

Original Study

A Comparison of Intravenous and Oral Transmucosal Administration of Pentobarbital Sodium for Humane Euthanasia in White Leghorn Chickens (*Gallus gallus domesticus*)

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Abstract: Euthanasia is a common veterinary medical procedure performed on terminally ill backyard chickens (*Gallus gallus domesticus*). To reduce stress for these animals, it is important to develop a euthanasia protocol that provides a rapid and humane death. For this prospective research investigation, we evaluated 2 different methods of euthanasia in chickens. Twenty-two adult female leghorn chickens were administered an intravenous (IV, n = 11) or oral transmucosal (OTM, n = 11) 602 mg/kg dose of pentobarbital sodium solution for humane euthanasia. Loss of consciousness (LOC) occurred in 95.4% (21/22) of the birds within 5 minutes. Both apnea and asystole were achieved in 100% (11/11) of the birds in the IV group and 54.5% (6/11) of the birds in the OTM group after 5 minutes; 45.5% (5/11) of the chickens in the OTM group had to be rescued. Birds administered the euthanasia solution IV were found to have faster times to LOC (Mann-Whitney U [MW]=110.5, $P < 0.001$), asystole (MW: 121, $P < 0.001$), and apnea (MW: 121, $P < 0.001$) compared with the OTM chickens. Overall, the OTM route for administering pentobarbital sodium in chickens resulted in a reliable LOC in all but 1 bird; however, it did not result in apnea and asystole for all birds within 5 minutes. Based on the results of this study, the OTM administration of pentobarbital sodium is not a reliable method to euthanize chickens.

Key words: avian, chicken, *Gallus gallus domesticus*, euthanasia, pentobarbital, transmucosal

INTRODUCTION

Euthanasia is a requested and recommended procedure in companion animal hospitals to reduce the pain and suffering of geriatric patients and those suffering from severe injury and terminal illness. There is an increasing emphasis on methods that are both humane for the patient and minimize stress for the veterinary staff and owners present with the animal when euthanized. The Guidelines for the Euthanasia of Animals established by the American Veterinary Medicine Association describe criteria for humane euthanasia, which include minimizing stress and pain and inducing a rapid loss of consciousness (LOC) followed by apnea and asystole.¹ Pentobarbital sodium, a barbiturate, is

the most frequently used agent for euthanasia of companion animal species and is labeled for intravenous (IV) use in all species.¹ Pentobarbital sodium affects the central nervous system by binding gamma-aminobutyric acid subtype A receptors along with inhibiting glutamate, thereby resulting in central nervous system depressant effects.^{1,2} Administration of pentobarbital sodium as a sole agent can result in muscle twitching, agonal breathing, urination, vocalization, and dysphoria in numerous species.^{1,2} For this reason, IV, intramuscular, subcutaneous, or inhalant sedative or anesthetic agents are commonly used before pentobarbital sodium injection.¹

Frequently, companion exotic animals and wild-life species pose unique challenges for euthanasia due to their small size, which precludes venous access. In larger species where IV access is possible, sedation or general anesthesia is often required to facilitate catheter placement. In cases where venous access is not feasible, the American Veterinary Medical

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Association-approved euthanasia alternatives in birds include physically traumatic methods, such as cervical dislocation or injection of the euthanasia agent into visceral organs (eg, intracardiac), a procedure that also necessitates prior general anesthesia.¹ The need for inhalant anesthesia or repeated injections can increase the stress for the patient, owner(s), and veterinary staff. They may also increase staff and client exposure to inhalant anesthetic agents. Investigating alternative methods of humane euthanasia that are minimally invasive but provide rapid and reliable LOC and asystole in birds offers an opportunity to improve euthanasia for patients, as well as the emotional experience for owners and veterinary staff present.

