Sedation is commonly used in birds to facilitate diagnostic and therapeutic procedures. Common drug combinations that are used in balanced sedation protocols for avian species include midazolam, ketamine, and butorphanol. The use of α-2 adrenoceptor agonists has been reported in some avian species. A drug in this class may be desirable as part of a sedation protocol since they are noncontrolled substances in the US and reversible with nonselective α-2 adrenoceptor antagonists, such as atipamezole. However, medetomidine and dexmedetomidine, when used alone or with ketamine, have been shown to provide minimal or unreliable

OBJECTIVE
To determine if sedation with medetomidine-vatinoxan (Zenalpha; Dechra Veterinary Products) and midazolam (Alvogen) (ZM) would cause less cardiovascular depression and maintain similar depth and duration of sedation in pigeons (Columba livia domestica) compared to dexmedetomidine and midazolam (DM).

METHODS
In a blinded crossover study, 15 healthy adult domestic pigeons were sedated IM with either dexmedetomidine (0.08 mg/kg) and midazolam (2 mg/kg) or medetomidine (0.16 mg/kg), vatinoxan (3.2 mg/kg), and midazolam (2 mg/kg) from November through December 2023. Each subject was monitored for 60 minutes, then the sedation was reversed with atipamezole (0.8 mg/kg) and flumazenil (0.1 mg/kg) as needed. Sedation scores, heart rates, and respiratory rates were compared.

RESULTS
There was no significant difference in the peak sedation score between DM and ZM groups, with both exhibiting median scores of 4 (heavy sedation). Mean heart rate was significantly higher for ZM than DM at 5, 10, 20, 30, 45, 60, and 65 minutes postinjection. Bradycardia occurred in both groups at 5 and 10 minutes postinjection and persisted for DM until reversal with atipamezole. Arrhythmias were auscultated in both groups. Bradypnea was not observed in either group, and all birds resumed normal behavior following recovery and the following day.

CONCLUSIONS
Medetomidine-vatinoxan-midazolam provides a similar depth of sedation to DM but with less incidence of bradycardia. Further study is needed to determine the clinical applicability of this sedative in birds.

CLINICAL RELEVANCE
Medetomidine-vatinoxan may be considered for short-term sedation and restraint in cardiovascularly stable pigeons.

Keywords: avian, dexmedetomidine, medetomidine, pigeon, vatinoxan
sedation in pigeons except at very high dosages.\textsuperscript{2–4} When used in a balanced protocol, the combination of dexmedetomidine and midazolam has been shown to produce effective sedation.\textsuperscript{5} Additionally, as α-2 adrenoreceptor agonists cause peripheral vasoconstriction and reduced cardiac output,\textsuperscript{6,7} dexmedetomidine and medetomidine administration in pigeons results in significant bradycardia, bradypnea, and hypothermia,\textsuperscript{2–5,8} which increases the risks of anesthetic complications but resolves with α-2 adrenoreceptor antagonism.\textsuperscript{8}

Vatinoxan is a peripheral α-2 adrenoreceptor antagonist that poorly crosses the blood-brain barrier in mammals.\textsuperscript{9} Vatinoxan has been shown to reduce the cardiovascular side effects of α-2 adrenoreceptor agonists while minimally affecting sedation in dogs, cats, sheep, and other species.\textsuperscript{10–15} The blood-brain barrier is well conserved across taxa, including birds, reptiles, and mammals,\textsuperscript{16} so it is likely that vatinoxan would not diffuse in the CNS in avian species. A veterinary commercial product containing medetomidine (0.5 mg/mL) and vatinoxan (10 mg/mL) (Zenalpha; Dechra Veterinary Products) has recently become available. A nonselective α-2 adrenoreceptor antagonist can still be administered at the end of the procedure for reversal of the sedative effects. Zenalpha provides a potential alternative solution for the use of α-2 adrenoreceptor agonists as part of an avian sedation protocol.

