Single oral or intravenous administration of voriconazole achieved recommended therapeutic minimum inhibitory concentrations against *Aspergillus* in the common raven (*Corvus corax*)

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OBJECTIVE
To determine the pharmacokinetics of voriconazole after single IV or orally administered boluses in common ravens (*Corvus corax*).

ANIMALS
8 healthy common ravens.

PROCEDURES
Voriconazole (5 mg/mL, 10 mg/kg IV) was administered to 8 birds, and then plasma voriconazole concentrations were measured at various time points by high-pressure liquid chromatography with mass spectrometry. Starting 6 months later in a randomized 3-treatment 3-period regimen, birds received a single oral dose of voriconazole suspension (10 mg/mL; 6, 12, and 24 mg/kg PO). The study period was May 2015 to March 2016.

RESULTS
Voriconazole (10 mg/kg IV) achieved an initial plasma concentration of 6.31 µg/mL when measured over 21 hours. After oral administration of voriconazole at 6, 12, and 24 mg/kg, the relative bioavailability was 67.5%, 209%, and 183%, respectively. For the 6-mg/kg dose, the maximum plasma concentration was reached at 30 minutes after administration and remained in the therapeutic range of 0.5 to 1 µg/mL for approximately 15 hours. The 12- and 24-mg/kg doses resulted in concentrations in a potentially toxic range.

CLINICAL RELEVANCE
Voriconazole was well tolerated. All 4 doses resulted in plasma concentrations of voriconazole > 0.5 µg/mL, which is the minimum inhibitory concentration recommended for pathogenic species of *Aspergillus* fungi known to affect birds. A single dose of voriconazole administered as 10 mg/kg IV or 6 mg/kg PO resulted in recommended target plasma concentrations. Administration of voriconazole 6 mg/kg PO 2 to 3 times daily may be adequate for treatment without exceeding the toxic range.

*A*spergillus fumigatus, *Aspergillus niger*, and *Aspergillus flavus* are the primary fungal species implicated in infections in birds, with *Aspergillus fumigatus* being the most common etiologic agent. Aspergillosis can result in high morbidity and mortality rates, particularly in birds of prey, waterfowl, and penguins. Such fungi are ubiquitous in the environment, and all species of birds are susceptible. Infiltrative and granulomatous involvement of any organ system is possible. Clinical signs that are commonly present are respiratory distress from obstruction of the syrinx, air sacculitis, or fungal pneumonia. Neurologic signs can manifest and are not limited to weakness in the limbs, ataxia, and dysphagia. Chronic weight loss and anemia reflect dysfunction of any organ.[1] Treatment is generally protracted and expensive. Economic losses can be great in commercial breeding operations such as poultry production units or exotic breeding farms.

Common ravens (*Corvus corax*) are one of the largest members of the order Passeriformes. They are intelligent[2] and gregarious members of the corvid family, which also includes crows and jays.[3] Ravens are frequently displayed in zoologic institutions, and present to wildlife rehabilitation centers for injury, disease, or toxicosis. Stress from confinement, trauma, or other comorbidities predisposes these birds to aspergillosis. A bird's potential response to stress and disease may be a consideration when treating a member of this species. The

clinical signs are typical of those observed with other birds affected with this mycosis. Anemia, weight loss, and neurologic dysfunction are common manifestations. Death is common.

The pharmacokinetics (PK) of voriconazole have been established in several species, including horses,[4,5] alpacas,[6] and cats[7] as well as humans, rodents, and dogs.[8] It is estimated that 10,000 species of birds exist.[9] To our knowledge, voriconazole has only been studied in 9 genera and 16 species of birds, include the Timneh parrot (*Psittacus timneh*),[10] Hispaniolan Amazon parrot (*Amazona ventralis*),[11] Japanese quail (*Coturnix japonica*),[12] domestic chicken (*Gallus gallus domesticus*),[13] pigeon (*Columba livia domestica*),[14] 6 penguin species,[15] mallard duck (*Anas platyrhynchos*),[16] 3 falcon species,[17,18] and the red-tailed hawk (*Buteo jamaicensus*).[1] The PK of voriconazole show marked variability among the species tested. Currently, voriconazole is the treatment of choice against systemic aspergillosis in humans because of to its wide tissue distribution, safety, and tolerance. Increasing resistance to commonly used azole drugs, such as itraconazole,[19,20] has prompted the use of voriconazole with increasing frequency in birds, such as parrots[10,11] and penguins,[15] with aspergillosis.

