

**Title:** Cefovecin pharmacokinetics after single-dose intramuscular administration in cheetahs (*Acinonyx jubatus*)

**Running Title:** Cefovecin pharmacokinetics in cheetahs

**Authors:** Jennifer H. Yu<sup>1</sup>, Mark G. Papich<sup>2</sup>, Rodrigo Garcés Torres<sup>3</sup>, Jessica Emerson<sup>4</sup>, Matthew E. Kinney<sup>5</sup>, Kelly Helmick<sup>6</sup>, Adrienne Crosier<sup>7</sup>, Carlos R. Sanchez<sup>8</sup>, Suzan Murray<sup>1</sup>

**Author Affiliations:**

<sup>1</sup> Global Health Program, Smithsonian's National Zoo and Conservation Biology Institute, Washington DC, USA

<sup>2</sup> Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina, USA

<sup>3</sup> Wildlife Safari, Winston, Oregon, USA

<sup>4</sup> White Oak Conservation, Yulee, Florida, USA

<sup>5</sup> San Diego Zoo Global, San Diego, California, USA

<sup>6</sup> Department of Veterinary Medicine, Smithsonian's National Zoo and Conservation Biology Institute, Front Royal, Virginia, USA

<sup>7</sup> Center for Species Survival, Smithsonian's National Zoo and Conservation Biology Institute, Front Royal, Virginia, USA

<sup>8</sup> Oregon Zoo, Portland Oregon, USA

**Keywords:** *Acinonyx*; Cefovecin; Cephalosporins; Pharmacokinetics

**Abstract:**

Cefovecin is a third-generation cephalosporin with potential value for use in exotic felids due to its long duration of action. A sparse sampling protocol was implemented with 18 zoo-housed cheetahs (*Acinonyx jubatus*) to evaluate the pharmacokinetics of cefovecin (Convenia<sup>®</sup>) after a single 8 mg/kg intramuscular injection. Blood was collected serially for 15 days following administration, and plasma cefovecin concentrations were determined using high-pressure liquid chromatography with ultraviolet detection. Pharmacokinetic parameters were estimated using population pharmacokinetic methods and non-linear mixed effects modeling (NLME). Cefovecin was well-tolerated by all cats, with no adverse effects observed. Peak plasma cefovecin concentration was 84.75 µg/mL, with a mean residence time of 207.9 hrs, and an elimination half-life of 144.1 hrs (6.00 days). Plasma concentrations of cefovecin were maintained > 7µg/ml in all individuals for the entire study duration (15 days). These concentrations are lower, and the half-life slightly shorter, than the values reported for domestic cats. Cefovecin was highly protein-bound (approximately 99.9%) in cheetah plasma, which is nearly identical to domestic cats. These results indicate that cefovecin is potentially useful as a long-acting antibiotic in cheetahs.

## 1. Introduction

Cefovecin is a third-generation cephalosporin currently approved for the treatment of skin infections in domestic dogs and cats in the United States (Stegemann et al., 2006a; Stegemann, Sherington, and Blanchflower, 2006b; Stegemann, Sherington, Coati, Brown, and Blanchflower, 2006c; Stegemann, Sherington, and Passmore, 2007), and additionally for feline urinary tract infections in other countries (Burke et al., 2017; Dokuzeylül et al., 2015; Passmore, Sherington, and Stegemann, 2008). Interpretive categories and breakpoints for cefovecin have been established by the Clinical Laboratory Standards Institute (CLSI) for testing pathogens from dogs and cats (CLSI, 2020). Cefovecin has the advantage of exhibiting a long terminal half-life of 5-7 days, which provides a long time above the minimal inhibitory concentration (MIC) which is essential for time-dependent antibiotics such as cephalosporins (Papich, 2014). For this class of antibiotics, a high degree of plasma protein binding is associated with a long terminal half-life and slow clearance. In domestic cats, cefovecin is highly protein-bound in plasma (> 99.5%–99.9%), and has a long elimination half-life of approximately 6.9 days and an effective dosing interval of 7–14 days, depending on the targeted pathogen (Stegemann et al., 2006c; Stegemann, Sherington, and Passmore, 2007).

