Aspergillosis is a major cause of mortality in captive birds and its prognosis is often poor due to treatment failure. Voriconazole is a novel triazole antifungal agent that may be useful for the treatment of this infection in birds as it has shown promise in other animal models of the disease. We examined the pharmacokinetic behaviour of voriconazole in racing pigeons (Columbia livia forma domestica). Intravenous, oral and aerosol administration were investigated in single (10 mg/kg BW PO; 10, 5, 2.5 mg/kg BW IV), multiple dose (10, 20 mg/kg BW PO q12h, q24h) and nebulization (15 min, 10 mg/ml NaCl 0.9%) experiments. Quantitative measurements of voriconazole in plasma, as well as in lung tissue, collected at several time points, were done with a validated high performance liquid chromatography method using ultraviolet detection. Designing a treatment schedule with voriconazole is complicated by dose-dependent pharmacokinetics and induction of its biotransformation. Moreover, hepatic changes were seen in the oral multiple dose regimen at 10 and 20 mg/kg BW twice a day. Taking all features into account our study suggests that the oral dosage schedules of 10 mg/kg BW twice a day or 20 mg/kg BW once a day could be most appropriate in treating pigeons with aspergillosis.

Keywords voriconazole, dose-dependent pharmacokinetics, antifungal treatment Aspergillus, avian
parrots (10 mg/kg BW PO q24h) and in falcons (12.5 mg/kg BW PO q12h for three days, then q24h for 18 to 87 days and 12.5 mg/kg BW PO q12h) [7–10]. Pharmacological studies of voriconazole have been conducted with chickens, falcons and African grey parrots [10–12]. None of these studies compared different routes of administration or voriconazole concentration in the lungs in addition to the plasma concentration.

The purpose of our study was to determine the pharmacokinetics of voriconazole in plasma and lung tissue of racing pigeons after intravenous, oral and aerosol administration in order to design a suitable treatment schedule for aspergillosis in racing pigeons as a model species of birds.

**Materials and methods**

**Chemicals**

The oral (tablets) and intravenous formulation of voriconazole (Vfend, Pfizer Global Pharmaceuticals,Ixelles, Belgium) were used for all experiments. The tablets were crushed into a fine powder, suspended in water and given by crop gavage using a 2.5 ml syringe and a 14 G 2 catheter. The powder residue in the syringe was suspended twice with water to maximize the precision of the dose. The total application volume was approximately 1.5 ml.

Pure voriconazole was used for the quantitative measurements of voriconazole in plasma and lung samples, with hydroxy-itraconazole employed as the internal standard. Solvents of high performance liquid chromatography (HPLC) grade were obtained from VWR (Leuven, Belgium) and Merck (Darmstadt, Germany) for use in HPLC analysis.

**Animals**

Clinically healthy adult male and female racing pigeons (*Columbia livia* forma domestica) (0.4–0.5 kg BW) received voriconazole in several experiments. During the studies, each bird was housed individually, in rooms maintained at 20–22°C with a 12-hour photoperiod. The birds received a commercial pigeon diet ad libitum and had free access to fresh drinking water. In the oral single dose experiment, food was withheld 12 h before administration of voriconazole.

**Salmonella spp. and endoparasites**

Prior to all tests, excreta were collected for five days from each pigeon (twice with two-week interval) and mixed. Bacteriological analysis was performed using direct plating on brilliant green agar and enrichment on buffered peptone water/brilliant green tetrathionate broth (BPW/BGA, Oxoid Ltd., Basingstoke, England). Parasitological analysis was performed using a saturated salt solution in water and microscopic examination. Serum samples were taken for the presence of agglutinating antibodies to *Salmonella* serovar Typhimurium. All tests were negative for the presence of *Salmonella* spp. and endoparasites ova.

**Collection of blood and lungs**

Blood (1 ml) was collected in heparin coated tubes (Microvette®, Sarstedt, Nümbrecht, Germany) from the medial metatarsal vein and centrifuged (2400 g for 10 min at room temperature) immediately after collection. Pigeons were euthanized for collecting the lungs by an intravenous injection of T61 (Intervet, Mechelen, Belgium) in the wing vein. Plasma and lung samples were stored at −20°C until further analysis.