Voriconazole is a synthetic derivative of fluconazole and is a member of the triazole class of antifungal drugs that also include itraconazole. They are part of a diverse group of antifungals known as ergosterol biosynthesis inhibitors. The triazoles are less toxic and better tolerated for systemic use than the imidazoles (ketoconazole, miconazole, clotrimazole) because of their greater affinity for fungal cytochrome (CYP) P450, in comparison to mammalian CYPs. In contrast to the polyenes (eg, amphotericin B), the triazoles are more water soluble, improving general availability in both the parenteral and oral preparations; have reduced nephrotoxicity (amphotericin B); and are not as cost prohibitive (lipophilic amphotericin B).[21,22] As with other members of its class, voriconazole exerts its effects by inhibition of ergosterol synthesis, thus resulting in loss of fluidity within the fungal cell membrane and prevention of cell growth.[23–27] Voriconazole is more potent and effective against a broad spectrum of molds and filamentous fungi as a result of the structural changes of the fluconazole molecule, making it both fungistatic and fungicidal. The fungicidal activity against many species of *Aspergillus* has made it the drug of choice against acute and chronic aspergillosis in humans.[24,28]

Veterinarians routinely extrapolate drug doses and administration periods from multiple sources when there are limited or no data regarding the species of concern. Intraindividual and interindividual serum voriconazole concentration can vary based on differences in absorption or elimination.[28] Because of such a nonlinear nature, plasma drug concentrations and elimination half-lives are dose dependent, making it impossible to predict their nature across species.[14] Empiric dosing can lead to detrimental effects as a result of subtherapeutic doses, inappropriate administration frequency, or toxicity.[15] Managing patients clinically can be challenging as a result.

The objective of our study was to demonstrate the PK of voriconazole after a single IV or PO bolus in common ravens. This species of bird was chosen because it is susceptible to aspergillosis, is commonly displayed in zoologic institutions, and is frequently treated at wildlife rehabilitation centers. Many facilities have monetary constraints, and commercially available voriconazole may be cost prohibitive. To our knowledge, this is the first PK study to assess the response to voriconazole administered IV and PO in ravens or Passeriformes in general.

Materials and Methods

Birds

Eight common ravens (4 males, 4 females) were included in this study. The birds were housed outdoors as pairs in separate enclosures (3 X 3 X 2 m) at La Paloma Animal Sanctuary in Peoria, AZ. The birds were provided by US Fish & Wildlife Service Region 2 and the Arizona Game & Fish Department. Their diet consisted of 25% commercial avian pellets (ZuPreem FruitBlend with Natural Fruit Flavors, ZuPreem), and 75% equal amounts of fresh or thawed frozen fruit and vegetables, cooked meats, cheese, pasta, bread, and hard-boiled eggs. Water was provided ad libitum for consumption and bathing. Environmental enrichment and beak conditioning were provided in the form of mealworms, frozen meatballs, and commercially prepared frozen rodents. The birds were examined physically and weighed at the start and conclusion of the study, and inspected visually twice daily. Hematologic and biochemical analyses, including resting bile acid concentrations (Idexx Laboratories, Inc), were conducted for each bird prior to the IV study to ensure all birds were healthy. PCV (CRITOCAPS, Oxford Labware) total protein concentration, resting bile acid concentration, and biochemical analyses were conducted at the conclusion of all 4 studies (VETSCAN VS2, original version; Abaxis). The birds were determined to be healthy and free of clinical disease based on examination and laboratory data. The study was approved by the Institutional Animal Care and Use Committee of Wildlife World Zoo & Aquarium, the US Fish & Wildlife Service Region 2, and the Arizona Game & Fish Department Wildlife Center.

Experimental design for IV administration

Eight birds (4 males, 4 females) were used in the IV study. Voriconazole (Voriconazole for Injection; Sandoz Inc) was reconstituted with sterile water per manufacturer instructions, yielding a solution concentration of 5 mg/mL. The birds underwent an overnight fast, and the study was initiated in the morning. Each bird was administered voriconazole (10 mg/kg IV) into the left medial tarsometatarsal vein given over 1 minute. They were fed 1 hour after receiving the injection. The volume of blood collected was based on a 1% body weight calculation. The birds were divided into 4 groups of 2 ravens each.

