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Cardiorespiratory dose-response relationship of isoflurane in Cinereous vulture (*Aegypius monachus*) during spontaneous ventilation

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ABSTRACT. Anesthesia is an inevitably important component of diagnosis and treatments examining the health condition of wild animals. Not only does anesthesia become an essential tool in minimizing stress of the patients and providing an opportunity to deliver accurate and safe procedures, but it also ensures the safety of the medical crew members. This study was conducted to investigate the dose-response cardiorespiratory effects of isoflurane during spontaneous ventilation in ten cinereous vultures. Each bird was administered isoflurane at initial concentration of 1.0, 1.5, 2.0, 2.5 and 3.0 and then an end-tidal isoflurane concentrations (ETiso) of 1.0% for an equilibration period of 15 min in the given order. At the end of the equilibration period, the direct blood pressure (BP), heart rate (HR), respiratory rate (RR) and end tidal CO_2 partial pressure ($P_{ET}CO_2$) were recorded, and blood gas analysis was performed. Increasing isoflurane concentrations during spontaneous ventilation led to dose-dependent increases in HR and $P_{ET}CO_2$, with minimal changes in RR, decreased arterial BP and respiratory acidosis. Overall, isoflurane for anesthesia of spontaneously breathing cinereous vultures is a suitable choice for diagnostic or surgical procedures.

KEY WORDS: *Aegypius monachus*, anesthesia, cardiorespiratory, Cinereous vulture, isoflurane

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The cinereous vulture (*Aegypius monachus*) is one of the rarest raptors in the world and has declined across its entire range in Europe and Asia [1]. This bird has been strictly protected since 1973 in the Republic of Korea as an endangered species and is a state-designated natural monument. Globally endangered cinereous vultures regularly migrate to the Republic of Korea for wintering, and they have frequently been rescued for various casualties, including exhaustion, starvation, orthopedic injuries and poisoning.

Chemical immobilization or general anesthesia allows veterinarians safely and rapidly to perform fluid administration, emergency procedures, blood collection and radiography, or accomplish invasive surgical procedures in avian patients [29]. Injectable anesthetics offer many advantages, including that they can be administered rapidly, require minimal equipment, are relatively inexpensive, and enable procedures involving the head and oral cavity without interruption from a face mask or endotracheal tube [7, 15]. However, the intramuscular or intraperitoneal injection of anesthetics is associated with disadvantages, such as induction time and prolonged metabolism [25]. In large raptors, such as cinereous vultures, their large body size and aggressive nature often necessitate anesthetic restraint to allow physical examination and diagnostic and surgical procedures. However, the birds can be very sensitive to the stress elicited by the restraint and handling [31]. Thus, inhalation anesthesia with oxygen has been used in the wildlife center, and isoflurane is the inhalant anesthetic agent, most commonly used in avian medicine because of its rapid induction, rapid recovery and easy control of anesthetic depth, as well as its moderate myocardial depressant effect [4, 18].

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The avian respiratory system is anatomically and physiologically different from the mammalian respiratory system in that it consists of para-bronchial lungs and air sacs that mechanically function as bellows for the lungs. These differences have fueled speculation that birds and mammals respond differently to inhalant anesthetics. The respiratory depression caused by inhalant anesthetics is one of the important disadvantages of spontaneously breathing avian patients. Inspiration and expiration occur by movement of the various respiratory muscles in avian species, and the muscle relaxation caused by inhalant anesthetics often leads to the respiratory depression via weakening of the respiratory muscles [13]. Hypercapnia caused by hypoventilation can have a variety of adverse effects on cardiopulmonary function through direct and indirect mechanisms [10]. Apnea and hypercapnia have been reported in ducks anesthetized with isoflurane during spontaneous ventilation [11]. Respiratory acidosis has been demonstrated in cranes [12], ducks [11] and pigeons [32] anesthetized with isoflurane during spontaneous ventilation. Moreover, in bald eagles [8], isoflurane resulted in significant progressive respiratory depression over time during spontaneous ventilation as indicated by increased carbon dioxide partial pressure ($PaCO_2$) values associated with a decrease in pH. In addition, a previous study [19] with sevoflurane anesthesia during spontaneous ventilation reported reduced pH and respiratory rate in chickens, while controlled ventilation prevented the increase in $PaCO_2$. A previous study conducted by Ludders *et al*. [11] demonstrated that the respiratory anesthetic index (AI; an index of anesthetic-induced apnea) of isoflurane for ducks is 1.65, while it is 2.51 in dogs and 2.33 in horses. These findings indicate that isoflurane serves as a more potent respiratory depressant in birds.

