (1983): Evaluation of laparoscopy for obtaining renal biopsy specimens from dogs and cats. Journal of the American Veterinary Medical Association 183, 677–679.

Hasson HM, Rotman C, Rana N, Asakura H (1993): Experience with laparoscopic hysterectomy. American Association of Gynecologic Laparoscopists 1, 1–11.

Hernandez-Divers SJ, Hernandez-Divers SM (2004): Avian diagnostic endoscopy. Compendium on Continuing Education for the Practicing Veterinarian 26, 839–851.

Hernandez-Divers SJ, Stahl S, Hernandez-Divers SM (2004): Coelomic endoscopy of the green iguana (Iguana iguana). Journal of Herpetological Medicine and Surgery 14, 10–18.

Hernandez-Divers SJ, Wilson GH, Lester VK, Hernandez-Divers SM, Latimer KS, Ritchie BW (2006): Evaluation of coelioscopic splenic biopsy and cloacoscopic bursa of fabricius biopsy techniques in pigeons (Columba livia). Journal of Avian Medicine and Surgery 20, 234–241.

Hernandez-Divers SJ, Stahl SJ, McBride M, Stedman NL (2007): Evaluation of an endoscopic liver biopsy technique in green iguanas. Journal of the American Veterinary Medical Association 230, 1849–1853.

Jekl V, Knotek Z (2006): Endoscopic examination of snakes by access through an air sac. Veterinary Record 158, 407–410.

Jekl V, Tukac V, Hauptman K, Knotkova Z, Knotek Z (2006): Endoscopic removal of a bullet from the cranial thoracic air sac of a peregrine falcon (Falco peregrinus). Journal of Avian Medicine and Surgery 20, 242–246.

Kehlet H (1999): Surgical stress response: Does endoscopic surgery confer an advantage? World Journal of Surgery 23, 801–807.

Kerwin SC (1995): Hepatic aspiration and biopsy techniques. Veterinary Clinics of North America – Small Animal Practice 25, 275–291.

Monnet E, Twedt DC (2003): Laparoscopy. Veterinary Clinics of North America – Small Animal Practice 33, 1147–1163.

Muller K, Gobel T, Muller S, Hermanns W, Brunnberg L (2004): Use of endoscopy and renal biopsy for the diagnosis of kidney disease in free-living birds of prey and owls. Veterinary Record 155, 326–329.

Orlando R, Lirussi F, Okolicsanyi L (1990): Laparoscopy and liver biopsy: Further evidence that the two procedures improve the diagnosis of liver cirrhosis. A retrospective study of 1,003 consecutive examinations. Journal of Clinical Gastroenterology 12, 47–52.

Proctor NS, Lynch PJ (eds) (1993): The urogenital and endocrine systems. In: Manual of Ornithology; Avian Structure and Function. 1st edn. Yale University Press, New Haven. 220–240.

Rawlings CA, Diamond H, Howerth EW, Neuwirth L, Canalis C (2003): Diagnostic quality of percutaneous kidney biopsy specimens obtained with laparoscopy versus ultrasound guidance in dogs. Journal of the American Veterinary Medical Association 223, 317–321.

Richter KP (2001): Laparoscopy in dogs and cats. Veterinary Clinics of North America – Small Animal Practice 31, 707–727.

Roth L (2001): Comparison of liver cytology and biopsy diagnoses in dogs and cats: 56 cases. Veterinary Clinical Pathology 30, 35–38.

Stetter MD (2002): Use of rigid laparoscopy in fish. In: Baer CK (ed.): Proceedings of the American Association of Zoo Veterinarians. 339–342.

Tams TR, Rawlings CA (eds) (2011): Small Animal Endoscopy. 3rd edn. Elsevier Mosby, St Louis. xiv + 659

doi: 10.17221/103/2016-VETMED

Taylor M (1994): Endoscopic examination and biopsy techniques. In: Ritchie BW, Harrison GJ, Harisson LR (eds): Avian Medicine: Principles and Application. Wingers Publishing, Lake Worth. 327–354.

Touzot-Jourde G, Hernandez-Divers SJ, Trim CM (2005): Cardiopulmonary effects of controlled versus spontaneous ventilation in pigeons anesthesized for coeliscopy. Journal of the American Veterinary Medical Association 227, 1424–1428.

Twedt DC, Monnet E (2005): Laparoscopy: Technique and clinical experience. In: McCarthy TC (ed.): Veterinary Endoscopy for the Small Animal Practitioner. 1st edn. Saunders Elsevier, St Louis. 357–385.

Vandervelpen GC, Shimi SM, Cuschieri A (1994): Diagnostic yield and management benefit of laparoscopy: A prospective audit. Gut 35, 1617–1621.

Wang KY, Panciera DL, Al-Rukibat RK, Radi ZA (2004): Accuracy of ultrasound-guided fine-needle aspiration of the liver and cytologic findings in dogs and cats: 97 cases (1990–2000). Journal of the American Veterinary Medical Association 224, 75–78.

Yu SY, Chiu JH, Loong CC, Wu CW, Lui WY (1997): Diagnostic laparoscopy: Indication and benefit. Zhonghua yi xue za zhi (Taipei) 59, 158–163.

Received: 2016–06–23
Accepted after corrections: 2016–09–24

Corresponding Author:

Seong-Chan Yeon, Gyeongsang National University, College of Veterinary Medicine, Jinju 52828, Republic of Korea
E-mail: scyeon@gnu.ac.kr