Groups were chosen to include 1 male and 1 female bird selected through randomization. Groups were assigned a specific testing point based blindly on numbers placed upside down on notecards and shuffled. Each bird was sampled at 4 time points, which minimized the blood volume taken per bird. Samples from all groups were collected at time 0. The samples from group 1 birds were collected at 0.08, 0.25, and 0.75 hour after drug administration; group 2 at 0.16, 0.5, and 1 hour; group 3 at 2, 4, and 8 hours; and group 4 at 12, 24, and 36 hours after injection. Blood samples were obtained from the right jugular vein and placed into blood tubes designed for small samples of blood, and with lithium heparin added. Ten minutes after being drawn, samples were centrifuged at 3,400 X g for 10 minutes. The plasma was then decanted and stored at –80°C until shipment to the laboratory on dry ice. Voriconazole solution was kept refrigerated at 4°C between administrations. The solution was frozen at the time of the last administration as described earlier for serum handling and submitted for analysis of concentration. This portion of the study was conducted in late April through early May 2015.

Experimental design for single oral dose

A randomized 3-treatment 3-period study was conducted using 4 birds (2 males, 2 females) for the 6- and 24-mg/kg doses, and 8 birds (4 males, 4 females) for the 12-mg/kg dose. The 4 birds were chosen randomly for the 6- or 24-mg/kg dose, as described earlier. The 6-mg/kg study was performed 6 months after the IV study. The 12- and 24-mg/kg studies were performed 1 and 4 months later, respectively. For each treatment, birds were fasted overnight. Voriconazole was administered as 6, 12, or 24 mg/kg. All testing was performed during the cooler months of the year (November 2015, December 2015, and March 2016).

Voriconazole tablets (Sandoz Inc; 10 X 50-mg tablets) were crushed and added to 37.5 mL of a suspending agent (ORA-Plus Suspending Vehicle, Paddock Laboratories) with 12.5 mL of deionized water to make a 10-mg/mL suspension. The suspension was mixed for 2 minutes by rapid agitation, then refrigerated overnight at 4°C and administered the following morning. The drug was shaken manually (2 shakes/second) for 1 minute prior to administration to the birds. This would represent the typical preparation of the drug in a clinical environment without access to a vortex mixer.

A 15-cm curved, 10-gauge stainless steel feeding needle with a ball tip was used to administer the suspension to the birds using a wooden dowel for a mouth gag. The volume capacity of the tube was determined prior to use. The allocated amount of drug given per each group was then calculated into the total volume of drug drawn into the tube prior to use, so that the required volume was administered.

Birds were administered the designated amount of drug and were then fed 1 hour later. Blood samples were taken at 0, 0.5, 1, 2, 4, 6, 8, 12, 24, and 36 hours after voriconazole administration. All birds were sampled at hour 0, and blood samples were collected from each bird at either 3 or 4 of the total time points from the right jugular or metatarsal veins. Time points were assigned randomly as described earlier. The volume of blood taken, processing, storage, and shipment were the same as for the IV study mentioned earlier.

Determination of drug sterility and stability

The sterility of the oral formulation was evaluated at 30 and 60 days after refrigeration (4°C) for the 6- and 12-mg/kg single-dose studies. The stability of the oral formulation was determined at days 0, 21, 30, and 60 after compounding.

Determination of drug concentration

Voriconazole concentrations were measured using high-performance liquid chromatography (HPLC) coupled tandem mass spectrometry (LC-MS/MS) in a manner described previously.[29] This study utilized a Thermo-Finnigan Surveyor HPLC (Thermo-Finnigan) connected to a mass spectrometer (Thermo-Finnigan TSQ Quantum Electrospray Ionization [ESI]–Mass Spectrometry Detector; Thermo Fisher Scientific). The equipment was capable of atmospheric pressure ionization, including ESI in either positive (M + H$^+$) or negative modes. Low pH in the HPLC mobile phase induced the presence of protonated voriconazole to give so-called M + H$^+$. Voriconazole was infused initially at 10 µg/mL in 0.1% formic acid (aq)-to-acetonitrile, 1:1, into the ESI (+)-mode mass spectrometer of the LC-MS/MS to determine product ions of the voriconazole m/z 350.3 M + H$^+$-positive ion. The resultant product ions, m/z 281.2 and 127.5, were each optimized for detection with the following settings: collision energy, 20 V; tube lens, 200 V. For extraction, 0.5 mL of plasma was protein-precipitated and extracted simultaneously by adding 1 mL acetonitrile. Standard dilutions in water were prepared, with concentrations typically ranging from 0.5 to 100 µg/mL, and a calibration curve was generated with standard software (Thermo-Finnigan Xcalibur Software, Thermo Fisher Scientific). Chromatography (Alltech Alltima 3-µm 2.1 X 50-mm C18 column, Thermo Fisher Scientific) involved a gradient with 0.1% formic acid in HPLC-grade water (solvent A) and HPLC-grade acetonitrile with 0.1% formic acid (solvent B) at 300 mL/minute throughout with variation as follows: 0 to 2 minutes, 90% solvent A/10% solvent B; 2 to 7 minutes, linear gradient to 10% solvent A/90% solvent B; 7 to 9 minutes, held at 10% solvent A/90% solvent B; 9 to 10 minutes, linear gradient to 90% solvent A/10% solvent B; and 10 to 13 minutes, held at 90% solvent A/10% solvent B. The linear or quadratic equation of the line resulting from the calibration plot was used to determine sample concentration by interpolating the peak area of each sample to the equation of the line.