Because of the long duration of plasma concentrations, cefovecin is an appealing therapeutic option for exotic species housed in zoo collections, with the potential to minimize repeated handling, injection, and anesthetic events, and circumvent non-compliance in food-averse patients (Bertelsen et al., 2010). However, its properties and efficacy can vary widely with taxon, necessitating rigorous evaluation in the target species to demonstrate its effectiveness and justify its use (García-Párraga et al., 2016; Lee et al., 2016; Nardini et al., 2014; Steeil et al., 2014; Sypniewski, Maxwell, Murray, Brandão, and Papich, 2017; Thuesen, Bertelsen, Brimer, and Skaanild, 2009). Cefovecin use has been reported in a variety of non-domestic felids – including caracals, tigers, lions, an ocelot, lynx, and bobcats – with minimal to no adverse effects reported (Bertelsen et al., 2010; DeFrancisco and Stern, 2013; Devesa-Garcia, Bañeres-De la Torre, Cabezas-Salamanca, Lucas-Lucas, and Rodriguez-Quiros, 2016; Marti et al., 2016; Mejia-Fava et al., 2015; Sadler et al., 2016; Schrader, Whiteside, Slater, and Black, 2012; Silva et al., 2013; Steeil, Schumacher, Seibert, and Tobias, 2012). In exotic felids, its uses have included conservative treatment of pyothorax, adjunctive therapy for septic peritonitis subsequent to pyometra, and post-operative antimicrobial prophylaxis. However, species-specific pharmacokinetic data are comparatively scarce, heretofore limited to only African lions and tigers (subfamily Pantherinae) among exotic felids (Cushing et al., 2017; Flaminio et al., 2019). In cheetahs, cefovecin has been used empirically without prior supportive pharmacokinetic evidence (Alves et al., 2018; Hartman et al. 2015; Stagegaard et al. 2017).

Species-specific pharmacokinetic information is needed to establish appropriate dosing intervals for the target species. Here, we provide pharmacokinetic data to support the efficacy and use of cefovecin in cheetahs (*Acinonyx jubatus*) (subfamily Felinae).

## 2. Materials and Methods

### 2.1 Animals

Adult cheetahs housed at six zoological institutions throughout the United States were selected based on temperament and tractability for training, behavioral restraint, hand injections, and phlebotomy. Each individual had been trained in advance using positive reinforcement to tolerate intramuscular injections and venipuncture, a common practice in zoos to facilitate regular veterinary procedures. The study population consisted of 12 male and 6 female cheetahs, ranging in age from 1–12 years (mean  $5.1 \pm 3.5$  SD). Weights ranged from 33.5–53.6 kg (mean  $44.3 \pm 5.7$  SD). One adult male was receiving benazepril for chronic renal disease, but all other selected animals were apparently healthy and were not receiving any additional medications during the course of the study. Animal handling and sampling protocols were reviewed and approved by the Institutional Animal Care and Use Committee for Smithsonian's National Zoological Park (protocol #19-25) and each respective holding institution.

### 2.2. Cefovecin administration and sample collection

Cefovecin (Convenia<sup>®</sup>, Zoetis Inc., Kalamazoo, MI 49007, USA) was reconstituted to 160 mg/ml. With the aid of prior behavioral training, each cheetah was isolated in a smaller area of their enclosure or a smaller apparatus like a squeeze cage, and administered a single dose of cefovecin (8 mg/kg) intramuscularly (IM) in the hindquarters. One animal moved at the time of administration, but received the full dose in two injections; otherwise, no problems with cefovecin delivery were encountered. Subsequently, 1.5–3.0 ml blood was collected from the lateral coccygeal vein into green-top heparin collection tubes (BD Vacutainer<sup>®</sup>, Franklin Lakes, NJ 07417, USA) at 12 specified timepoints post-administration: 30 mins, 60 mins, 90 mins, 2 hrs, 6 hrs, 24 hrs, 72 hrs, 5 days, 7 days, 10 days, 12 days, and 15 days. One sample was inadvertently collected as serum, but was included in the analysis. Because repeated sampling would be stressful and unpractical in these animals, we used a sparse-sampling design with the goal of obtaining at least three samples per individual cheetah, plus a sample obtained prior to drug administration from two animals. The sampling times were spread out over a grid covering 0 to 360 hours (15 days) to account for at least three samples per timepoint. Due to variability in compliance, each individual contributed between 1–7 blood samples. The final collection yielded a range of 2–9 samples per time point (median 5). Within one hour of collection, samples were centrifuged for 15 mins at 1000–2000 g, and the resultant plasma collected and stored at -80 – -40 °C until shipped to North Carolina State University for analysis. A total of 61 samples across 12 timepoints was collected and analyzed. Cheetahs were monitored closely by husbandry staff for any adverse effects following cefovecin administration and sample collection. Additionally, banked cheetah plasma collected and stored from previous unrelated veterinary events was also used as blank samples.

