When managing pain in avian species, opioids have been the most widely utilized class of analgesics in the veterinary field. Of the opioids, butorphanol has historically been one of the most commonly used opioid analgesics in birds, particularly among psittacines such as Hispaniolan Amazon parrots (Amazona ventralis), grey parrots (Psittacus erithacus), and green-cheeked conures (Pyrrhura molinae). Butorphanol's ability to significantly reduce the minimum alveolar concentration of isoflurane when administered to species such as cockatoos (Cacatua alba) and grey parrots makes it a favorable agent to include in anesthetic protocols. When anesthetized with sevoflurane following preoperative butorphanol administration, Hispaniolan Amazon parrots did not show significant anesthetic or cardiopulmonary changes. Readily available access to butorphanol and its low cost have additionally cast this analgesic in a favorable light when creating analgesic plans for avian patients.

Despite its popularity as an analgesic, the standard butorphanol tartrate formulation does have its limitations of use, the most significant being its limited effect over severe pain and its short duration of action in avian species. The terminal half-life of butorphanol tartrate in Hispaniolan Amazon parrots was very short (0.5 hours approximately at a dose times), making it unsuitable for long-term pain management.

OBJECTIVES
To determine the pharmacokinetics of butorphanol tartrate incorporated into poloxamer 407 (P407) after subcutaneous administration to orange-winged Amazon parrots (Amazona amazonica). 

ANIMALS
Six orange-winged Amazon parrots, ages 28 to 45 years.

PROCEDURES
A sterile formulation of butorphanol in P407 (But-P407) as a 25% gel was created to produce a concentration of 8.3 mg/mL. The formulation was administered SC at a dose of 12.5 mg/kg to all birds. Blood samples were collected at baseline prior to injection (time 0) and then at 0.08, 0.5, 1, 1.5, 4, 8, and 12 hours after drug administration. Butorphanol concentrations were quantitated via liquid chromatography–tandem mass spectrometry. Pharmacokinetic analysis was performed using noncompartmental analysis and a commercially available software program.

RESULTS
Plasma concentrations of butorphanol remained > 100 ng/mL for > 4 hours for some birds (3/5) but were < 100 ng/mL for all birds by the 8-hour mark. Cmax and tmax were 346.9 ± 233.7 ng/mL and 1.3 ± 0.274 hours, respectively. Half-life was 1.56 ± 0.445 hours. No adverse effects were detected.

CLINICAL RELEVANCE
Butorphanol was absorbed from the But-P407 25% by the majority of the orange-winged Amazon parrots in this study (3/5), although to a lesser extent compared to Hispaniolan Amazon parrots. Absorption followed a pharmacokinetic profile compatible with a sustained-release drug. A dose of 12.5 mg/kg, SC, would be expected to provide antinociception for 4 to 8 hours, although pharmacodynamic studies in this species using this formulation have not demonstrated this.
of 5 mg/kg), with half-life minimally increasing in larger species like red-tailed hawks (0.9 hours) and great-horned owls (1.8 hours). Oral bioavailability of butorphanol tartrate in Hispaniolan Amazon parrots is poor (5.9%), thereby ideally requiring that this analgesic be administered via injection or continuous rate of infusion to be most effective. Although provision of appropriate and adequate analgesia can greatly improve an avian patient’s welfare and recovery, the added stress of frequent handling, injection-induced discomfort, local trauma to repeatedly used injection sites, and need for personnel to provide frequent administrations must be considered.