Information regarding differences in responses to inhalation anesthetics among species has been established in a variety of mammalian and non-mammalian species, but there is limited information available for birds, especially large raptors. Therefore, this study was conducted to investigate the dose-response cardiorespiratory effects of isoflurane during spontaneous ventilation in cinereous vultures.

MATERIALS AND METHODS

Animals

This study was approved by the Animal Care and Use Committee at Gyeongsang National University (approval number: GNU-140128-E0006). Ten clinically healthy cinereous vultures were used in this study. These birds were hospitalized in the wildlife center in the province of Gyeongsangnam-do, Republic of Korea, due to various orthopedic injuries and had received appropriate medical treatments to recover their health. All vultures in this study were suspected of being between 1 and 2 years of age. Body weights and lengths of the birds were ranged from 7.5 to 9.0 kg (Mean ± SD of body weight, 8.27 ± 0.40 kg) and from 98 to 104 cm (Mean ± SD of body length, 100.9 ± 2.0 cm), respectively. Although these cinereous vultures were recovered to normal health, they had not been released because of disability. The health status of ten cinereous vultures was assessed by physical examination, diagnostic imaging, CBC and serum biochemical analyses. In addition, no clinical diseases were verified other than their disability caused by the previous orthopedic injuries. After recovering to normal health conditions, the birds were acclimated to the rehabilitation program for at least 7 days prior to the study. The birds were then transferred to temporary individual housing units, and food was withheld for 12 hr prior to anesthesia, although they were allowed access to water *ad libitum*.

Anesthesia and monitoring

The experimental protocol was modified based on dose-related effects obtained from a previous study by Naganobu and Hagio [18]. Differences based on gender were not investigated, because cinereous vultures do not show sexual dimorphism. Anesthesia was induced with 5% isoflurane (Ifran®, Hana Pharm., Seoul, Korea) of an isoflurane vaporizer (Multiplus, Royal Medical Co., Ltd., Pyeongtaek, Korea) setting in 100% oxygen (3 *l*/min) delivered via a mask. A non-rebreathing anesthetic circuit (Modified Jackson Rees anesthesia circuit) was used in this study. When voluntary movement of the nictitating membrane was absent for 30 sec, the trachea was intubated with an uncuffed endotracheal tube. The endotracheal tubes with internal diameter of 8.0 mm (n=8) and 7.5 mm (n=2) were chosen to prevent leakage of the anesthetic agent according to the size of cinereous vultures. Each bird was restrained in dorsal recumbency to minimize the impairment of sternal motion. After endotracheal intubation, anesthesia was maintained by spontaneous ventilation with isoflurane in 100% oxygen (3 *l*/min). The end tidal CO_2 partial pressure ($P_{ET}CO_2$) and the inspired and end-tidal isoflurane concentrations (ETiso) and respiratory rate (RR) were monitored with a calibrated multigas monitor (AS3®, Datex-Ohmeda Division Instrumentarium Corp., Helsinki, Finland). Initial anesthesia was maintained at 1.0% ETiso. To measure the systolic arterial blood pressure (SAP), diastolic arterial blood pressure (DAP), and mean arterial blood pressure (MAP) and obtain arterial blood samples for the blood gases and acid-base analysis, a sterile 24-gauge catheter (Angiocath Plus, Becton Dickinson, Sandy, UT, U.S.A.) was inserted aseptically through a small cut-down incision into the superficial ulnar artery. The arterial catheter was connected to a blood pressure transducer (Transpac IV Monitoring Kit®, Abbott Critical Care Systems, North Chicago, IL, U.S.A.) and a pressure line filled with saline, which was connected to the monitor for heart rate (HR) and direct blood pressure (BP) monitoring. The values of the blood pressure were zeroed to the atmospheric pressure at the level of the chest. Oxyhemoglobin saturation was also monitored continuously with a pulse oximeter. A lead II electrocardiogram (ECG) was monitored using the monitor described above during the procedure. Body temperature (BT) was also recorded with an oral probe linked to the monitor. Throughout the procedure, the BT was maintained at 39–40°C with a circulating water blanket (Medi-Therm®, Gaymer Industries Inc., Ochard Park, NY, U.S.A.) and a forced warm air blanket (Warm Air Hyperthermia System®, Cincinnati Sub-Zero Products Inc., Cincinnati, OH, U.S.A.). Next, 0.9% normal saline (Greenflex, Daihan Pharm Co., Ltd., Ansan, Korea) was administered via an intravenous 24-gauge catheter (Angiocath Plus, Becton Dickinson) placed in the ulnar vein during the procedure at a rate of 10 m*l*/kg/hr. The environmental temperatures of the operating room were adjusted to between 20°C and