Method validation

The LC-MS/MS method was examined for chromatographic area responses in the range 0.1 to

100 µg/mL voriconazole, and the signal-to-noise was calculated at each concentration. Interpolation to a signal-to-noise ratio of 3.0 and averaging over 4 such examinations provided a lower limit of detection of 0.080 µg/mL and a lower limit of quantitation of 0.240 µg/mL. Standard curves were fit to quadratic relationships and linearity was demonstrated by the average coefficient of determination, R^2, at 0.9982 ± 0.0010. Instrumental carryover was assessed by examining matrix blanks analyzed immediately after the highest calibrator, and was found to be minimal at 0.2%. Precision was determined by the measurement of 1-µg/mL spikes over 4 days, yielding an average of 0.65 ± 0.03 µg/mL for a coefficient of variation (CV) of 4.9%. Accuracy was assessed by refit of calibrators to the standard curve of 0.5 to 200 µg/mL, which gave an average recovery of 101.4% ± 8.3% for a CV of 8.1%. If applied to the 0.2- to 200-µg/mL range, the average result broadened to 113.3% ± 35% for a CV of 31%, illustrating the decreased reliability of values at or less than the calculated lower limit of quantitation.

Pharmacokinetic calculations

Data analyses were performed using a 1-compartment model with standard software (PKSolver, version 2.0; China Pharmaceutical University) to determine standard pharmacokinetic parameters, including elimination half-life ($t_{1/2}$), time to reach maximum plasma concentration (t_{max}), maximum serum drug concentration (C_{max}), initial concentration at time 0 (C_0), clearance rate corrected for bioavailability (Cl/F), volume of distribution adjusted for bioavailability (Vd/F), mean residence time, and area under the concentration curve from time 0 to infinity ($AUC_{0-\infty}$). The program assumed first-order kinetics based on linearity of the terminal portion of the semilogarithmic concentration–time plots. Bioavailability (F) was calculated by use of the following standard equation: $F = (AUC_{PO}/AUC_{IV}) \times (Dose_{IV}/Dose_{PO})$, where AUC_{PO} and AUC_{IV} are the $AUC_{0-\infty}$ for the oral and IV administrations, respectively, and $Dose_{IV}/Dose_{PO}$ represent the IV dose-to-PO dose ratio.[30]

Results

Birds

Eight common ravens (4 males, 4 females) weighing 0.77 to 0.91 kg (mean, 0.847 kg) were included in our study. A total of 12 ravens were part of a permanent collection as a result of disfigurement, blindness, or imprinting. All the birds resided at the sanctuary for more than 1 year prior to the start of the study. Inclusion in the study required the birds to be free of clinical disease and to have normal physical examination findings (excluding physical handicaps) and normal laboratory values. All 12 birds passed the inclusion requirements. Eight birds were the maximum number used in the study at a given time based on financial constraints. Eight birds in place of 4 were used in the 12-mg/kg study because of simulated concentration–time profiles. There were no unfavorable effects observed in any of the birds participating in the study. Physical examinations, clinicopathologic variables, and weights were consistent with pretreatment evaluations.

Drug stability

The reconstituted IV solution of 5 mg/mL measured 5.55 ± 0.510 mg/mL. The compounded concentration of the suspension for oral use averaged 9.392 ± 0.803 µg/mL, with a CV of only 8.5% across the 60-day period, indicating little or no degradation. Cultures failed to show any microbial growth.