### 2.3 Plasma cefovecin assays

Plasma cefovecin concentrations were determined by high-pressure liquid chromatography (HPLC) paired with ultraviolet (UV) detection with a method previously validated in the investigator's laboratory for another study (Messenger and Papich, 2013). Because the assay was previously validated for a canine study, we performed a partial validation for this study in cheetahs by fortifying blank (control) cheetah plasma with nominal concentrations of cefovecin to ensure that the assay met our acceptance criteria for this study.

### 2.4 Pharmacokinetic analysis

A naïve averaged pooled analysis using a one-compartment model was used to obtain initial estimates. From these initial estimates, and using a single bolus input, the non-linear mixed effects (NLME) model was fitted to these data (Phoenix NLME<sup>™</sup> version 8.4, Certara Inc., St. Louis, Missouri 63105, USA). Compartmental analysis of the data from the cefovecin injection in cheetahs was calculated using a 1-compartment model according the following formula:

$$C(T) = \frac{D}{V} \times e^{(-K_{10} \times T)} \quad (1)$$

where  $C$  is the cefovecin concentration,  $D$  is the dose,  $V$  is the apparent volume of distribution,  $K_{10}$  is the elimination rate constant, and  $T$  is time. Secondary parameters calculated include the elimination half-life ( $T_{1/2}$ ), area-under-the-curve (AUC), systemic clearance (Cl), and mean residence time (MRT).

Various models were tested with different error structures to determine the best fit base model. The final model was selected after an examination of the visual predictive plot, and examination of the plots of the conditional weighted residuals (CRWES) vs independent variable (time) and vs the predicted concentrations. The models were parameterized as described above after testing other models. The models were run with the Quasi-Random Parametric Expectation Maximization (QRPEM) engine in Phoenix. Model selection was based on goodness of fit plots, diagnostic plots of residuals, scatter plots of predicted vs. observed values, and statistical significance between models using -2LL (twice the negative log likelihood), Akaike information Criterion (AIC), obtained in Phoenix, and CV% of parameter estimates. Inter-individual (between subject) variability (variance of a parameter among different subjects) was expressed using an exponential error model according to the equation:

$$P_i = \theta P \times \exp(\eta_i P) \quad (2)$$

where  $P$  is the pharmacokinetic parameter of interest for the individual  $i$ ,  $\theta P$  is  $\theta$  (theta), or the typical value (fixed effect) for the population estimate of the parameter of interest, and  $\eta_i P$  is the  $\eta$  (eta, random effect) for the inter-individual (between subject) differences of the parameter of interest. The  $\eta$  values were assumed to be independent and have a normal distribution with a mean of zero and variance of  $\omega^2$ . A multiplicative model described the residual random variability ( $\varepsilon$ ) of the data, where  $\varepsilon$  is the residual intra-subject (within subject) variability with a mean of zero and a variance of  $\sigma^2$ , according to the equation:

$$C_{\text{obs}} = C_{\text{pred}} \times (1 + \varepsilon_i) \quad (3)$$

where  $C_{\text{obs}}$  is the observed concentration for the individual, and  $C_{\text{pred}}$  is the model predicted concentration plus the error value ( $\varepsilon$ ).

## 2.5 Protein-binding analysis

Degree of protein-binding was determined using methods previously used in our laboratory for cephalosporin antibiotics (Papich, et al. 2010). Three replicate blank cheetah plasma samples were fortified with a stock solution of cefovecin prepared from a reference standard to 50  $\mu\text{g/mL}$ . The percent protein binding was calculated based on the following equation:

$$\text{Percentage bound} = \frac{\text{Total concentration} - \text{unbound concentration}}{\text{Total concentration}} \times 100 \quad (4)$$

## 3. Results

### 3.1 Effects of cefovecin on study animals

In the immediate 10–15 mins following drug administration, animals were monitored for indications of allergic reactions and anaphylaxis, but none were observed. Animals were also observed over the course of the study for other clinical signs related to antibiotic administration, including anorexia, lethargy, or antibiotic-associated diarrhea. Cefovecin appeared well-tolerated in the study population, with no adverse effects observed following administration. One individual in the study experienced recurrent phlebitis following serial venipuncture but no other complications.