The purposes of this study were to evaluate medetomidine-vatinoxan as a sedative for use in avian medicine and to evaluate the cardiovascular effects in comparison to dexmedetomidine in pigeons when used with midazolam to facilitate sedation in a balanced sedation protocol. The authors hypothesized that medetomidine-vatinoxan and midazolam would cause less cardiovascular depression but maintain similar sedation depth and duration in pigeons (Columba livia domestica) compared to dexmedetomidine and midazolam.

**Methods**

This study was reviewed and approved by the University of Georgia IACUC (Animal Use Protocol A2023 08-012-A4). Twenty adult domestic pigeons (C / domestica) were selected for the study. All birds were housed in laboratory caging (152 X 76 X 147 cm), with 2 to 6 pigeons per cage. The cages and the food and water bowls were cleaned daily. The ambient temperature ranged 22 to 25 °C, and there was a typical 12:12-hour light:dark cycle. Free access to commercial pigeon food (Classic Pigeon Food Blend Racing Pigeon Food; Versele-Laga) and water was available at all times except when fasted for sedation as described below.

After a 7-day acclimation period to the laboratory housing, each bird was assigned a number (1 through 20) based on unique identification (ie, band color, band number, and/or feather color pattern). A complete physical examination was performed on each bird at that time, and any birds with ausculted heart murmurs were excluded.

The study subjects were divided into 2 groups. Seven pigeons received 0.08 mg/kg of dexmedetomidine (Dexdomitor; Zoetis; 0.5 mg/mL) combined with 2 mg/kg of midazolam (Alvogen; 5 mg/mL) (DM) IM in the right pectoral muscle. After a washout period of 5 days, the same 7 pigeons then received 0.16 mg/kg of medetomidine and 3.2 mg/kg of vatinoxan (Zenalpha) combined with 2 mg/kg of midazolam (ZM) IM. The other 8 pigeons received the same drug protocols in the opposite order. The dosages were extrapolated from published data in pigeons and parrots.\textsuperscript{5,17} Given that medetomidine is a 50:50 racemic mixture of active dexmedetomidine and inactive levomedetomidine,\textsuperscript{18,19} equipotent dosages of medetomidine and dexmedetomidine were chosen for this study. Each bird was randomly assigned to a treatment order using a random number generator, and the treatment was administered by 1 author (NSP) who was not blinded, whereas the authors who collected and recorded the subjective parameters (AJ, LB, and SS) looked away while each bird was injected and were blinded to the treatment administered. Data collection was completed from November through December 2023. If any 1 person performing data collection (AJ, LB, and SS) was uncertain as to how to classify a subjective parameter, the other authors were consulted until a consensus was agreed. Dosage calculations were based on the weight on the day of treatment. All birds were fasted for at least 2 hours prior to sedation.

Immediately prior to administration of the assigned treatment, baseline (time 0 [T0]) parameters were collected, which included respiratory rate, heart rate, ulnar refill time, and sedation score (as defined by Pollock et al\textsuperscript{15}). Respiratory rate was measured by counting keel excursions. Heart rate was measured via auscultation. Ulnar refill time was measured by digitally compressing the ulnar vein and counting the time to refill in seconds. In brief, sedation was scored as follows: 0 was defined by normal behavior; 1, minimal sedation with fluffed feathers, stooped broad-based stance, ataxia, and resisting manual restraint; 2, mild sedation with sternal recumbency, closed eyes, and resisting manual restraint; 3, moderate sedation with allowing placement in dorsal recumbency but retaining righting reflex; and 4, heavy sedation with absence of righting reflex and no resistance to wing extension.

After drug administration, the bird was placed in a cage by itself and monitored continuously. The aforementioned parameters were continuously monitored and recorded at 5, 10, 20, 30, 45, and 60 minutes postinjection. Bradycardia was defined as a heart rate lower than 150 beats per minute.\textsuperscript{2} Any abnormalities were also recorded, such as arrhythmias or heart murmurs, regurgitation, spontaneous arousal or movement, and abnormal breathing pattern. The time from injection to initial sternal position was recorded.