All experiments were performed with the permission of the Ethical Committee of the Faculty of Veterinary Medicine, Merelbeke, Ghent University, Belgium (EC 2006/074, EC 2006/075).

**Biochemical blood parameters**

To assess the effect of voriconazole on liver and kidney parameters, the levels of aspartate aminotransferase (AST), bile acids, creatine kinase and uric acid were determined in plasma using the VetScan VS2® (Abaxis Europe, Darmstadt, Germany).

**High performance liquid chromatography of plasma and lung tissue**

Plasma concentrations of voriconazole were measured using a HPLC method with ultraviolet (UV) detection based on the method of Khoschsorur *et al*. [13]. A Thermo Separations Product (TSP, Fremont, CA, USA) HPLC system containing a P-4000 pump, model AS 3000 autosampler and a Focus Forward scanning UV-detector set at 255 nm, run by PC 1000 software, was used. The chromatographic separation was performed using a 4.6-mm x 150-mm reverse-phase column (Waters Symmetry® C18, 5μm). The mobile phase comprised 0.06% acetic acid in water/acetonitrile (45/55, v/v). An isocratic solvent program was run. The flow rate was 0.5 ml/min.

Samples were prepared by pipetting 250 μl plasma into a 5-ml screw-capped tube, followed by the addition of 25 μl of internal standard (hydroxy-itraconazole in methanol, 100 μg/ml) and 1.5 ml of extraction solvent hexane-isooamyl alcohol (90/10, v/v). The mixture was
vortexed briefly, extracted on a mechanical rolling device for 10 min and centrifuged at 2,500 g for 10 min. The organic layer was transferred to a clean screw-capped tube and evaporated under nitrogen at 40°C. After evaporation of the organic extract, the residue was dissolved in 250 µl of the mobile phase, vortexed and 100 µl was injected onto the HPLC column. Voriconazole eluted at approximately 6.1 min and the internal standard at 10.9 min.

This method was validated prior to the start of the analysis. The selectivity of the method was shown, since no interfering peaks from endogenous compounds in the blank pigeons plasma sample were observed with the same retention time as voriconazole and hydroxyitraconazole in the chromatograms of blank samples.

Calibration curves were prepared from a pool of blank pigeon plasma spiked with eight concentrations of voriconazole between 100–10,000 µg/l. Correlation coefficients (r) were >0.99 for calibration curves. The limit of detection was determined as three times the signal/noise ratio at the time of elution and was 78.12 µg/l. The limit of quantification was calculated as the lowest concentration for which the method is validated with trueness and precision that fall within the ranges recommended by the EU and was 100 µg/l. The accuracy was within -20 to +10% and the within-run precision was within two-thirds of the values calculated according to the Horwitz equation [R.S.D. = 2(1+0.3 log C)], where C is the concentration at which the plasma was fortified. For between-run imprecision, the maximum tolerances are equal to the values of the Horwitz equation. These ranges are specified by the European Commission [14].

Lung concentrations of voriconazole were measured using a HPLC method with UV detection based on the method of Koks et al. [15] and the same HPLC system as for the plasma samples.

Lung samples were homogenized first. One gram of each sample was fortified with 25 µl of internal standard (hydroxyl-itraconazole in methanol, 100 µg/ml), followed by the addition of 3 ml of phosphate buffer (pH 8.0) and 8 ml diethylether. The samples were vortexed for 15 sec, introduced in an ultrasonic bath for 5 min, shaken for 10 min and centrifuged at 3,500 g for another 10 min. The organic layer was transferred to a clean screw-capped tube and evaporated to dryness at 40°C under a gentle nitrogen stream. After dissolving the dry residue in 3 ml 0.4 M aqueous hydrochloric acid, the acidified layer was brought onto a strong cation exchange (SCX) solid phase extraction column (Isolute SCX 100 mg/3 ml, International Sorbent Technology LTD, Hengoed, UK), after subsequently conditioning it with 3 ml of methanol and 3 ml of 1.1% (v/v) perchloric acid in water (pH 1.5). The samples were allowed to slowly pass through the sorbent bed. Afterwards the column was washed with 3 ml of 0.01 M hydrochloric acid in methanol, 3 ml of 0.1 M phosphate buffer (pH 6.0) and 3 ml of distilled water. The column was dried for 10 min under vacuum. The components were eluted off the column with 3 ml of methanol. The elution solvent was evaporated to dryness at 40°C under a gentle nitrogen stream. The dry residue was dissolved in 250 µl of the mobile phase and 100 µl injected onto the HPLC system.