### 3.2 Pharmacokinetics and protein-binding

Cefovecin plasma concentration-time curves were plotted (Fig. 1), and select population-based cefovecin pharmacokinetic parameters are depicted in Table 1. Following IM administration, cefovecin achieved a maximum mean plasma concentration of  $97.58 \pm 39.64 \mu\text{g/mL}$  at approximately two hours post-injection. This was followed by a gradual decline in mean plasma concentrations for the duration of the collection period. Cefovecin was detected at levels  $> 7 \mu\text{g/mL}$  for the duration of the collection period (15 days post-administration), with a mean concentration of  $12.70 \pm 4.32 \mu\text{g/mL}$  at the final timepoint (Table 2). However, a high degree of variability was observed in this study as observed in the Figure 1 spaghetti plots. We attempted to explore the source of the variability using a covariate analysis and including, age, sex, and dates of collection as covariates; however, addition of these covariates did not show a significant effect in our analysis. We were able to identify significant differences associated with the site (zoo) where samples were collected, but all zoos used the same protocol. Thus, we were unable to identify the source of variation in our population analysis. Cefovecin was found to be highly protein-bound in cheetah plasma, at approximately 99.9%.

## 4. Discussion

The present study provides evidence for the long-acting nature of cefovecin in cheetahs and supports its use in this species. Cefovecin was highly protein-bound in plasma (99.9%), which is almost identical to domestic cats at a similar concentration (Stegemann, et al. 2006c). High protein binding accounts for slow renal clearance and a long terminal half-life (Stegemann et al. 2006c; Valitutto et al. 2011). The bound fraction also functions as a reservoir that slowly releases that active unbound drug over time. In our study, cefovecin had a long elimination half-life of 144.1 hrs (approximately six days) based on the typical value from our NLME model.

For cephalosporins, the therapeutic efficacy against microbial pathogens is time-dependent, and the duration that free drug concentrations in plasma and other biological fluids exceed the MIC of the targeted pathogen is used as a predictor of efficacy (Craig, 1995; Papich, 2014; Stegemann et al. 2006c). Throughout our 15-day study period, the cheetahs in our population maintained plasma cefovecin concentrations  $> 7 \mu\text{g/mL}$ , with a mean plasma concentration of  $12.70 \pm 4.32 \mu\text{g/mL}$  at the 15-day study endpoint. These concentrations represent both bound and unbound concentrations and were above the concentrations designated as effective for 90% of the feline *Staphylococcus intermedius* and *Pasteurella multocida* (0.06–0.25  $\mu\text{g/ml}$ ) isolates in domestic cats (Stegemann et al. 2006a). Although these concentrations appear to persist for up to seven days in our population, we have not specified a dose interval from these observations because in most instances this agent would likely be administered only as a single dose.

Cefovecin had a high protein binding in our population of cheetahs, as it does in domestic cats. Only the free drug concentration is microbiologically active. In domestic cats, the free (unbound) concentrations in tissues were higher than in plasma (Stegemann et al. 2006c). We cannot confirm that this also occurs in cheetahs without further undue study, but it is a likely possibility. If cefovecin disposition is similar in cheetahs and domestic cats, unbound cefovecin concentrations may be over 3 times greater in transudate compared to plasma 8 hrs post-injection to the end of the sampling interval (Stegemann et al. 2006c). Sampling the transudate fluid in zoo-housed cheetahs presents significant challenges and this observation from domestic cats could not be confirmed in our cheetah population. We cannot confirm the clinical efficacy of cefovecin for treating infections caused by susceptible bacteria without further study in clinical patients.

Compared to other felids, the pharmacokinetic profile of cefovecin in cheetahs appears to be intermediate between domestic cats and lions (Table 3). In cheetahs, the peak plasma cefovecin concentration ( $C_{\text{max}}$ ), elimination half-life ( $T_{1/2}$ ), and MRT were greater than values for lions, but less than those for domestic cats. Because the extent of protein-binding was highly similar among all three species, it is unlikely that this was a source of the observed differences from other felids. In all three species, animals were awake when blood samples were collected; therefore, there were no confounding effects of anesthesia. However, the route of administration differed, IM in cheetahs and SC in domestic cats and lions, which may contribute to the rate and extent of absorption, but not the elimination.