IV study

Plasma voriconazole concentrations over time after a single dose of voriconazole (10 mg/kg IV) were determined from a 1-compartment model **(Figure 1)**. A C_0 of 6.31 µg/mL was attained immediately after administration, and the concentration then decreased to less than 1 µg/mL by approximately 15 hours. The $t_{1/2}$
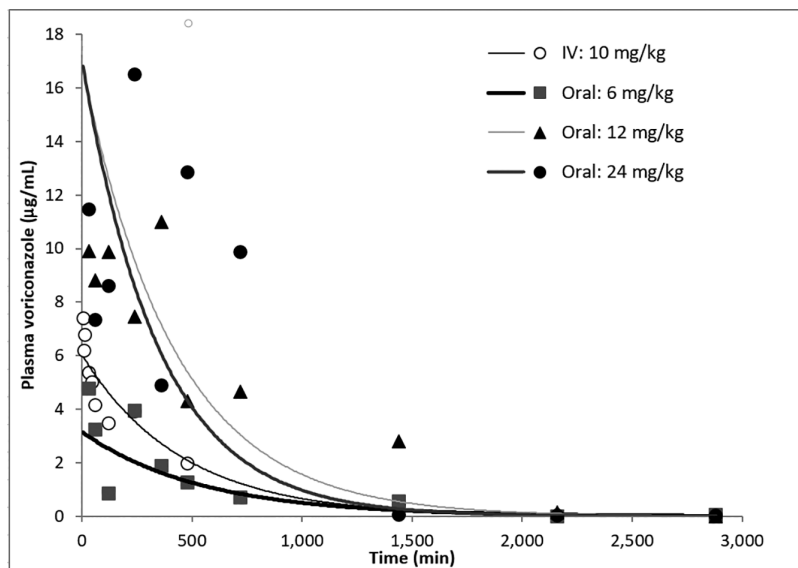

Figure 1—Plasma concentrations of voriconazole in 8 healthy, nonreleasable, common ravens (*Corvus corax*) at 0.5, 1, 2, 4, 8, 12, 36, and 48 hours after receiving voriconazole at a dose of 10 mg/kg IV (n = 8, open circles), 6 mg/kg PO (n = 4, squares), 12 mg/kg PO (n = 8, triangles), or 24 mg/kg PO (n = 4, filled circles). Birds were fed 1 hour after treatment. There was a 6-month washout period between the IV treatment and the 6-mg/kg PO treatment, followed by a 1-month washout period before the 12-mg/kg PO treatment, and then a 3-month washout period before the 24-mg/kg PO treatment. Plasma concentrations were generally less than the lower limit of quantification 36 hours (2,160 min) after treatment. Each symbol represents a result for a raven. The curved lines represent the mean plasma concentrations of voriconazole for each treatment group over the sampling times.

Table 1—Pharmacokinetic parameters determined from the 1-compartment model for voriconazole administered IV at a dose of 10 mg/kg or PO at a dose of 6, 12, or 24 mg/kg to 8 healthy, nonreleasable, common ravens (*Corvus corax*) permanently housed at an animal sanctuary.

Parameter	10 mg/kg IV (n = 8)	6 mg/kg PO (n = 4)	12 mg/kg PO (n = 8)	24 mg/kg PO (n = 4)
$t_{1/2}$ (h)	3.92	0.76	1.59	2.83
Vd/F (mL/kg)	1,585.80	970.94	444.37	690.14
Cl/F (mL/h/kg)	280.11	415.16	133.72	152.50
$AUC_{0-\infty}$ (µg/mL/h)	35.70	14.45	89.74	157.38
MRT (h)	5.66	3.43	5.61	8.61
t_{max} (h)	—	1.56	2.74	4.30
C_{max} (µg/mL)	—	3.17	11.84	13.46
C_0 (µg/mL)	6.31	—	—	—
F (%)	—	67.5	209	183

— = Not calculated. $AUC_{0-\infty}$ = Area under the plasma concentration-versus-time curve extrapolated to infinity. C_0 = Concentration at time 0 for an initial IV bolus dose. C_{max} = Maximum plasma concentration. Cl/F = Clearance corrected for bioavailability. F = Bioavailability. MRT = Mean residence time. $t_{1/2}$ = Half-life of elimination phase. t_{max} = Time to maximum concentration. Vd/F = Apparent volume of distribution adjusted for bioavailability.

Birds were fed 1 hour after treatment. There was a 6-month washout period between the IV treatment and the 6-mg/kg PO treatment, followed by a 1-month washout period before the 12-mg/kg PO treatment, and then a 3-month washout period before the 24-mg/kg PO treatment.

was 3.92 hours, the Vd/F was high at 1585.80 mL/kg, and Cl/F was 280.11 mL/hour/kg **(Table 1)**.