The utilization of sustained-release opioid analgesics would allow for a reduction in dosage frequency and patient handling, both of particular benefit in avian patients recovering from severe pain scenarios like those of orthopedic and extensive soft tissue procedures. Sustained-release liposome-encapsulated butorphanol formulation in parrots and osmotic pumps used to slowly deliver butorphanol to common peafowl (Pavo cristatus) have been evaluated, but neither of these applications are readily available. The use of hydrogels that allow for sustained-release drug delivery has held greater promise, with Poloxamer 407 being a particular hydrogel of recent interest. Poloxamer 407 is liquid at room temperature and can easily be mixed with therapeutic agents and then formulated for routine injection. Following injection into homeothermic animals, Poloxamer 407 compounds become a gel via micellar packing, which subsequently allows for slow dissolution and sustained release of both hydrophilic and hydrophobic drug mixtures.

Both pharmacokinetic studies in Hispaniolan Amazon parrots and pharmacodynamic studies in orange-winged Amazon parrots evaluated the use of butorphanol tartrate in a long-acting Poloxamer 407 gel (But-P407). In the study evaluating the pharmacokinetics of But-P407 in Hispaniolan Amazon parrots, both intramuscular (IM) and subcutaneous (SC) administration of But-P407 was performed in preliminary experiments at equal doses of 8.3 mg/kg, with the mean plasma butorphanol concentration for birds receiving the formulation SC being twice that of birds given the same dose and formulation IM. Based on these preliminary experiments, a 12.5-mg/kg, SC, dose was given for the primary pharmacokinetic study and was well absorbed by the Hispaniolan Amazon parrots and the pharmacokinetic profile was compatible with a sustained-release drug.

With the promising pharmacokinetic profile of the 12.5-mg/kg, SC, dosage of But-P407 in the Hispaniolan Amazon parrots, a pharmacodynamic study was performed to evaluate the analgesic abilities of this formulation in a larger species, the orange-winged Amazon parrot. Given the similarities between Hispaniolan and orange-winged Amazons parrots, it was hypothesized that But-P407 would reach sufficient concentrations in the orange-winged Amazon parrots to provide longer thermal antinociceptive effects compared to standard butorphanol tartrate. Surprisingly, this study resulted in significant differences in thermal thresholds between control saline and treatment birds for the IM butorphanol tartrate but not for either trial of the SC But-P407. This then raised the question if the pharmacokinetic profile for But-P407 is similar in Hispaniolan compared to orange-winged Amazon parrots.

The objective of this study was to determine the pharmacokinetic profile of a sustained-release But-P407 25% butorphanol formulation following parenteral administration to orange-winged Amazon parrots. It was hypothesized that plasma concentrations of butorphanol considered to be therapeutically (> 100 ng/mL) would be reached following But-P407 administration and the resulting pharmacokinetic profile would be similar to the Hispaniolan Amazon parrot study.

Materials and Methods

Animals
Initially, six adult male orange-winged Amazon parrots (Amazona amazonica) 28 to 45 years of age were used in this study. The mean ± SD body weight of the birds was 404.1 ± 35.0 g. All birds were healthy based on previous history and physical examination prior to this study. However, during the study period, one bird developed a heart murmur and was eliminated from the remainder of the study. During pharmacokinetic challenge, birds were housed individually in hanging wire cages (36 [length] X 23.5 [width] X 36 [height] inches) with ad libitum access to a pelleted diet formulated for psittacine birds (Roudybush Inc) and water. Each cage contained one perch and hanging toy. All birds were exposed to a photoperiod of 16 hours of light and 8 hours of darkness and the temperature of the room maintained at 24 °C.

Preparation of But-P407

The But-P407 formulation was prepared using the cold method as previously described. The amount of Poloxamer 407 sufficient to yield a 25% gel (% weight/weight) was added to previously refrigerated (5 °C) butorphanol (Torb Gegsic; Fort Dodge Animal Health) in a syringe case. The syringe case, surrounded by ice packs, was placed on a BenchMixer (Benchmark Scientific Inc) vortex mixer (3,200 rpm on “touch” mode) for 5 minutes until a homogenous solution was obtained, after which it was placed in a refrigerator at 4 °C for 5 to 10 minutes until clear of bubbles. The solution was then sterilized by filtration through a 0.22-μm microfilter (Milllex GP filter unit, Millipore Express) and injected directly into a sterile rubber topped glass tube. The solution was kept on ice and used within 2 h.