Our study showed the large variation in pharmacokinetic profiles in a population of zoo-housed animals. Compared to small groups of uniform research animals used for most pharmacokinetic studies (often with six animals per group), we observed high variability in a larger population of animals maintained at different zoological facilities (Fig. 1). By accounting for interindividual (between subject) variability in our population, we were able to identify the typical pharmacokinetic variables for our population (fixed effect in our model) (Table 1), which improved the fit for the pharmacokinetic model (Fig. 1, right side plot). The highest variation among our population occurred at the 2-hr timepoint, where the highest plasma cefovecin concentration recorded for an individual was  $150.3 \mu\text{g/mL}$ , higher than the timepoint mean ( $97.58 \pm 39.64 \mu\text{g/mL}$ ) and the peak calculated from the model ( $C_{\text{max}} 84.75 \mu\text{g/mL}$ ).

In order to explore sources of interindividual variability in our population, we examined covariates to identify possible factors that could explain the variability in pharmacokinetic parameters. No significant effects of demographics (i.e. sex or age) were found, but categorical grouping by location (housing institution) and dates of sampling yielded three distinct and significant groupings. However, because all zoos used the same protocol for drug administration and blood sampling for their cheetahs, without additional undue analysis, this study did not explain why these factors alone would result in such variation in cefovecin pharmacokinetics. Other individual-level factors such as variation in physical activity level post-injection, local massage at the injection site, or differences in muscle groups injected may affect circulation to the targeted tissues and thus absorption of the drug. However, because these factors were not monitored in our study, the contribution of these factors to the observed variability is undetermined. Inter-individual variability in cefovecin disposition was also observed in a pharmacokinetic study in tigers, but similarly remained unexplained (Cushing et al. 2017).

We used a dose of 8 mg/kg because it is the approved label dose for domestic cats, but other investigators used different doses. Flaminio et al. suggested that a 4 mg/kg dose in African lions was sufficient to maintain efficacious plasma concentrations for 14 days (2019), while Cushing et al. demonstrated that plasma cefovecin levels exceeded 3.1 µg/mL for at least 49 days in tigers (2017). Our study reported here was not designed to examine the effects of different doses on the pharmacokinetics; therefore, the effect of other doses, routes of administration or sample collection duration in these cheetahs is undetermined. Additionally, culture and susceptibility testing are recommended prior to treatment whenever possible, to identify if the target pathogen is susceptible to cefovecin using clinical breakpoints published for domestic cats (CLSI, 2020).

### **Acknowledgements**

The authors are greatly thankful for the assistance of all animal husbandry, behavior, and veterinary staff who were vital to the completion of this project. Thanks are also due to Zoetis, who provided cefovecin for this study. The authors thank Delta R. Dise of the North Carolina State University Clinical Pharmacology Laboratory for her assistance with sample analysis. Additional support for this project and manuscript preparation was provided by the Smithsonian Institution, the Morris Animal Foundation and Dennis and Connie Keller through a training partnership, and James and Jamie Coss. This content has not been reviewed or endorsed by the Morris Animal Foundation, and the views expressed herein do not necessarily reflect the views of the Foundation, its officers, directors, affiliates, or agents.

### **Conflict of Interest**

One of our authors (MGP) has received gifts, honoraria, and research support from Zoetis, the sponsor of cefovecin (Convenia®). Zoetis has also provided cefovecin used in this study.

### **Author Contributions**

JHY, MGP, and SM contributed to study design, coordination, and project management, with input from KH and AC. SM and MGP contributed to fund acquisition. RGT, JE, MK, CRS, KH, and AC contributed to coordination, supervision, and collection of samples. MGP was responsible for drug and data analysis and pharmacokinetic analysis. JHY and MGP led manuscript preparation and editing. All authors have reviewed and approved the final manuscript.

### **Animal Welfare and Ethics Statement**

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The authors confirm that they have adhered to US standards for the protection of animals used for scientific purposes.

### **References**

1. Alves, S.E., Joyner P.H., Aitken-Palmer, C., Crosier, A.E., & Ware, L. (2018). Full-term pregnancy with vaginal birth following dystocia and caesarean section in two cheetahs (*Acinonyx jubatus*). *Veterinary Record Case Reports*, 6(2), 1–5.
2. Bertelsen, M.F., Thuesen, L.R., Bakker, J., Hebel, C., Grøndahl, C., Brimer, L., & Skaanild, M.T. (2010). Limitations and usages of cefovecin in zoological practice. *Proceedings of the International Conference of Diseases of Zoo and Wild Animals*, 140–141.
3. Burke, S., Black, V., Sánchez-Vizcaíno, F., Radford, A., Hibbert, A., & Tasker, S. (2017). Use of cefovecin in a UK population of cats attending first-opinion practices as recorded in electronic health records. *Journal of Feline Medicine and Surgery*, 19, 687–692.
4. CLSI. *Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals*. 5th ed. CLSI supplement VET01S. Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA, 2020.