Table 1. Dose-response effects on temperature, cardiovascular and respiratory parameters following isoflurane anesthesia with spontaneous ventilation in cinereous vultures (Aegypius monachus)

Variables	1.0% ETiso (first) (n=10)	1.5% ETiso (n=10)	2.0% ETiso (n=10)	2.5% ETiso (n=10)	3.0% ETiso (n=7)	1.0% ETiso (second) (n=10)
HR (beats/min)	99 ± 23	124 ± 35	170 ± 54[b)]	198 ± 60[b,d)]	199 ± 49[b,e)]	100 ± 22
RR (breaths/min)	17 ± 12	13 ± 10	12 ±4	13 ± 5	14 ± 5	11 ± 5
SAP (mmHg)	157 ± 22	154 ± 21	148 ± 18	138 ± 15	127 ± 7[c)]	156 ± 15
MAP (mmHg)	130 ± 18	124 ± 21	117 ± 20	106 ± 15	97 ± 8[c)]	122 ± 13
DAP (mmHg)	109 ± 20	103 ± 24	96 ± 25	84 ± 19	71 ± 10[c)]	92 ± 15
$P_{ET}CO_2$ (mmHg)	41 ± 8	46 ± 8	54 ± 7[b)]	58 ± 9[b,e)]	62 ± 11[b,d)]	48 ± 5
BT (°C)	39.8 ± 0.6	39.7 ± 0.5	39.7 ± 0.5	39.7 ± 0.4	39.8 ± 0.4	39.7 ± 0.4

a) Values are presented as the mean ± SD. Abbreviations: HR, heart rate; RR, respiratory rate; SAP, systolic arterial blood pressure; MAP, mean arterial blood pressure; DAP, diastolic arterial blood pressure; $P_{ET}CO_2$, end tidal carbon dioxide partial pressure; BT, body temperature. b) $P<0.01$, Significant difference compared with the values at the first application of 1.0% ETiso. c) $P<0.05$, Significant difference compared with the values at the first application of 1.0% ETiso. d) $P<0.01$, Significant difference compared with the values of 1.5% ETiso. e) $P<0.05$, Significant difference compared with the values of 1.5% ETiso.

23°C, by the facilities and plants engineering department of the wildlife center.