The oral transmucosal (OTM) route for administering medication has been used in both human and veterinary medicine. The OTM drug interface offers several benefits, including the potential for a rapid onset of action and reduced drug breakdown in the gastrointestinal tract compared with the oral route.³ Once a drug is placed in the oral cavity, the compounds enter the body through a transmucosal process that relies on transcellular or paracellular passive transport and favors molecules that are small, lipophilic, and nonionized.³ Other factors that may influence drug absorption include saliva volume, the pH of the oral cavity, and contact time with the mucosa.³ The OTM route has shown promise as a method of drug administration for several medications, including buprenorphine, in cats, dogs, and guinea pigs, where it was reported to reach therapeutic plasma concentrations and exhibit similar efficacy to IV administration in cats.^{4–6} Pentobarbital sodium administered via the OTM route has previously been used with success for sedation in human pediatric patients and in the euthanasia of conscious wild birds, where it resulted in a reliable and rapid means of euthanasia in avian patients belonging to several taxa.^{7,8} To the authors' knowledge, a direct comparison between the IV and OTM routes for pentobarbital sodium administration in chickens (*Gallus gallus domesticus*) before this study has not been investigated.

The goal of this prospective investigation was to determine if pentobarbital sodium delivered via the OTM route provided similar end-of-life results to IV drug administration in white leghorn chickens. The specific objectives of this study were to measure and compare the time to LOC, asystole, and apnea between the 2 routes of administration. The authors hypothesized that IV administration of pentobarbital sodium would result in a more rapid LOC, asystole, and apnea compared with the OTM route; however,

both routes would result in LOC and asystole within 5 minutes of drug administration.

MATERIALS AND METHODS

Case selection

Twenty-two adult female intact white leghorn chickens were used in the study. Approval of this study was obtained from the Louisiana State University Institutional Animal Care and Use Committee (protocol number 21-123-01). All chickens were acquired from the Louisiana State University Poultry Unit, where they were housed in accordance with state and university housing and welfare regulations. The chickens used for the study were transported to the Louisiana State University School of Veterinary Medicine, and physical examinations were performed upon their arrival to ensure they were healthy. Bodyweight, heart and respiratory rates, and any abnormalities found on physical examination were recorded. Exclusion criteria included any birds with physical examination or vital parameter abnormalities suggestive of systemic illness. All birds were deemed healthy based on the absence of abnormalities on physical examination, a heart rate between 200 and 380 beats per minute, and a respiratory rate between 15 and 80 breaths per minute.

Study design

The 22 chickens were randomly divided using a random number generator (random.org) into 2 groups to receive a dose of 602 mg/kg of pentobarbital sodium (FatalPlus, Vortech Pharmaceuticals LTD, Dearborn, MI, USA) via either the IV or OTM route. A standard metal poultry identification wing band (National Brand & Tag Co, Newport, KY, USA) was placed in the right or left patagium of each chicken during examination for identification purposes. Thorough external physical examinations were conducted before initiating the study, as previously described. Chickens were placed in a transportation crate for approximately 2 hours after the physical examination to condition the birds before initiating the study. Each chicken was placed under general anesthesia using 1–2% isoflurane (Fluriso, VetOne, MWI, Boise, ID, USA) in oxygen at 1–1.5 L/min via a tight-fitting face mask for placement of a 24-G IV catheter (Surflo, Terumo Medical Products, Somerset, NJ, USA) in the right or left basilic vein. In 2 birds from the OTM group, catheter placement in a basilic vein was not possible, and a catheter was instead placed in a right or left medial metatarsal vein. Birds were recovered