5. Cox, S., Martin, A., Hamill, M., Pistole, N., Steeil, J., & Schumacher, J. (2014). Development of a method for the determination of cefovecin in plasma by HPLC. *Biomedical Chromatography*, 28, 1126–1130.
6. Craig, W.A. (1995). Interrelationship between pharmacokinetics and pharmacodynamics in determining dosage requirements for broad-spectrum cephalosporins. *Diagnostic Microbiology and Infectious Disease*, 22, 89–96.
7. Cushing, A.C., Ramsay, E.C., Steeil, J., & Cox, S. (2017). Pharmacokinetic parameters of cefovecin sodium (Convenia) in captive tigers (*Panthera tigris*). *Journal of Zoo and Wildlife Medicine*, 48, 1188–1192.
8. DeFrancisco, A. & Stern, A.W. (2013). Bilateral thyroid follicular atrophy in a young Canadian lynx (*Lynx canadensis*). *Journal of Veterinary Science and Medical Diagnosis*, 2, 1000108.
9. Devesa-García, N., Bañeres-De la Torre, A., Cabezas-Salamanca, M.A., Lucas-Lucas, N., & Rodríguez-Quiros, J. (2016). Surgical correction of traumatic patellar luxation in an Eurasian lynx (*Lynx lynx*). *Journal of Zoo and Wildlife Medicine*, 47, 890–894.
10. Dokuzeylül, B., Kahraman, B.B., Bayrakal, A., Siğirci, B.D., Çelik, B., İkiz, S., Kayar, A., & Or, M.E. (2015). Bacterial species isolated from cats with lower urinary tract infection and their susceptibilities to cefovecin. *Irish Veterinary Journal*, 68, 2.
11. Flaminio, K.P., Christensen, J.M., Alshahrani, S.M., & Mohammed, S.M. (2019). Pharmacokinetics after subcutaneous administration of a single dose of cefovecin sodium in African lions (*Panthera leo*). *American Journal of Veterinary Research*, 80, 230–234.
12. García-Párraga, D., Gilabert, J.A., García-Peña, F.J., Álvaro, T., Ros-Rodríguez, J.M., Vallis M., & Encinas, T. (2016). Long-lasting concentrations of cefovecin after subcutaneous and intramuscular administration to Patagonian sea lions (*Otaria flavescens*). *The Veterinary Journal*, 208, 65-69.
13. Hartman, M.J., Kirberger, R.M., Tordiffe, A.S.W., Boy, S., & Schoeman, J.P. (2015). Laparoscopic removal of a large abdominal foreign body granuloma using single incision laparoscopic surgery (SILS) and extraction bag in a cheetah (*Acinonyx jubatus*). *Veterinary Record Case Reports*, 3(1), 1–5.
14. Lee, E.A., Byrne, B.A., Young, M.A., Murray, M., Miller, M.A., & Tell, L.A. (2016). Pharmacokinetic indices for cefovecin after single-dose administration to adult sea otters (*Enhydra lutris*). *Journal of Veterinary Pharmacology and Therapeutics*, 39, 625-628.
15. Marti, I., Pisano, S.R.R., Wehrle, M., Meli, M.L., Hofmann-Lehmann, R., & Ryser-Degiorgis, M-P. (2019). Severe conjunctivitis associated with *Chlamydia felis* infection in a free-ranging Eurasian lynx (*Lynx lynx*). *Journal of Wildlife Diseases*, 55, 522–525.
16. Mejia-Fava, J., Mayer, J., Divers, S.J., Cohen, E.B., Schmiedt, C., & Holmes, S.P. (2015). Focused assessment with sonography as an aid for the diagnosis of gastrointestinal perforation in a bobcat (*Felis rufus*). *Journal of Zoo and Wildlife Medicine*, 46, 921–924.
17. Messenger K, Papich M. [Abstract P-2] The Influence of Cefovecin on The Enantioselective Pharmacokinetics Of Carprofen In Dogs. *Journal of Veterinary Internal Medicine*. 2013 May;27(3).
18. Nardini, G., Barbarossa, A., Dall’Occo, A., Di Girolamo, N., Cagnardi, P., Magnone, W., Bielli, M., Roncada, P., & Zaghini, A. (2014). Pharmacokinetics of cefovecin sodium after subcutaneous administration to Hermann’s tortoises (*Testudo hermanni*). *American Journal of Veterinary Research*, 75, 918–923.
19. Papich MG. Pharmacokinetic–pharmacodynamic (PK–PD) modeling and the rational selection of dosage regimes for the prudent use of antimicrobial drugs. *Veterinary Microbiology*. 2014 Jul 16;171(3-4):480-6.
20. Papich MG, Davis JL, Floerchinger AM. Pharmacokinetics, protein binding, and tissue distribution of orally administered cefpodoxime proxetil and cephalixin in dogs. *American Journal of Veterinary Research*. 2010 Dec;71(12):1484-91.