Oral single-dose study

Pharmacokinetic parameters were calculated using a 1-compartment method. Plasma concentration-versus-time curves were developed from 3 single-dose studies (Figure 1). Doubling the dose of voriconazole from 6 to 12 and then to 24 mg/kg resulted in a 620% and 175% increase in the $AUC_{0-\infty}$ (14.45 µg/mL/h for the 6-mg/kg PO dose vs 89.74 µg/mL/h for the 12-mg/kg dose, 89.74 µg/mL/h for the 12-mg/kg dose vs 157.38 µg/mL/h for the 24-mg/kg dose, respectively, Table 1). The C_{max} increased by 373% (3.17 µg/mL vs 11.84 µg/mL) for the 6- versus the 12-mg/kg dose, and then by 114% (11.84 µg/mL vs 13.46 µg/mL) for the 12- versus the 24-mg/kg dose. Bioavailability was high at 67.5% at 6 mg/kg (range, 67.5% to 209% across all doses), indicating that voriconazole was well absorbed from the birds' intestinal tracts. C_{max} values were > 0.5 µg/mL in all 3 groups, with values ranging from 3.17 to 13.46 µg/mL. These C_{max} values were similar to those obtained by the 10-mg/kg IV dose, which yielded a C_{max} of 5.8 µg/mL. The t_{max} ranged from 1.56 to 4.3 hours, and the $t_{1/2}$ was between 1 hour and 3 hours. These values attained the minimum inhibitory concentration (MIC) reported for 100% of pathogenic *Aspergillus* spp.[17,18,23]

Discussion

Voriconazole is currently the standard of care for antifungal treatment in human medicine[28,31,32] and is frequently prescribed for birds infected with *Aspergillus*. The large volume of distribution (4.6 L/kg) in humans makes it a favorable choice because of its increased concentration in the respiratory, ocular, and central nervous systems.[33,34] These are the systems commonly affected by *Aspergillus* colonization in many species, and especially in avian patients. The Vd/F for the IV and 6-mg/kg single PO dose were

greater than those for the 12- and 24-mg/kg PO doses, indicating that more drug was present within the plasma and not the tissues. The short $t_{1/2}$ of all 3 PO studies, combined with a reduced Vd/F, might indicate rapid metabolism by the hepatic system. Because of the nonlinear PK, the dose-dependent $t_{1/2}$ was not useful in predicting the accumulation or elimination of the drug in humans.[35] This was probably true for our study as well. The measured t_{max} ranged from 2 to 4.5 hours, indicating rapid gastric emptying. This was 1 to 2 times that observed in humans,[8] and similar to the findings in hawks,[1,33] horses,[4,5] cats,[7] parrots,[10,11] ducks,[16] dogs, and most rodent species.[8]

The relative bioavailability was 67.5% for the 6-mg/kg PO dose, and this exceeded the values observed for most avian species studied to date. The bioavailability in humans was high (96%) within 2 hours of absorption.[25,32] The bioavailability in other species tested (mouse, rabbit, guinea pig) is more than 75%, with 159% in rats and 138% in dogs after single, multiple, and IV dosing.[8] The absolute bioavailability calculated in the ravens approximated that observed in rats (156% for the 6-mg/kg dose) and dogs (132% for the 24-mg/kg dose).[8] The bioavailability reported for avian species tested showed that ducks approximated the results observed in some mammals at 61%[16]; pigeons[13] and chickens[14] showed far less (44% and < 20%, respectively). The bioavailability was 95% and 135.75% in 2 studies with horses[4,5] but less than 23% in alpacas.[6] The poor oral bioavailability in the alpaca study[6] was thought to be associated with binding to ingesta. Ruminal microbe degradation, slow gastric emptying, and poor permeability across the cell membranes were also possibilities. Reduced plasma voriconazole concentration has also been observed in falcons that were administered the drug with food.[17] Food has been shown to reduce the oral bioavailability in humans by 22%, and the absorption time in humans[27,36] and hawks.[1] Red-tailed hawks in another study[33] had a relative bioavailability of 97% when voriconazole was given through a gavage tube separately, followed by gavage of a liquid diet.