from general anesthesia while being provided oxygen at 1–1.5 L/min via face mask and monitored for a complete return to consciousness and baseline heart and respiratory rates before administering the pentobarbital sodium. A minimum of 10 minutes passed before the euthanasia trial was started. A dose of 602 mg/kg of pentobarbital sodium was used in this study. The dose was selected based on published results that found 602 mg/kg resulted in LOC and apnea faster than 430 mg/kg or 516 mg/kg when administered via the OTM route to wild birds.⁸ In both chicken groups, doses of pentobarbital sodium were administered over 60 seconds either IV through the catheter or OTM, where the medication was placed within the oral cavity. While orally administering the pentobarbital sodium, it was spread over the mucosal surface for maximum contact while avoiding the glottis. Birds were continuously auscultated with a stethoscope and monitored visually and via electrocardiogram (VetSpecs KM100 ECG; VetSpecs, Canton, GA, USA) for asystole and apnea. Three leads were placed on the right patagium, left patagium, and left foot of each chicken. Chickens were also monitored for complete LOC, which was defined as cessation of response to external stimuli, lack of wing withdrawal, and muscle relaxation with ventroflexion of the head and neck. Time to LOC, apnea, and asystole were recorded. The number of vocalization and spontaneous muscle movement events that occurred after LOC were also recorded for each bird during humane euthanasia. Vocalization events were defined as vocalization preceded and followed by at least 5 seconds of silence. In contrast, muscle movement events were described as movement of the head, neck, wings, or body preceded and followed by at least 5 seconds of stillness. Movement of the keel during respiration was not counted as muscle movement. All birds were monitored until asystole or for a maximum of 5 minutes, at which point a second dose of pentobarbital sodium at 602 mg/kg was administered IV if asystole had not yet been achieved. The study was conducted over 2 days after the arrival of the chickens at the Louisiana State University School of Veterinary Medicine. Eleven of the birds were euthanized on the same day. The remaining 11 birds were housed together overnight and the remainder of the study was conducted the following morning. Chickens that were kept overnight were housed in a newspaper-lined concrete pen approximately 1.5 × 1.5 m (5 × 5 ft) in size and provided with chicken scratch feed and water.

Statistical analysis

The distributions of the continuous data were evaluated using the Shapiro-Wilk test, skewness, kurtosis, and q-q plots. Data for body weight, drug volume, and baseline heart and respiratory rates by the group were normally distributed and are reported by the mean ± SD and minimum-maximum (min-max) values. Independent samples *t*-tests were used to compare the body weights, drug volumes, and baseline heart and respiratory rates between the IV and OTM groups. Levene's test was used to assess the equality of variances and equal variances were found for all comparisons. Because data in the OTM group for the times to LOC, apnea, and asystole, and number of muscle movements and vocalizations, were not normally distributed, nonparametric testing (Mann-Whitney *U* test [MW]) was used. All data were reported as median, interquartile range (25–75%), and min–max. Mann-Whitney *U* tests were also used to determine whether times to LOC, asystole, and apnea differed in chickens within the OTM group that did or did not experience aspiration. A Fisher exact test was used to determine if an IV rescue was required for either group following the administration of the original euthanasia solution. A commercial software (SPSS 28.0, IBM Statistics, Armonk, NY, USA) was used to analyze the data. A $P < 0.05$ was used to determine statistical significance.

RESULTS

There was no significant difference in the body weights ($t = -0.12$, $P = 0.91$), the volume of pentobarbital sodium administered ($t = 0.194$, $P = 0.848$), or baseline heart ($t = 0.65$, $P = 0.519$) and respiratory rates ($t = 0.61$, $P = 0.551$) between the IV and OTM groups (Table 1). There were significant differences in the times to LOC (MW = 110.5, $P < 0.001$), apnea (MW = 121, $P < 0.001$), and asystole (MW = 121, $P < 0.001$) between the IV and OTM groups, with the IV group achieving these physiologic measures faster than the OTM group (Table 2). Chickens in the IV group were also significantly more likely to vocalize (MW = 20, $P = 0.013$) and have muscle movements (MW = 10, $P = 0.001$) compared with the OTM group (Table 2). Chickens in the OTM group (45.4%, 5/11) were significantly ($P = 0.035$) more likely to require an IV rescue of pentobarbital sodium compared with the IV group (0%, 0/11). Chickens in the OTM group that aspirated had significantly shorter times to LOC (MW = 4, $P = 0.026$), asystole (MW = 21, $P = 0.008$), and

Table 1. Descriptive statistics for body weights, volume of pentobarbital sodium, and baseline heart and respiratory rates for female white leghorn chickens (*Gallus gallus domesticus*) divided into 2 treatment groups: intravenous (n = 11) and oral transmucosal (n = 11).