Experimental design
All birds were injected subcutaneously with a dose of 12.5 mg/kg butorphanol in 25% P407, as described above. Under manual restraint, 0.3 mL of blood was collected via right jugular vein using a 29-gauge needle attached to a 0.3-mL plastic syringe for the following time points: 0.08, 0.5, 1, 2, 4, and 6 hours.
4, 8, and 12 h. Needles were removed from syringes and blood transferred to lithium heparin microtubes. Microtubes were stored on ice for < 1 hour until centrifugation at 3,800 X g for 10 minutes. Plasma was harvested into microcentrifuge tubes and stored at −80°C until analysis approximately 2 months later. The birds received approximately 50 mL/kg Ringer’s lactate solution SC in the left inguinal region following final sample collection. The birds were visually monitored subjectively for adverse effects like sedation-agitation and nausea-like behavior during the duration of the study.

Measurement of plasma butorphanol concentration

The concentration of the dosing formulation was determined using liquid chromatography tandem-mass spectrometry (LC-MS/MS) as described below for plasma samples. Prior to injection on the LC-MS/MS instrument, 2 aliquots of the dosing solution were serially diluted 100-fold 2 times in water. Butorphanol concentrations were quantitated in orange-winged Amazon parrot plasma by LC-MS/MS analysis of protein-precipitated samples, using modifications of a previously published method and butorphanol-d₆ (Toronto Research Chemicals) as the internal standard. A partial validation was performed using orange-winged Amazon parrot plasma as a matrix. The response for butorphanol was linear and gave a correlation coefficient of better than 0.99. Quality control samples (at 3 concentrations within the standard curve) were included as an additional check of accuracy. Accuracy was reported as percent nominal concentration and precision as percent relative standard deviation. For butorphanol, accuracy was 111% for 0.3 ng/mL, 107% for 5 ng/mL, and 111% for 80 ng/mL. Precision was 6% for 0.3 ng/mL, 4% for 5 ng/mL, and 4% for 80 ng/mL. Accuracy and precision for both assays were considered acceptable based on FDA guidelines for bioanalytical method validation. The assay was optimized to provide a limit of quantitation of 0.1 ng/mL and a limit of detection of 0.01 ng/mL.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed on plasma butorphanol concentrations using non-compartmental analysis and a commercially available software program (Phoenix WinNonlin Version 8.0; Certara). The maximum measured plasma concentration (C_max) and time to maximal plasma concentration (t_max) were obtained directly from the plasma concentration data. The area under the curve from 0 to 24 hours (AUC Last) was calculated using the log-linear trapezoidal method. Pharmacokinetic parameters for butorphanol are reported as individual and mean (± SD) values.

Results

Mean plasma butorphanol concentrations were > 100 ng/mL by 0.5 hours postadministration for 4 out of 5 birds. At 4 hours postadministration, the mean plasma butorphanol concentration for the birds was 110.8 ng/mL, remaining > 100 ng/mL in 3 out of 5 birds. Mean plasma concentrations decreased to < 100 ng/mL by the 8-hour time point for all birds (Figure 1). One bird never attained a plasma concentration > 100 ng/mL (maximal plasma concentration = 61.1 ng/mL). No adverse effects were detected in any of the birds during the course of this study.

Pharmacokinetic parameters determined for the 5 orange-winged Amazon parrots are listed in Table 1. The t_max was 1.3 hours, while C_max was 346.9 ± 233.7 ng/mL.

Table 1—Pharmacokinetic parameters following subcutaneous administration of 12.5 mg/kg of butorphanol in a Poloxamer 407 25% base to orange-winged Amazon parrots (n = 5).