Immediately after collecting the T60 monitoring parameters, each subject received 0.8 mg/kg of atipamezole (Antisedan; Zoetis; 5 mg/mL, IM) in the left pectoral muscle to antagonize the α-2 adrenoreceptor agonist. Monitoring parameters were...
recorded at T65, T70, and T80. At T80, 0.1 mg/kg of flumazenil (Hikma Pharmaceuticals; 0.1 mg/mL, IM) was administered in the left pectoral muscle to antagonize midazolam only if the subject did not receive a score 0 for sedation at that timepoint. Monitoring parameters continued to be measured until a sedation score of 0 was achieved after reversal. The total time to standing and time to complete recovery (sedation score 0) were recorded. The time from injection to complete recovery (sedation score 0) was only recorded at a predefined timepoint (ie, T90, T105, T120) and not at a timepoint in between measurements. Once the bird was completely recovered, it was returned to group housing.

During data collection, thermal support was provided to all birds via a patient-warming pad (HotDog Patient Warming System; Augustine Surgical Inc) set to 40 °C, and supplemental oxygen at 0.5 to 1 L/min was administered via a loose-fitting face mask if tolerated. One bird (#4) was treated with 45 ml/kg, SQ, of Lactated Ringer solution (Vetivex) in the inguinal fold due to mildly prolonged recovery after treatment with ZM.

Statistical analysis
The sample size was chosen based on power analysis (G*Power, version 3.1.9.6; Heinrich-Heine-Universität Düsseldorf), which assumed a significance threshold of 0.05, power of 0.80, and 2-sided tests, suggesting a sample size of 10 birds; this was increased to 20 based on review of previous studies2,5,10–14 and to account for any individuals that may have needed to be excluded due to a health status that might interfere with results of this study.

All further analyses were performed using standard software (SAS, version 9.4; SAS Institute Inc). A significance threshold of 0.05 was used. The assumption of normality was evaluated via inspection of quantile-quantile and probability-probability plots, histograms, and skewness. Normally distributed variables were summarized descriptively with mean and SD, and non-normally distributed variables were reported as median and IQR.

Generalized linear mixed models (GLMM) were utilized to test for effects of treatment on sedation score and peak sedation score (ordinal logistic GLMMs), heart rate and respiratory rate (negative binomial GLMMs), pathological arrhythmias, and panting (logistic GLMMs). The models for heart rate, respiratory rate, and sedation score each had fixed factors for treatment, time, and a treatment-by-time interaction and a pigeon- and treatment-specific baseline covariate (for heart and respiratory rates only) as well as random intercepts for each pigeon and each treatment within pigeon. The models for peak sedation score, pathological arrhythmias, and panting had a fixed factor of treatment and a random intercept for each pigeon. The Satterthwaite degrees of freedom method and residual pseudo-likelihood estimation were used in all GLMMs. At 90 minutes, there were all zero values for sedation score for a treatment, which caused the GLMM to fail to converge and so were alternatively analyzed with Wilcoxon signed-rank tests. A Cox proportional hazards frailty model with a random factor of pigeon was used to test for the effects of treatment on time-to-event data. The presence of pathological arrhythmias and panting was compared between treatments using a McNemar test.

Results
Five birds were excluded from the study due to auscultation of heart murmurs. The remaining 15 birds were deemed clinically healthy, which still provided a study population within the means of the performed power analysis. Ten male and 5 female healthy adult pigeons were included in this study. Mean ± SD body weight was 435 ± 47 g during the study.