Parameter	Group	Mean	SD	Min–Max	<i>t</i>	<i>P</i>
Body weight, kg	IV	1.5	0.22	1.2–1.9	–0.12	0.91
	OTM	1.5	0.13	1.4–1.8		
Volume of pentobarbital sodium, mL	IV	2.3	0.28	1.9–2.9	0.194	0.848
	OTM	2.3	0.13	2.1–2.5		
Heart rate (baseline beats/min)	IV	309	30.1	260–360	0.65	0.519
	OTM	299	40.6	210–350		
Respiratory rate (baseline breaths/ min)	IV	44	12.4	30–70	0.61	0.551
	OTM	41	12.2	20–60		

Abbreviations: IV, intravenous; OTM, oral transmucosal; Min, minimum; Max, maximum.

There were no significant differences in these parameters between the 2 groups (all $P > 0.05$).

apnea (MW = 21, $P = 0.008$) compared with birds that did not aspirate (Table 3).

DISCUSSION

Intravenous administration of pentobarbital sodium is considered the reference standard for euthanasia in companion animals due to its ability to reliably induce rapid LOC, apnea, and asystole. Administration of IV pentobarbital sodium has been shown to result in brain death confirmed via electroencephalograph (EEG) in isoflurane-anesthetized laying hens at a dose of 0.5 mL/kg (approximately 195 mg/kg) within 15 seconds.⁹ Likewise, brain death confirmed via EEG in turkeys administered an approximately 87 mg/kg dose of pentobarbital sodium IV was noted within 30 seconds of drug administration.¹⁰ While EEG monitoring was not implemented in the current study, loss of posture and muscle tone, along with the cessation of spontaneous blinking, were used to

determine loss of consciousness. These parameters have previously been described as reliable markers of LOC in birds and were noted at a median time of 41 seconds in birds receiving IV pentobarbital sodium in the current study.¹¹ The discrepancy in time to LOC noted between studies despite a higher dose of pentobarbital sodium being used in the present study may reflect a lag between EEG cessation of brain activity and outwardly perceptible signs indicating LOC. Alternatively, variations in dose, administration rate, or pentobarbital sodium formulation may contribute to differences in LOC.

As hypothesized, there were significant differences between time to LOC, asystole, and apnea between the OTM and IV groups. In addition, approximately half of the birds receiving the OTM dose required an additional IV dose because a heartbeat and respirations were still present after 5 minutes. This suggests that the OTM route at the dose used is not reliable as

Table 2. Descriptive statistics for the times to loss of consciousness, apnea, asystole, and the number of muscle fasciculations and vocalizations in female white leghorn chickens (*Gallus gallus domesticus*) administered pentobarbital sodium intravenously (n = 11) or via the oral transmucosal route (n = 11).

Parameter	Group	Median	25–75%	Min–Max	MW	<i>P</i>
LOC, s	IV	45	20–64	15–76	110.5	<0.001
	OTM	90	60–172	50–360		
Time to apnea, s	IV	58	28–90	21–105	121	<0.001
	OTM	349	257–360	192–360		
Time to asystole, s	IV	60	31–90	18–107	121	<0.001
	OTM	354	260–360	210–360		
Vocalizations (number/5 min)	IV	1	0–2	0–3	20	0.013
	OTM	0	0–0	0–0		
Muscle movement (number/5 min)	IV	2	1–5	0–8	10	0.001
	OTM	0	0–0	0–0		

Abbreviations: LOC, loss of consciousness; IV, intravenous; OTM, oral transmucosal; 25–75%, interquartile range; MW, Mann-Whitney statistic; Min, minimum; Max, maximum.

Significant differences were found for all 5 parameters between the 2 groups (all $P < 0.05$).