The ravens in our study were fasted before treatment to determine the biologic availability at doses that have been extrapolated from selected avian studies in which the species did not show toxic effects. Under most circumstances in a clinical setting, patients with clinical signs are anorexic. Because food can influence the therapeutic effects of voriconazole as a result of delayed absorption or reduced bioavailability, it is best to provide voriconazole separately, until the pharmacokinetic effects can be evaluated in response to feeding in this species. Of interest is the greater bioavailability of voriconazole in the omnivorous and carnivorous species studied compared with granivorous ones. Increased rates of gastric emptying and intestinal absorption may be the result of differences in gastrointestinal length and shorter transit times. There was also potential for differences between generic and brand-name formulations; however, this was probably minimal because manufacturers of generic products must show with a 90% CI that their drug is within the limits of 80% to 125% bioavailability when compared with the brand-name product, thus being pharmaceutically equivalent.[37] Last, bioavailability values can be skewed when using compartmental and noncompartmental methods to evaluate nonlinear drugs.[11]

Bioavailability is also influenced by solubility and permeability across lipid-rich cell membranes. Solubility can be pH dependent, as has been observed with some members of the azole subclass triazole (itraconazole) and imidazole (ketoconazole), but not as much with voriconazole. The increased solubility in water made it easier to compound. The drug will stay in solution if compounded between 1 mg/mL and 10 mg/mL.[4,31,32,38] A concentration of 10 mg/mL of solution yielded a reasonable volume per weight based on a 6-mg/kg PO dose for tube feeding in most avian species. The concentration can be adjusted based on extremes in size of the patient, but is limited by solubility and volume capacity in the crop. Some antifungal drugs such as itraconazole require the addition of an acid during compounding to enhance the solubility, thereby improving bioavailability.[39,40] The benefits of not using a compounded acidic formulation are a reduction in the time of preparation, fewer required supplies, and a decrease in the risk of regurgitation or vomiting in clinically ill patients, making it a more favorable option over itraconazole against aspergillosis. Furthermore, the stability of the generic voriconazole suspension tested well.

Voriconazole in adult humans[8] and many other species such as Timneh parrots,[10] pigeons,[14] and falcons[17,18] showed nonlinear PK. Nonlinearity was indicated by the C_{max} and AUC increase that was disproportionate to the administered dose resulting from saturation of metabolic processes involving the CYP enzyme system.[11,36] The AUC increase observed for the 6- versus the 12-mg/kg dose and the 12- versus the 24-mg/kg dose was 6- to 11-fold. Also, the lack of a convex shape of the semilog curves supported nonlinearity. Hepatic CYP groups CYP2C and 3A are responsible for voriconazole metabolism in humans.[41] These CYPs and others have also been identified in avian species.[16] Genetic variation, gender, and age influence the plasma concentration and elimination of voriconazole in humans. Induction of metabolism has been observed after multiple dosing or dose escalation in mice, rats, dogs[8], Timneh parrots,[10] and pigeons.[14] PK can vary between IV and single and multiple doses of voriconazole administered. Dose extrapolation is not advised, particularly for extended treatment, unless specific studies have been performed for that species.[11]

It has been suggested in human medicine to maintain serum trough concentrations of voriconazole between 1 μg/mL and 4 μg/mL to ensure efficacy.[28] In avians, 59 A fumigatus isolates have been obtained from 12 orders, including 5 representatives from the Passeriformes order. The MIC range was 0.13 to 8 μg/mL, MIC_{50} was 0.25 μg/mL, and MIC_{90} was 0.5 μg/mL, with 4 resistant isolates of A fumigatus identified that had MIC values 8 to 16 times higher.[28] Recommendations are currently based on results from a study[17] involving 4 species of Aspergillus [A fumagatus (61%), A flavus (20%), A niger (12%), and Aspergillus terreus (8%)] from 117 falcons. All isolates were sensitive to voriconazole at ≤ μg/mL, and 97.4% were sensitive at ≤ 0.5 μg/mL (median MICs in micrograms per milliliter are 0.25 and 0.5 for A fumigatus or A terreus and A flavus or A niger, respectively). The ravens in our study had a C_0 of 6.31 μg/mL after voriconazole IV injection. The plasma concentration stayed in the therapeutic range of 0.5 to 1 μg/mL between 15 hours and 21 hours, making a single IV injection every 24 hours potentially effective for this species. This would be the initial treatment of choice in a clinically ill raven, given the high bioavailability, C_0, and large volume of distribution after IV administration.