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Mean ± SD</th>
<th>Range</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_max (ng/mL)</td>
<td>346.9 ± 233.7</td>
<td>61.1–608.0</td>
<td>56.7–637.2</td>
</tr>
<tr>
<td>t_max (h)</td>
<td>1.3 ± 0.274</td>
<td>1.0–1.5</td>
<td>0.96–1.64</td>
</tr>
<tr>
<td>lambda_z (1/h)</td>
<td>0.444 ± 0.102</td>
<td>0.291–0.553</td>
<td>0.317–0.57</td>
</tr>
<tr>
<td>Half-life lambda_z (h)</td>
<td>1.56 ± 0.445</td>
<td>1.25–2.38</td>
<td>1.09–2.2</td>
</tr>
<tr>
<td>AUC Last (h X ng/mL)</td>
<td>1157 ± 699.1</td>
<td>330.8–1987.4</td>
<td>288.6–2024.8</td>
</tr>
</tbody>
</table>

All parameters were generated using noncompartmental analysis. C_max, maximum plasma concentration; t_max, time of maximal plasma concentration; lambda_z, terminal slope of plasma concentration curve; half-life lambda_z, half-life of terminal slope; AUC Last, area under the curve to the last time point collected.
Discussion

Previous studies with Hispaniolan Amazon parrots determined that plasma concentrations of butorphanol and metabolites required for antinociception ranged from > 55 ng/mL to > 80 ng/mL based on exposure to thermal and electrical stimuli, respectively.4 However, the assay used in this cited study did not differentiate between the parent compound and its presumed active metabolites. Plasma concentrations of 100 ng/mL expected to result in antinociception, were reached 0.5 hours post-administration of But-P407 in 4 out of the 5 orange-winged Amazon parrots in this study. It is intriguing that one bird never reached a plasma concentration greater than 61.1 ng/mL throughout the duration of the study despite being of comparable size and age to the other birds. All orange-winged Amazon parrots reached their maximal plasma concentration either 1 or 1.5 hours following administration of But-P407.

In comparing the 2 species of Amazon parrots that have undergone pharmacokinetic testing of But-P407, the pharmacokinetic profiles for the 12.5-mg/kg, SC, dose highlighted similarities in $t_{\text{max}}$ but differences in maximal plasma concentration and the terminal half-life. The $t_{\text{max}}$ was reached at 1.31 hours for the Hispaniolan Amazon parrots14 and at 1.3 hours for the orange-winged Amazon parrots. However, mean Cmax reached approximately 452.3 ng/mL in the Hispaniolan Amazons14 and 346.9 ng/mL in the orange-winged Amazons, and the terminal half-life was considerably shorter in the orange-winged Amazons compared to the Hispaniolan Amazons (1.56 hours versus 3.41 hours, respectively). When considering species differences, orange-winged Amazon parrots tend to have larger average body size compared to Hispaniolan Amazon parrots,19,20 with this holding true for the populations used for the published But-P407 pharmacokinetic studies. The average Hispaniolan Amazon parrot population weight was 305.5 grams14 versus the average orange-winged Amazon parrot population weight of 404.1 grams. In studies evaluating the pharmacokinetics of levofloxacin in sheep, sheep with a higher body mass were found to have lower drug clearance and a longer elimination half-life compared to lighter counterparts.21,22 The Hispaniolan Amazons were also of younger age (11 to 27 years)14 than the orange-winged Amazons utilized in this study (28 to 45 years), which could result in potential differences in drug absorption and clearance secondary to differences in cardiovascular, hepatic, or renal capabilities. Along similar lines, only male individuals were used from this orange-winged Amazon parrot population, whereas both males and females (6 males and 5 females) were included in the population of Hispaniolan Amazon parrots for that respective But-P407 pharmacokinetic study.14 Differences in morphine metabolism and antinociceptive effects between male and female rats have been reported,23,24 as have differences in butorphanol metabolism when comparing young to adult mammalian species such as horses.17