Table 3. Descriptive statistics for times to loss of consciousness, apnea, and asystole in female white leghorn chickens (*Gallus gallus domesticus*) administered pentobarbital sodium via the oral transmucosal route (n = 11) based on suspected aspiration status.

Parameter	Group	Median	25–75%	Min–Max	MW	P
LOC s	Did not aspirate	172	92.5–323	80–360	4	0.026
	Aspirated	74.5	57.5–110	50–170		
Time to apnea s	Did not aspirate	360	360–360	360–360	21	0.008
	Aspirated	260	237.5–317.5	190–349		
Time to asystole s	Did not aspirate	360	360–360	360–360	21	0.008
	Aspirated	261.5	261.5–318.7	210–354		

Abbreviations: LOC, loss of consciousness; MW, Mann-Whitney statistic; Min, minimum; Max, maximum; 25–75%, interquartile range.

There were significant differences in all 3 parameters between the 2 groups (all $P < 0.05$).

a sole method of euthanasia. These results are inconsistent with those of wild birds who received a 602 mg/kg pentobarbital sodium dose, where rapid loss of consciousness was observed along with asystole and apnea in most cases by 5 minutes.⁸ The reasons for this difference may be related to a variety of factors, including the signalment of birds, body size and mass, and volumes of distributions of the drugs between species. While the present study enrolled captive, apparently healthy domestic chickens from a limited-pathogen facility, previous results were documented in wild birds of various species, all of which were euthanized based on the presence of underlying medical conditions. Health status may have contributed, at least in part, to differences in efficacy of OTM pentobarbital sodium between these studies.

Drug considerations may have further contributed to the lack of efficacy of OTM pentobarbital sodium in this study as a means of achieving asystole and apnea. In the current study, Fatal Plus solution was used, which contains pentobarbital sodium as the sole active ingredient. This formulation was selected because it does not contain phenytoin sodium, which can have actions on cardiac function before the onset of sedation when administered via a non-IV route.¹² Other euthanasia solution formulations are known to contain phenytoin sodium, which theoretically reduces the time to asystole via class 1b antiarrhythmic effects. It is unknown whether the use of a euthanasia solution containing additional active ingredients aside from pentobarbital sodium would influence the time to LOC, asystole, and apnea when administered via the OTM route, although potential adverse effects of pentobarbital sodium combinations should also be considered.

Owing to the alkaline pH of pentobarbital sodium, it has previously been described to cause discomfort with injection into the peritoneal cavity in rats. It may also cause discomfort when in contact with the oral

mucosa.^{13,14} An aversive taste of the medication has also been reported in people and mice and may explain why, in the current study, all chickens in the OTM group demonstrated signs of aversion to the drug being placed into the oral cavity, including head shaking and attempts to move away from the syringe.^{7,15} While chickens have fewer taste buds than domestic mammalian species, such as dogs and cats, responses to the palatability of feed have been documented, and it is speculated that chickens likewise experience aversion to feed tainted with medication.^{16,17} Whether the responses documented in this study were due to discomfort, adverse flavor, or simply the presence of foreign material placed into the oral cavity is unknown. It is important to note that drugs may be expelled from the mouth during head shaking and pose an exposure risk for nearby people or animals. Similarly, a consideration for pet birds administered OTM pentobarbital sodium is that the medication may be present on the beak and face and pose a risk for drug exposure via direct contact as well.