Figure 1—Box and whisker plots of median sedation scores on a scale from 0 (normal behavior) to 4 (heavy sedation, with an absence of righting reflex and no resistance to wing extension) for 15 healthy adult domestic pigeons (Columbia livia domestica) immediately before (time = 0) and 5, 10, 20, 30, 45, 60, 65, 70, 80, 90, and 105 minutes after IM administration of dexmedetomidine (0.08 mg/kg) and midazolam (2 mg/kg) (DM treatment [blue]) versus IM administration of medetomidine (0.16 mg/kg), vatinoxan (3.2 mg/kg), and midazolam (2 mg/kg) (ZM treatment [red]). All birds had sedation reversed with atipamezole (0.8 mg/kg, IM; time = 60) and as-needed flumazenil (0.1 mg/kg, IM; time = 80). For each box and whisker plot, the solid line within the box represents the median; the lower and upper limits of the box represent the IQR, respectively; the whiskers delimit the range; and circles represent outliers.
first set of sedation episodes, and 437 ± 50 g during the second set of sedation episodes, after the 5-day washout period.

There was no significant difference in the peak sedation score between ZM or DM groups (P = .708), and both had a median peak score of 4 (IQR, 3 to 4). The median duration of peak sedation was longer for DM (50 minutes; IQR, 30 to 55 minutes) than ZM (20 minutes; IQR, 15 to 40 minutes), but this difference was not significant (P = .088). Median sedation scores over time are displayed in Figure 1. At T5, the median sedation score was significantly higher for ZM than DM (P < .001). There was no significant difference in the median sedation score at T10 (P = .125) or T20 (P = .946). Dexmedetomidine-midazolam had a significantly higher sedation score at T30 (P = .017), T45 (P = .001), T60 (P = .001), T65 (P = .014), and T70 (P = .009). There was no significant difference in sedation score at T80 (P = .108) or T90 (P = .500). There was no significant difference in time to sternal position postinjection (DM: 6 minutes; IQR, 4 to 7 minutes; ZM: 3.1 minutes; IQR, 2 to 6 minutes; P = .196), time to peak sedation (DM: 10 minutes; IQR, 10 to 20 minutes; ZM: 5 minutes; IQR, 5 to 10 minutes; P = .106), time to standing postatipamezole (DM: 22 minutes; IQR, 6 to 25 minutes; ZM: 7 minutes, IQR, 0 to 23 minutes; P = .815), or time to sedation score 0 postatipamezole (DM: 31 minutes; IQR, 30 to 32 minutes; ZM: 30 minutes; IQR, 29 to 31 minutes; P = .848). Three birds that received ZM did not require reversal with flumazenil due to the rapidity of return to sedation score 0 after receiving atipamezole.
Mean heart rates over time are displayed in Figure 2. The mean heart rate was significantly higher for ZM than DM at T5 (P = .003), T10 (P < .001), T20 (P < .001), T30 (P < .001), T45 (P < .001), T60 (P < .001), and T65 (P = .032). Mean heart rate was lower than 150 beats per minute at T5 and T10 for ZM and at T5, T10, T20, T30, T45, and T60 for DM. There was no significant difference in heart rate between the groups at T70 (P = .245), T80 (P = .960), or T90 (P = .590). Two birds that received ZM developed a low-grade (grade 3/6 or less) heart murmur during the sedation period, which resolved prior to or upon recovery. There were more birds in the DM group (n = 9) that had an auscultated pathologic arrhythmia (ie, “dropped beats”) event than in the ZM group (n = 4), but the difference was not significant (P = .059). Without ECG monitoring, it was not possible to characterize the auscultated arrhythmias; thus, those described as “dropped beats” were labeled as second-degree atrioventricular block.

Median respiratory rates over time are displayed in Figure 3. The median respiratory rate was significantly higher for ZM than DM at T20 (P = .003) and T30 (P = .004). The median respiratory rate was significantly higher for DM than ZM at T65 (P < .001), T70 (P = .005), and T80 (P = .024). The median respiratory rate was always greater than 28 breaths per minute in both groups. In both groups, there were episodes of abnormal respiratory pattern noted (ZM n = 7; DM n = 8), characterized as “panting,” and there was no difference in occurrence between the groups (P = .655).

Three birds that received DM regurgitated during the sedation or recovery period. All birds maintained an ulnar refill time of less than 1 second at every timepoint. All birds were behaving normally following recovery and the following day.