21. Passmore, C.A., Sherington, J., & Stegemann, M.R. (2008). Efficacy and safety of cefovecin for the treatment of urinary tract infections in cats. *Journal of Small Animal Practice*, 49, 295–301.
22. Sadler, R., Schumacher, J., Ramsay, E., McCleery, B., Baine, K., Thomas, W., Nobrega-Lee, M., Henry, G.A., & Newman, S.J. (2016). Progressive syringohydromyelia and degenerative axonopathy in a bobcat (*Lynx rufus*) following surgical correction of a Chiari-like malformation. *Journal of Zoo and Wildlife Medicine*, 47, 329–332.
23. Schrader, G.M., Whiteside, D.P., Slater, O.M., & Black, S.R. (2012). Conservative management of pyothorax in an Amur tiger (*Panthera tigris altaica*). *Journal of Zoo and Wildlife Medicine*, 43, 425–429.
24. Silva, R.O.S., D'elia, M.L., de Magalhães Soares, D.F., Cavalcanti, Á.R., Leal, R.C., Cavalcanti, G., Lithg Pereira, P.L., & Faria Lobato, F.C. (2013). *Clostridium difficile*-associated diarrhea in an ocelot (*Leopardus pardalis*). *Anaerobe*, 20, 82–84.
25. Stagegaard, J., Kurth, A., Stern, D., Dabrowski, P.W., Pocknell, A., Nitsche, A., & Schrick, L. (2017). Seasonal recurrence of cowpox virus outbreaks in captive cheetahs (*Acinonyx jubatus*). *PLoS ONE*, 12(11), e0187089.
26. Steeil, J.C., Schumacher, J., George, R.H., Bulman, F., Baine, K., Cox, S. (2014). Pharmacokinetics of cefovecin (Convenia®) in white bamboo sharks (*Chiloscyllium plagiosum*) and Atlantic horseshoe crabs (*Limulus polyphemus*). *Journal of Zoo and Wildlife Medicine*, 45, 389–392.
27. Steeil, J., Schumacher, J., Seibert, R., & Tobias, K. (2012). Cefovecin (Convenia) for the treatment of septic peritonitis in a female lion (*Panthera leo*). *Journal of Zoo and Wildlife Medicine*, 43, 678–681.
28. Stegemann, M.R., Passmore, C.A., Sherington, J., Lindeman, C.J., Papp, G., Weigel, D.J., & Skogerboe, T.L. (2006a). Antimicrobial activity and spectrum of cefovecin, a new extended-spectrum cephalosporin, against pathogens collected from dogs and cats in Europe and North America. *Antimicrobial Agents and Chemotherapy*, 50, 2286–2292.
29. Stegemann, M.R., Sherington, J., & Blanchflower, S. (2006b). Pharmacokinetics and pharmacodynamics of cefovecin in dogs. *Journal of Veterinary Pharmacology and Therapeutics*, 29, 501–511.
30. Stegemann, M.R., Sherington, J., & Passmore, C. (2007). The efficacy and safety of cefovecin in the treatment of feline abscesses and infected wounds. *Journal of Small Animal Practice*, 48, 683–689.
31. Stegemann, M.R., Sherington, J., Coati, N., Brown, S.A., & Blanchflower, S. (2006c). Pharmacokinetics of cefovecin in cats. *Journal of Veterinary Pharmacology and Therapeutics*, 29, 513–524.
32. Sypniewski, L.A., Maxwell, L.K., Murray, J.K., Brandão, J.L., Papich, M.G. (2017). Cefovecin pharmacokinetics in the red-eared slider. *Journal of Exotic Pet Medicine* 26: 108–113.
33. Thuesen, L.R., Bertelsen, M.F., Brimer, L., Skaanild, M.T. (2009). Selected pharmacokinetic parameters for cefovecin in hens and green iguanas. *Veterinary Pharmacology and Therapeutics* 32: 613–617.
34. Valitutto, M., Raphael, B.L., Calle, P.P., Sykes, J.M., Moore, R.P., & Papich, M.G. (2011). Protein-binding of cefovecin (Convenia®) in 25 zoological species: a predictor for extended duration of action. *Proceedings of the 42nd American Association of Zoo Veterinarians (AAZV) Conference, Kansas City, MO, USA*: 48.