Overall, the OTM route showed fewer instances of adverse effects during euthanasia when compared with the same dose of pentobarbital sodium administered IV. In the IV group, 64% (7/11) of the chickens vocalized, and 82% (9/11) of the chickens had spontaneous muscle movement after losing consciousness. Although these are well-documented effects of pentobarbital sodium, it does not diminish the emotional distress to the veterinary staff and pet owners present when vocalization and/or spontaneous muscle movement occurs after the pentobarbital sodium injection. Of note, none of the chickens that were part of the OTM group demonstrated muscle movement or vocalization, including birds that subsequently received an IV dose following initial OTM administration. This difference may be due to a slower rate of systemic absorption from the OTM route compared

with the IV route. It may have allowed for deeper sedative effects before cardiorespiratory depression. In dogs administered propofol before pentobarbital sodium, the incidence of spontaneous muscle movement was significantly reduced, which supports that LOC before pentobarbital sodium administration IV may help to alleviate these effects during euthanasia.¹⁸ In a previous study evaluating OTM pentobarbital sodium in birds, adverse effects were still noted in a small number of birds, including vocalization, excitation, head shaking, wing flapping, and tremors.⁸ Overall, the OTM route was shown to have a significant reduction in these overt physical effects compared with IV administration, even when administered over 60 seconds.

Unfortunately, 1 adverse effect suspected in several birds within the OTM group was drug aspiration, which occurred in 54% (6/11) of the birds. Drug aspiration was suspected in the OTM group based on sneezing or opening of the beak during respiration, along with the presence of crackles noted on continuous auscultation after administering pentobarbital sodium. Although aspiration is a risk with any substance placed into the oral cavity, the increased number of suspected aspiration events in the current study was likely influenced by the large volume of the drug and the relatively low viscosity of the Fatal Plus solution. In the previous OTM study in wild birds, possible aspiration was only suspected in 1.8% (2/110) of the cases.⁸ Another contributing factor may have been that chickens have a relatively large body weight compared with the size of the head and speculated surface area of the oral cavity than other avian species. Although the occurrence of aspiration was documented as an adverse effect, there was a significant difference in time to LOC, apnea, and asystole between birds that had suspected aspiration events and those that did not within the OTM group. This may have been due to increased systemic drug uptake through the respiratory tract. Regardless, aspiration remains a potential risk with OTM administration of pentobarbital sodium, and caution should be taken to avoid the glottis when administering any medication via this route.

Although this study did not support the use of OTM pentobarbital sodium as a sole means of euthanasia in chickens, it was reliable in most cases as a means of inducing LOC. It may be useful as part of a multistep protocol for euthanasia in this species. Multistep euthanasia protocols that involve heavy sedation or anesthesia before euthanasia with IV pentobarbital sodium have been shown to speed up

the time to asystole and minimize side effects such as involuntary muscle movement and vocalization.¹⁹ In species where EEG changes during euthanasia with pentobarbital sodium have been studied, brain death characterized by loss of brainstem reflexes typically occurs before asystole and apnea.²⁰ Although the time to brain death with OTM pentobarbital sodium in chickens is an area requiring additional research, all birds in the current study showed evidence of loss of posture and muscle tone along with spontaneous blinking as markers of unconsciousness before actuable asystole. Additional studies evaluating different formulations and doses of OTM pentobarbital sodium are warranted to confirm the efficacy of this method as a means of sedation before euthanasia, as well as its use as a sole euthanasia method in other avian species.

Limitations of this study include that the investigators were not blinded when evaluating the euthanasia methods, which may have contributed to bias during data collection, especially regarding determining suspected aspiration events because these were not confirmed postmortem. The lack of postmortem evaluation remains a drawback of this study, as this information may have provided additional insight into the overall health of the enrolled chickens. The postmortem examination of both the IV and OTM birds may have helped determine if there were any histologic tissue differences associated with the different routes of administration, including potential tissue artifacts from the Fatal Plus solution. This study also enrolled only adult female white leghorn chickens and does not provide insight into potential differences that may exist because of sex, breed, or species. Future studies to evaluate the efficacy of OTM pentobarbital sodium in avian species should be considered to investigate its use in avian euthanasia protocols. We found pentobarbital sodium at a dose of 602 mg/kg via the OTM route in chickens was not an effective sole agent for humane euthanasia in this study. Given that most chickens experienced LOC within 5 minutes of medication administration, OTM pentobarbital sodium may be considered as part of a multistep euthanasia process to achieve LOC; however, use should be weighed against the potential risk of aspiration.

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