Discussion

Similar to the findings of previous studies evaluating α-2 adrenoreceptor agonists in combination with midazolam, both groups in the present study achieved heavy sedation. As there was no difference in the peak sedation score between ZM and DM, it is likely that in pigeons, similar to mammals, vatinoxan does not cross the blood-brain barrier and thereby does not reverse the central sedative effects of the α-2 adrenoreceptor agonist.12,21 ZM produced deeper sedation than DM at T5, whereas DM had a higher sedation score at T30 through T70. Although the difference in duration of sedation was not statistically significant, it is possible that a larger sample size would have shown a significant difference in duration of sedation, with ZM being likely shorter than DM. However, considering that sedation is used for short and minimally invasive procedures in clinical settings, a drug that provides a quicker onset of deeper sedation, such as medetomidine-vatinoxan, might be most suitable for these types of procedures.

Although there was not a significant difference in time to recovery after atipamezole between the groups, 3 birds receiving ZM did not require flumazenil as they achieved normal behavior quickly after atipamezole. This is similar to the findings in sheep, in which sedation scores declined faster after atipamezole for the medetomidine-vatinoxan group than the medetomidine group.12 Adam et al12 discuss a possible phenomenon in ruminants whereby atipamezole briefly increases plasma medetomidine via displacement of medetomidine from highly perfused organs back into circulation, which may prolong sedation compared to medetomidine-vatinoxan.

The mean heart rate was significantly higher for ZM than DM at most timepoints postinjection, which is similar to findings in dogs and cats.10,22 However, birds in both groups were bradycardic at T5 and T10. The heart rates with ZM trended up over time during sedation and were above the bradycardia threshold from T20 onward, whereas heart rates with DM did not trend upwards nor exceed the threshold until after atipamezole administration. This suggests some cardiovascular relief with ZM compared to DM, although cardiovascular depression, as represented by the bradycardia during the early timepoints, still occurred. Alternatively, given that the mean depth of sedation was lower in the ZM group than the DM group from T30 to T70, lighter depth may have caused stress during handling and therefore higher heart rates.

Type II second-degree atrioventricular blocks were noted in birds in both groups in this study and are also common in dogs receiving dexmedetomidine, and it appears to occur in a dose-dependent pattern due to an increased reduction in sympathetic tone at higher doses.23 Arrhythmias are reported in a pigeon treated with 0.08 mg/kg medetomidine2 and in a budgerigar (Melopsittacus undulatus) treated with DM at 0.08 mg/kg dexmedetomidine, but not at the lower 0.04 mg/kg or 0.01 mg/kg dosages.24 Further studies in pigeons may allow elucidation as to whether a larger sample size or lower dosage of medetomidine-vatinoxan would result in an occurrence of arrhythmias that was statistically significant between the groups.

The 2 birds that developed low-grade systolic heart murmurs during treatment with ZM did not have auscultable heart murmurs prior to sedation, and the murmurs resolved by the end of the sedation period. In dogs, dexmedetomidine causes mitral regurgitation and can also cause pulmonic, tricuspid, and aortic valvular regurgitation due to cardiovascular changes, including increased afterload, increased preload, increased end diastolic volume, bradycardia, and hypertension.25 In the present study, the heart murmurs were auscultated in birds receiving ZM, which contradicts the hypothesis that vatinoxan reduces the cardiovascular impact of α-2 adrenoreceptor agonists in pigeons. Further research comparing echocardiograms between birds receiving ZM and DM to determine if there is an objective difference in valvular regurgitation between the protocols is needed.