No interfering peaks from endogenous compounds in the blank pigeons lung were observed, although a peak occurred with a retention time of 5.3 min closely before the retention time of voriconazole. The peak did not interfere with the analysis. Calibration curves were prepared from a homogenized pool of blank pigeon’s lung spiked with six concentrations of voriconazole between 250–2000 µg/l. Correlation coefficients (r) were >0.99 for calibration curves. The limit of detection was 42.53 µg/l and the limit of quantification was 250 µg/l. The accuracy precision was within the required ranges [14].

**Single dose experiments**

Twenty-four pigeons were used in a cross over design receiving voriconazole intravenously and orally (10 mg/kg BW) with a wash out period of 14 days. Blood was taken before administration (blank), at 15 and 30 min and at 1, 1.5, 2, 4, 8, 12, 24 and 48 h after administration.

Fifteen pigeons were given voriconazole intravenously at a dose of 5 mg/kg BW and fifteen pigeons at a dose of 2.5 mg/kg BW. Blood was taken at 0.5, 1, 2, 4, 6, 8, 10, 12, 16 and 24 h after administration.

Six groups of pigeons were nebulized with the intravenous formulation of voriconazole for 15 min at a concentration of 10 mg/ml NaCl 0.9%. The birds were placed in a closed 25 × 30 × 47 cm box and nebulised using a PARI LC plus™ nebuliser driven by PARI turboboy N® compressor (mass median diameter 3.5 µm; 68% <5 µm) (Pari GmbH, Starnberg Germany). After nebulization blood was collected after 0, 0.5, 1.5, 2, 4, 6, 10, 12, 24 and 30 h and their lungs were collected after 0, 1, 6, 10, 24 and 30 h.

**Multiple dose experiments**

Twelve pigeons received voriconazole orally q24h for three days at a dose of 10 mg/kg BW and 12 pigeons received voriconazole orally q24h for 10 days at a dose of 20 mg/kg BW. Blood was collected prior to
administration (C_{min}) and 2 h after administration (C_{max}, selected from oral single dose experiment (Table 1)). At every time point blood was taken from six pigeons. In the first multiple dose experiment (10 mg/kg BW PO q24h), six pigeons were euthanized 2 h after the final administration and 24 h after the final administration to measure the concentration of voriconazole in the lungs at C_{max} and C_{min}. After the final administration in the second multiple dose experiment (20 mg/kg BW PO q24h), blood was collected of six pigeons to measure AST and uric acid values.

Twelve pigeons received voriconazole orally q12h for four days at a dose of 10 mg/kg BW and 12 pigeons received voriconazole orally q12h for four days at a dose of 20 mg/kg BW. Blood was collected prior to administration (C_{min}) and 2 h after administration (C_{max}). At every time point blood was taken of four pigeons. Prior to euthanasia blood was taken to measure AST and uric acid values. Ten to 12 h after the final administration pigeons were euthanized and necropsied. Lungs were collected for HPLC analysis. Samples from the liver were fixed in phosphate buffered formaldehyde solution, sectioned and stained with Haematoxylin & Eosin (H&E) and Von Gieson’s staining.