## Tables

**Table 1.** Pharmacokinetic parameters of cefovecin in cheetahs, as determined by non-linear mixed effects modeling. Typical values for the population ( $\theta$ , theta) are depicted, as well as the following parameters: V, volume of distribution;  $K_e$ , elimination rate constant; AUC, area under the drug concentration curve;  $C_{max}$ , peak plasma cefovecin concentration; Cl, systemic clearance; MRT, mean residence time; and  $T_{1/2}$ , elimination half-life. Note that because the injection was intramuscular instead of intravenous, the V and Cl are expressed as per fraction absorbed (V/F and Cl/F, respectively).

Parameter	Value	Units
$\theta V$	0.094	L/kg
$\theta K_e$	0.005	1/hr
AUC	17618.906	$\mu\text{g}\cdot\text{hr}/\text{mL}$
$C_{max}$	84.747	$\mu\text{g}/\text{mL}$
Cl	0.454	$\text{mL}/\text{kg}/\text{hr}$
MRT	207.899	hr
$T_{1/2}$	144.105	hr

**Table 2.** Mean plasma cefovecin concentrations at each timepoint post-injection.

Time	n	Mean Total Plasma Cefovecin ( $\mu\text{g}/\text{mL}$ )
0.5 hr	3	57.44 $\pm$ 11.15
1 hr	2	64.82 $\pm$ 12.71
1.5 hr	3	72.61 $\pm$ 3.54
2 hr	4	97.58 $\pm$ 39.64
6 hr	5	52.98 $\pm$ 10.75
24 hr	5	53.68 $\pm$ 15.11
72 hr	9	67.79 $\pm$ 28.10
5 days	5	39.27 $\pm$ 21.89
7 days	9	50.98 $\pm$ 23.53
10 days	8	32.38 $\pm$ 16.29
12 days	5	18.25 $\pm$ 4.81
15 days	5	12.70 $\pm$ 4.32

**Table 3.** Comparative pharmacokinetics of cefovecin (dosed at 8 mg/kg) in domestic and exotic felids.

<b>Parameter (Units)</b>	<b>Domestic cats (<i>Felis catus</i>) Stegemann et al. 2006c</b>	<b>Tiger (<i>Panthera tigris</i>) Cushing et al. 2017</b>	<b>African lion (<i>Panthera leo</i>) Flaminio et al. 2019</b>	<b>Cheetah (<i>Acinonyx jubatus</i>) Present study</b>
Route	SC	IM	SQ	IM
AUC ( $\mu\text{g}\cdot\text{hr}/\text{mL}$ )	22,700 $\pm$ 3450	64,463.3 $\pm$ 12,409.5	1,855.5 $\pm$ 276.3	17,618.906
C <sub>max</sub> ( $\mu\text{g}/\text{mL}$ )	141 $\pm$ 12	214.2 $\pm$ 81.4	18.35 $\pm$ 0.94	84.747
T <sub>max</sub> (hr)	2.0 $\pm$ 2.0	22.3 $\pm$ 58.9	4.00 $\pm$ 0	N/A*
MRT (hr)	256	301.1 $\pm$ 50.1	153.5 $\pm$ 49.9	207.899
T <sub>1/2</sub> (hr)	166 $\pm$ 18	227.8 $\pm$ 29.3	115.1 $\pm$ 35.7	144.105
Protein-binding	99.8%	98%	99%	99.9%

\* T<sub>max</sub> not able to be calculated in this present study.

## Figure Legends

**Figure 1.** Plots of ceftiofur plasma concentrations in cheetahs over time. Left panel shows individual cheetahs with model fitted to the data. Right panel shows all cheetahs fitted to the model after incorporation of random effects.