After instrumentation, each bird was held initially at 1.0, 1.5, 2.0, 2.5 and 3.0% ETiso and then at 1.0% ETiso for an equilibration period of 15 min in the given order. At the end of the equilibration period, the direct BP, HR, RR and $P_{ET}CO_2$ were recorded, and 0.4 m*l* arterial blood was collected for pH, partial pressure of arterial oxygen (PaO_2), partial pressure of arterial carbon dioxide ($PaCO_2$), bicarbonate (HCO_3^-) and base excess (BE) analyses. Blood gas analysis (Vetstat®, Idexx Laboratories, Inc., Westbrook, ME, U.S.A.) was performed using a 1 m*l* heparin washed syringe immediately after the blood was sampled. When arrhythmia or respiratory instabilities occurred during the procedure, the anesthetic concentration was immediately decreased to 1.0% ETiso. After the procedure, the arterial catheter was removed, hemostasis was performed by manual compression, and the skin was sutured. The vaporizer was then turned off, and the birds were allowed to breathe 100% oxygen until extubation, which was performed when the presence of a swallowing or cough response was observed. Time to induction and extubation, and total anesthesia time were recorded for each anesthetic procedure. Time to induction was defined as the time from the beginning of isoflurane inhalation to intubation. Time to extubation was defined as the time from the cessation of isoflurane administration to extubation. Total anesthesia time was defined as the time from the intubation to the cessation of isoflurane administration.

Statistical analysis

All statistical analyses were performed using SPSS Statistics 21® (IBM Corp., Armonk, NY, U.S.A.). Repeated measures analysis of variance (ANOVA) and the Bonferroni correction technique for multiple comparisons were used to compare the HR, RR, direct BP, $P_{ET}CO_2$, BT and blood gas data at each designated anesthetic concentration. A $P<0.05$ was considered statistically significant.

RESULTS

Mean ± SD induction and extubation time were 297.2 ± 33.9 sec and 308.1 ± 37.8 sec, respectively. Total anesthesia time was 98.0 ± 9.5 min (Mean ± SD). Three of ten vultures developed adverse cardiorespiratory effects as the isoflurane concentration was increased from 2.5% to 3.0% ETiso. Specifically, sinus arrhythmia occurred in one vulture, and two vultures showed difficulty breathing. As soon as symptoms were observed, the anesthetic concentration was immediately decreased to 1.0% ETiso, at which time their arrhythmias and dyspnea disappeared. Following the procedure, all vultures recovered from the anesthesia uneventfully, and no more cardiorespiratory problems were observed during the recovery period. All of the vultures tested in this experiment were treated and put through the rehabilitation program.

Dose-response effect on temperature, cardiovascular and respiratory parameters

The mean HR, RR, arterial BP (SAP, MAP and DAP) and $P_{ET}CO_2$ of cinereous vultures at various concentrations of isoflurane are summarized in Table 1. The BT remained constant throughout the procedures. The mean HR, arterial BP and $P_{ET}CO_2$ changed significantly with changes in isoflurane concentration. When the vultures were subjected to 2.0, 2.5 and 3.0% ETiso, the HR and $P_{ET}CO_2$ increased significantly relative to the value obtained at the first application of 1.0% ETiso. In addition, there were significant increases in HR and $P_{ET}CO_2$ from 2.0 to 2.5% and from 2.5 to 3.0% ETiso. When the vultures were subjected to 3.0% of ETiso, the arterial BP decreased significantly compared to the value obtained at the first application of 1.0% ETiso. However, the mean RR did not change significantly with different isoflurane concentrations.

The mean arterial blood gas and acid-base changes of cinereous vultures at various isoflurane concentrations are summarized in Table 2. The mean arterial pH, PaO_2 and $PaCO_2$ changed significantly with changes in the isoflurane concentration. When the vultures were subjected to 2.0, 2.5 and 3.0% ETiso, the arterial pH decreased significantly compared to the value obtained at the first application of 1.0% ETiso. In addition, there were significant decreases in arterial pH from 2.0 to 2.5% and from 2.5 to 3.0% ETiso. When vultures were subjected to 3.0% ETiso, the arterial PaO_2 decreased significantly relative to the value obtained at the