A C_{max} of 3.17 μg/mL was reached at 1.56 hours after a single oral dose of 6 mg/kg voriconazole. The plasma voriconazole concentration stayed above 1 μg/mL for 8 hours, and above 0.5 μg/mL for approximately 15 hours. A twice- to 3-times-daily dosing schedule is a consideration based on these findings. The C_{max} of the 12-mg/kg single oral dose of voriconazole was 11.84 μg/mL, reached in 2.74 hours with a $t_{1/2}$ of 1.59 hours. The plasma voriconazole concentration remained above 5.0 μg/mL for approximately the first 12.5 hours after administration. In humans, values exceeding 5.0 μg/mL are potentially toxic, based on observed side effects. The birds in our study showed no untoward effects and, theoretically, a dosing frequency of twice daily may be appropriate. The C_{max} after the 12- or 24-mg/kg dose of voriconazole was in the toxic range for other species, making this dose range less favorable. Nevertheless, there were no undesirable effects noted in the birds. This species may be able to tolerate doses considered toxic in others. The therapeutic efficacy is dependent on the amount of time that the Aspergillus organism is exposed to a particular concentration of voriconazole. The higher doses resulted in an increase in the AUC. These higher doses are potentially more effective than the 6-mg/kg dose. Because these were single-dose studies, it

is also possible that toxic effects could manifest with chronic administration. The $t_{\frac{1}{2}}$ increased with the dose administered in the 3 study groups. At the same time, the clearance rates decreased. Clearance is dependent on metabolism by hepatic CYP enzymes.[5] This is indicative of saturation of metabolism.

The birds in our study displayed no adverse effects after the administration of the voriconazole. Behavioral, hematologic, and biochemical parameters remained clinically normal. The limited number of single-dose studies in other avian species have also reflected this. Anorexia and neurologic signs have been reported in penguins,[15] and emesis and hepatopathy have been observed by one of the authors (SDJ) in an African fish eagle (*Haliaeetus vocifer*). Toxicoses in other species have included miosis and hypersalivation in domestic cats,[7] gastrointestinal upset,[15] as well as visual disturbances, skin reactions, and hepatopathies in humans.[10,26] In avians and mammals, the PK are variable and difficult to predict because of a saturable metabolism, nonlinear PK, and poor correlation to weight-based dosing.[42] The presence of food also affects the PK.[1,17,24] Extrapolating a dose and frequency of administration of voriconazole can lead to subtherapeutic treatment or toxicity. Biochemical, histologic, or clinical abnormalities were reported in studies with pigeons[14] and Timneh parrots,[10] but not in hawks,[1] ducks,[16] chickens,[13] or falcons.[17]

Possible autoinduction of CYP enzymes has also been a potential cause for decreased plasma voriconazole levels after multidose studies in Timneh parrots,[10] Hispaniolan Amazon parrots,[11] pigeons,[14] mallard ducks,[16] falcons,[17,18] as well as some mammalian species.[8] Voriconazole is metabolized extensively by the liver. Metabolites have been identified in several species, and the N-oxide compound was the most readily observed, but it was 100-fold less effective than voriconazole and was not considered to have any antifungal activity.[8] A specific CYP enzyme in ravens has not been identified.

The limitations faced during this study included a limited testing window associated with warm temperatures that required the cessation of strenuous activities with the birds, resulting in sampling delays that could have potentially influenced certain variables that could not be detected. Also, achieving a balance between experimental design for a study taking place in a harsh environment while evaluating the response in a species with known intelligence and cognitive abilities presented challenges in obtaining data without infringing on animal welfare because of potential awareness of stress. A randomized 3-treatment 3-period regimen was chosen over a crossover study design to limit the duration of the testing schedule to prevent chronic stress and blood loss, and to reduce the potential for the confounding of effects.[43] The small population of birds available also presented a limitation of data, and vessel fragility and available blood volume reduced the number of time points that could be sampled per individual.

The data presented in our study support that a single dose of either 10 mg/kg IV or 6 mg/kg PO administered 2 to 3 times daily was sufficient to reach the target MIC (0.5 to 1 µg/mL) for the most common pathogenic *Aspergillus* species known to infect birds without exceeding the potential toxic range ($\geq$ 5.0 µg/mL).

We stress that all birds are not created equal metabolically and that our data should be evaluated in consideration of other references provided, resulting in awareness for the need to proceed with extreme caution when extrapolating from dose regimens with this drug during extra-label use. Because the disposition of voriconazole shows complex intra- and interindividual variability, because of its nonlinear PK, further investigation with multidose administration of voriconazole is warranted to determine the long-term safety, efficacy, metabolism, and tissue accumulation in this species.

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