The baseline respiratory rate of the pigeons in this study was higher than reported ranges of 34 ± 6 breaths per minute,25 which may be explained by...
handling and stress.\textsuperscript{1,17} Similar to Hispaniolan Amazon parrots (\textit{Amazona ventralis}) receiving midazolam\textsuperscript{17} and pigeons receiving medetomidine or dexmedetomidine,\textsuperscript{3,5} the respiratory rate decreased 10 and 20 minutes postinjection for DM and ZM, respectively, although the median respiratory rate was higher for ZM than DM at T20 and T30. Again, given that the mean depth of sedation was lower in the ZM group than the DM group from T30 to T70, lighter depth may have caused stress during handling and therefore higher respiratory rates. Although this overall decrease with both treatment groups could be interpreted as respiratory depression, it is more likely that the sedation alleviated the stress from handling since the respiratory rate never decreased below the reported resting respiratory rate in pigeons\textsuperscript{26} at any timepoint. Pigeons in both groups also exhibited a “panting” behavior during the sedation episode. A similar behavior was described in black-cheeked lovebirds after receiving medetomidine-midazolam.\textsuperscript{27} In humans receiving dexmedetomidine, the respiratory rate increased to compensate for decreased tidal volume in a dose-dependent fashion,\textsuperscript{28} which could explain this behavior in the present study. Other causes of tachypnea or exaggerated breathing effort under sedation or anesthesia include the patient being in a light plane of sedation or anesthesia, hypoxemia, hypercapnia, hyperthermia, or hypotension.\textsuperscript{29} However, most of the panting episodes occurred shortly after the injection of sedative or reversal drugs, which might reflect a stress response or pain response secondary to intramuscular injection. In the future, studies should consider additional monitoring equipment, such as capnography, temperature, and blood pressure, although these values may be challenging to accurately obtain in a sedated bird, especially if in a light plane of sedation.\textsuperscript{30}

The 3 birds that regurgitated during the sedation period received DM. A side effect of α-2 adrenoceptor agonists is emesis, which has been reported in pigeons receiving medetomidine in a previous study.\textsuperscript{2} Emesis results via activation of α-2 adrenoceptor receptors in the chemoreceptor trigger zone,\textsuperscript{31} which is not protected by the blood-brain barrier.\textsuperscript{32} This likely explains why birds in the DM group but not the ZM group regurgitated as vatinoxan likely antagonizes receptors in the chemoreceptor trigger zone to prevent vomiting or regurgitation.

A limitation of this study was the lack of a pilot study to determine the lowest effective dose of medetomidine-vatinoxan in pigeons. This was not performed as the dosing was based on the dosing of medetomidine or equipotent doses of dexmedetomidine in pigeons in previous studies,\textsuperscript{2,5} although evaluation of the lowest effective dose would be an area for future study. Blood pressure is affected by α-2 adrenoceptor agonists and antagonists,\textsuperscript{6,11,12,22} therefore, the lack of blood pressure monitoring was a limitation of this study. Previously, systemic blood pressure was significantly lower in sheep (\textit{Ovis aries}), cats (\textit{Felis domesticus}), and Patagonian mara (\textit{Dolichotis patagonum}) treated with medetomidine-vatinoxan than medetomidine alone.\textsuperscript{11,12,22} Due to this, blood pressure measurement was considered; however, indirect blood pressure measurement in birds is generally inaccurate, and direct blood pressure is invasive.\textsuperscript{30} Therefore, this variable was excluded due to the anticipated challenges of acquiring accurate and safe blood pressure measurements in a sedated pigeon and the impact that the increased handling would have on confounding the sedation scoring. Similarly, another limitation was the lack of ECG monitoring, which was also excluded due to the anticipated impact on sedation data collection. ECG monitoring would have allowed characterization of auscultated arrhythmias and definitive differentiation between pathologic and physiologic arrhythmias.

In conclusion, based on the present study, ZM produces a similar depth of sedation to DM in pigeons but with less incidence of bradycardia and emesis. Further studies are needed to determine the clinical applicability and the cardiovascular effects of medetomidine-vatinoxan in birds. Caution should be undertaken when considering medetomidine-vatinoxan in birds, especially those with underlying cardiovascular disease, until further evidence is available but may be considered for short-term sedation and restraint in cardiovascularly stable pigeons.

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