Table 2. Dose-response effects on arterial blood gas and acid-base balance following isoflurane anesthesia with spontaneous ventilation in cinereous vultures (Aegypius monachus)

Variables	1.0% ETiso (first) (n=10)	1.5% ETiso (n=10)	2.0% ETiso (n=10)	2.5% ETiso (n=10)	3.0% ETiso (n=7)	1.0% ETiso (second) (n=10)
pH	7.50 ± 0.05	7.46 ± 0.05	7.41 ± 0.06[b)]	7.33 ± 0.06[b,d)]	7.24 ± 0.06[b,d,f)]	7.48 ± 0.05
PaO_2 (mmHg)	518 ± 31	522 ± 26	512 ± 20	502 ± 18	457 ± 88[c,e)]	527 ± 19
$PaCO_2$ (mmHg)	38 ± 7	41 ± 6	48 ± 9	60 ± 12[b,e)]	76 ± 15[b,d,f,g)]	39 ± 6
HCO_3^- (mmol/*l*)	26.8 ± 3.6	26.9 ± 2.7	27.8 ± 2.7	28.8 ± 2.9	29.8 ± 3.4	26.0 ± 2.7
BE (mmol/*l*)	4.3 ± 2.9	3.5 ± 2.2	2.7 ± 1.7	1.5 ± 1.9	0.1 ± 2.8[c)]	3.1 ± 2.3

a) Values are presented as the mean ± SD. Abbreviations: PaO_2, partial pressure of arterial oxygen; $PaCO_2$, partial pressure of arterial carbon dioxide; HCO_3^-, calculated bicarbonate concentration; BE, calculated base excess. b) $P<0.01$, Significant difference compared with the values at the first application of 1.0% ETiso. c) $P<0.05$, Significant difference compared with the values at the first application of 1.0% ETiso. d) $P<0.01$, Significant difference compared with the values of 1.5% ETiso. e) $P<0.05$, Significant difference compared with the values of 1.5% ETiso. f) $P<0.01$, Significant difference compared with the values of 2.0% ETiso. g) $P<0.05$, Significant difference compared with the values of 2.5% ETiso.

first application of 1.0% ETiso. When the vultures were subjected to 2.5 and 3.0% ETiso, the arterial $PaCO_2$ increased significantly relative to the value obtained at the first application of 1.0% ETiso. In addition, there were significant increases in arterial $PaCO_2$ as the ETiso increased from 2.5 to 3.0%. The mean BE values changed significantly at 3.0% ETiso relative to the value obtained at the first application of 1.0% ETiso. However, the mean HCO_3^- values did not change significantly with changes in isoflurane concentration.

DISCUSSION

In raptors, the anesthesia with isoflurane resulted in rapid induction and recovery, and the anesthetic depth was easily controlled [8]. Even allowing for reported adverse cardiac effects of isoflurane in raptors, such as tachycardia, hypertension and arrhythmias [8], this anesthetic agent was considered as a reasonable selection to anesthetize the birds, because of its evident cardiorespiratory stability. Thus, the results of the present study indicate that it is possible to safely use isoflurane by controlling the anesthetic concentration. ETiso concentrations of 1.5 to 2.0% were considered adequate for anesthetic maintenance with minimal adverse effects in cinereous vultures.

According to previous studies [17, 18, 23], although the minimum alveolar concentration (MAC) is not appropriate as an index of potency for inhalant anesthetics in birds, the minimum anesthetic concentration as a similar index has been used to determine the anesthetic dose. The minimum anesthetic concentration for isoflurane in ducks is 1.3% [13], while that for cockatoos is 1.44% [3], and for captive thick-billed parrots, it is 1.07% [16]. Factors that may influence the minimum anesthetic concentration include age, circadian rhythm, methodology used to determine the minimum anesthetic concentration, severe hypercapnia, severe hypoxemia, changes in body temperature, severe hypotension and acidemia or alkalemia [16, 24]. Accordingly, different species of birds show great variations in the minimum anesthetic concentration. In addition, this value may differ within the same species of birds. Therefore, in the present study, the clinical ETiso concentration was used as an index for inhalant anesthetics dose.