This pharmacokinetic study was investigated in part to determine an explanation for the lack of significant effect exhibited during thermal threshold evaluation of a 12.5-mg/kg, SC, dose of But-P407 in orange-winged Amazon parrots.15 Assuming provision of thermal antinociception is achieved with a butorphanol tartrate plasma concentration of > 55 ng/mL,4 all 5 birds in this study did reach those concentrations within 1.5 hours or sooner postadministration of But-P407 SC. Even if extrapolated to require a higher therapeutic threshold between 150 and 450 ng/mL in orange-winged Amazon parrots as described in previous studies,10,15 all but 1 of the 5 birds in this study reached and maintained butorphanol levels above 150 ng/mL from 0.5 to 1.5 hours postadministration. Despite this, significant effect in thermal threshold withdrawal were not appreciated at any of the time points assessed for the But-P407 pharmacodynamic study, although effects did exist 0.5 hours after administration of butorphanol tartrate alone at a dose of 5 mg/kg, IM.15

As the absorption and subsequent plasma concentrations of But-P407 in the orange-winged Amazon parrots here demonstrate that desired target plasma concentrations are reached following administration, the effectiveness of P407 as a vehicle given the concurrent pharmacodynamic findings previously published is therefore questionable.25 While it cannot be concluded from this study that P407 hinders the release or absorption of butorphanol in orange-winged Amazon parrots, it is still possible that P407 may interfere with butorphanol’s ability to exert an antinociceptive effect when administered within that vehicle. As an example, P407 has been documented to cause alterations in lipid metabolism in rabbits and rodents.25–27 While it is unlikely that this would affect the metabolism of butorphanol itself, it may alter lipid transport across the blood brain barrier while concurrently altering transport of the butorphanol to target opioid receptors. Given its nature as a hydrophilic substance, the blood-brain barrier in mammals can further restrict access of vehicles such as P407 due to the lack of fenestrations between endothelial cells of the blood-brain barrier.28 It is currently unknown as to whether similar lipid metabolism changes exist in psittacine species given this vehicle. To date, it likewise has not been indicated in human or animal models that P407 blocks or otherwise hinders opioid receptors when given, although poor blood-brain barrier permeability is a leading cause of ineffectiveness in CNS-active drugs in human medicine.29

In completing this study, the authors would like to acknowledge the limitation of having a small number of birds. Given that only 5 orange-winged Amazon parrots were evaluated, this allows for individual variation to have a greater effect on overall group results, particularly in terms of the single bird that did not reach the target butorphanol plasma concentration of 100 ng/mL. The birds used in this study were also older than those used in both the Hispaniolan Amazon parrot But-P407 study14 and the pharmacodynamic study22 of this drug in orange-winged Amazon parrots, which may make direct comparison of these study populations challenging should variations in drug metabolism exist.
between age groups. Additionally, only male orange-winged Amazon parrots were utilized in this study, bringing into question whether the pharmacokinetic profile would have been altered had females also been included in the study population as they were in previous evaluations of But-P407.14,15

Results obtained from the present study indicate that absorption of butorphanol from a P407 25% base administered SC to orange-winged Amazon parrots follow a pharmacokinetic profile consistent with that of a sustained release drug. However, although a dose of 12.5 mg/kg, SC, would be expected to provide antinociception in this species for at least 4 to 8 hours, it has been determined that such effect is not present when evaluated via thermal nociception.15 Although butorphanol tartrate in its standard form provides antinociception in this species, it may be that But-P407 is ineffective in these birds due to either underlying species variation or confounding effects of the poloxamer base. Based on the findings in both this study and that evaluating thermal antinociceptive effects of But-P407 in orange-winged Amazon parrots, this method of butorphanol administration needs further evaluation before being recommended to use with butorphanol. Further pharmacodynamic studies testing other nociceptive stimuli may shed additional light on the usefulness of But-P407 in orange-winged Amazon parrots.

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