Many previous studies [8, 18, 19, 21, 32] of avian anesthesia have not included measurement of awake cardiovascular parameters. Although comparison of the awake and anesthetized parameters is important to evaluate the significance of the adverse effects caused by the anesthetics, birds can be extremely sensitive to the stress induced by handling [31]. In birds that are restrained or excited, the HR can be 184 to 401% higher than that of birds that are caged, free from excitement or at rest [14]. In our pilot study, although measurement of the cardiorespiratory parameter was attempted while the vultures were awake, it was not possible to maintain stable enough conditions to collect the data normally.

Sinus arrhythmia is defined as a patterned, irregular sinus rhythm with alternate slowing and acceleration of the HR. In the current study, three of the cinereous vultures developed cardiorespiratory problems (sinus arrhythmia and respiratory instability), while attempting to increase the ETiso from 2.5 to 3.0%. These adverse effects disappeared without any immediate treatment after the isoflurane concentration was decreased. Therefore, it can be assumed that the anesthetic index is more than 2.5. This is higher than the value for ducks, dogs and cats [11]. The sinus arrhythmia at high HR identified via ECG was identified in a vulture of this study. The anesthesia of 3.5% ETiso is associated with cardiac arrhythmia, such as second degree atrioventricular block in bald eagles, probably because of an increase of vagal tone [8]. Premature ventricular contractions have been described in isoflurane anesthetized ducks, however, the arrhythmias resolved when isoflurane concentration was reduced [11], which might suggest that higher anesthetic concentrations can more easily induce arrhythmia in birds.

In avian species, anesthetic dose-related changes in cardiorespiratory parameters have been reported during inhalational anesthesia. Spectral Doppler-derived cardiac parameters, including HR and blood flow velocities, were found to decrease significantly in common buzzards anesthetized with isoflurane [31]. In chickens [17, 18], dose-related decreases in arterial BP were observed, but HR did not differ when the chickens were anesthetized with isoflurane or sevoflurane during controlled ventilation. When ducks were anesthetized with isoflurane during spontaneous ventilation, there was no significant dose-dependent difference between the means of either HR or arterial BP [11]. However, another study [9] demonstrated that there was a significant dose-dependent increase in HR when ducks were anesthetized with halothane during spontaneous ventilation. In this study, a

dose-dependent increase in HR and decrease in arterial BP were observed in cinereous vultures anesthetized with isoflurane during spontaneous ventilation. Therefore, the patterns of the dose-dependent changes in cardiorespiratory parameters may vary depending on the species of bird, type of inhalant anesthetic and ventilation conditions. In addition, as reported in mammals [30], a decrease in systemic vascular resistance caused by inhalant anesthetics seems to be the most probable cause of the hypotension observed in the current study.

Respiratory depression caused by anesthesia with isoflurane was investigated in different bird species during spontaneous ventilation [11, 12, 25, 32]. Species-dependent characteristics, such as the absence of a diaphragm, restriction of sternum excursions by dorsal recumbency and decreased responsiveness of intrapulmonary chemoreceptors to CO_2 caused by inhalant anesthetics, make birds more sensitive to respiratory depression caused by inhalant anesthetic [5, 10, 22]. In the present study, an increase in $P_{ET}CO_2$ and $PaCO_2$ and decrease in pH were associated with the respiratory depression caused by anesthesia with isoflurane. In a previous study [26], inhalant-induced hypoventilation resulted in increased $P_{ET}CO_2$, $PaCO_2$ and HCO_3^- and decreased pH. An FiO_2 >40% has been shown to lead to hypoventilation in ducks anesthetized with isoflurane, likely due to depression of O_2-sensitive chemoreceptors and a depressed ventilator drive. In addition, previous studies [11, 18, 19, 26] reported respiratory acidosis in ducks anesthetized with isoflurane and chickens anesthetized with sevoflurane during spontaneous ventilation. Moreover, increased sympathetic activity and circulating concentration of catecholamines, peripheral vasodilation and myocardial depression were caused by hypercapnia in humans [2]. When the things above are taken into consideration, the respiratory and cardiovascular effects by anesthesia with isoflurane seem to affect the cardiorespiratory system complexly.

Normal arterial pH in awake birds varies between 7.4 and 7.6 [23]. In mammals, depression of the central nervous system may be associated with a pH below 7.2 [4]. Moreover, the decreased pH may lead to organ damage and failed activity of some enzymes, causing alterations in metabolism [27]. Dose-dependent decreases in arterial pH were observed during isoflurane anesthesia in the present study. A non-rebreathing anesthetic circuit, such as the Modified Jackson Rees anesthesia circuit used in this study, is considered to be ideal for the use in birds, because it provides minimal resistance to patient ventilation [10]. However, inhalant anesthetics leading to respiratory depression seems to be much more significant in birds than mammals. This may justify the fact that the thoracic musculature in birds plays significantly important roles in ventilation and that they relax these muscles during anesthesia [6]. When aerobic energy metabolism is degraded or there is a low hematocrit or lactic acidemia, a negative BE is expected as an indicator of acid/base status [26]. Since the BE was > −2 mmol/L in the present study, the disturbance in pH was more likely due to respiratory rather than metabolic changes [20]. A previous study investigated that saline administration results in dilutional acidosis [28]. In the present study, 0.9% normal saline was administered during the procedure at a rate of 10 m*l*/kg/hr. Although each vulture was administered about 100–150 m*l* of normal saline, the values of pH, $PaCO_2$ and HCO_3^- were within the normal range during the anesthetic phase at the second application of 1.0% ETiso. Therefore, the present study indicates that the dilution associated with saline administration at the rate of 10 m*l*/kg/hr did not affect pH of the birds.

In the current study, the duration of anesthesia and the specific concentration of anesthetics may be significant factors influencing changes in cardiorespiratory parameters. A previous study [21] investigated whether anesthesia with isoflurane did not cause a significant change in the arterial BP of spontaneously ventilating anesthetized hispa-niolan Amazon parrots. The same study reported that a decrease of HR was noted only after 45 min of anesthesia. Conversely, another study conducted by Joyner *et al*. [8] reported that the HR and RR significantly decreased over time, whereas the arterial BP significantly increased over time in bald eagles anesthetized with isoflurane during spontaneous ventilation. However, since there was very limited information describing the correlation between the duration of anesthesia and changes in cardiorespiratory parameters of birds, it is difficult to define exactly how the prolonged period of anesthesia would affect changes in cardiorespiratory parameters of the birds. In the present study, while dose-dependent increases in $P_{ET}CO_2$ and $PaCO_2$ were measured, the RR taken at the second application of 1.0% ETiso appeared to be slightly lower than that taken at the first application of 1.0% ETiso, but these differences were not statistically significant. Based on the results, although spirometry was not used to measure breathing capacity, respiratory depth was not able to be maintained because of anesthetic induced muscle relaxation. Although the duration of anesthesia had no significant effects on the cardiorespiratory parameters investigated in the current study, it is difficult to be certain that the duration of anesthesia would not have any effects on the cardiorespiratory parameters of the cinereous vulture under different clinical settings.

In conclusion, when the isoflurane concentration was increased during spontaneous ventilation in cinereous vultures, a dose-dependent increase in HR and $P_{ET}CO_2$ with minimal changes in RR, a decrease in arterial BP and respiratory acidosis were observed. Overall, isoflurane for anesthesia of spontaneously breathing cinereous vultures is a suitable choice for diagnostic or surgical procedures. However, careful controls of anesthetic dose and ventilation conditions are recommended for this species.

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