Pharmacokinetic calculations

Analyses were performed on the average data sets. The pharmacokinetic parameters were calculated with a computer program (Winnonlin Version 5.01 (Pharmasight), Mountain View, CA, USA) using both compartmental and non-compartmental methods. In the compartmental analysis, the best fitting model was selected based on curve appearance and on a smaller value for the Akaike information criterion [16]. The following equation was used to describe the concentration-time curve for voriconazole after intravenous and oral administration and nebulization: 

\[ C = A_1 e^{-\beta t} + A_2 e^{-\gamma t} \]

where \( A_1 \) and \( A_2 \) are the mathematical coefficients, \( \beta \) is the elimination rate constant, \( \gamma \) the absorption rate constant, \( t \) the time and \( A_1 \) and \( A_2 \) mathematical coefficients. The bioavailability (F) of the oral administration (10 mg/kg BW) was calculated according to formula of AUC_{PO}/AUC_{IV}, using the IV 10 mg/kg BW data. Predicted concentrations were determined in the multiple dose experiments 10 mg/kg q24h and q12h by using the non-parametric superposition tool in Winnonlin based on the pharmacokinetic parameters calculated by one compartmental analysis of the single oral bolus data.

Statistical analysis

Observed and predicted values in the steady state experiments 10 mg/kg BW q24h for three days and 10 mg/kg BW q12h for four days were statistically analyzed using One-Sample T test (statistical software program SPSS 15).

Table 1: Pharmacokinetic parameters of voriconazole after oral bolus administration (10 mg/kg BW) and intravenous bolus administration (10 mg/kg BW, 5 mg/kg BW and 2.5 mg/kg BW) calculated with one compartmental (noncompartmental) data analysis on average data (n = 6) per time point.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter (Unit)</th>
<th>PO 10 mg/kg</th>
<th>IV 10 mg/kg</th>
<th>IV 5 mg/kg</th>
<th>IV 2.5 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean dose (mg)</td>
<td>4.73 (4.73)</td>
<td>4.73 (4.73)</td>
<td>2.4 (2.4)</td>
<td>1.07 (1.07)</td>
</tr>
<tr>
<td>AUC (h × µg/l)</td>
<td>57070.04 (55081.02)</td>
<td>130780.36 (131733.42)</td>
<td>57928.16 (60229.16)</td>
<td>18824.45 (18480.92)</td>
</tr>
<tr>
<td>K01 (1/h)</td>
<td>1.52</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>K10 (1/h)</td>
<td>0.067 (0.075)</td>
<td>0.043 (0.043)</td>
<td>0.061 (0.059)</td>
<td>0.105 (0.113)</td>
</tr>
<tr>
<td>t1/2 el (h)</td>
<td>10.32 (9.29)</td>
<td>16.25 (16.18)</td>
<td>11.33 (11.82)</td>
<td>6.62 (6.15)</td>
</tr>
<tr>
<td>t1/2 ab (h)</td>
<td>0.456</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cmax (µg/l)</td>
<td>3317.87 (3649.90)</td>
<td>5576.72 (6408.57)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>2.15 (2)</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>F (%)</td>
<td>43.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CI/F (1/u × kg) oral</td>
<td>0.176 (0.18)</td>
<td>0.076 (0.076)</td>
<td>0.086 (0.083)</td>
<td>0.12 (0.12)</td>
</tr>
<tr>
<td>CI (1/u x kg) intravenous</td>
<td>2.60 (2.44)</td>
<td>1.79 (1.77)</td>
<td>1.41 (1.42)</td>
<td>1.11 (1.046)</td>
</tr>
<tr>
<td>V/F (l/kg) oral</td>
<td>3065069.87 (2955207.58)</td>
<td>946870.38 (100939.95)</td>
<td>179823.88 (165119.5)</td>
<td>16.35 (16.76)</td>
</tr>
<tr>
<td>AUMC (h × h × µg/l)</td>
<td>23.44 (22.43)</td>
<td>16.35 (16.76)</td>
<td>9.55 (8.93)</td>
<td></td>
</tr>
</tbody>
</table>

AUC: area under the curve, K01: absorption constant, K10: elimination constant, t1/2 el: elimination half life, t1/2 ab: absorption half life, Cmax: maximum plasma concentration, Tmax: time point of maximum plasma concentration, F: bioavailability, CI/F: clearance, V/F: distribution volume AUMC: area under the first moment curve, MRT: mean residence time.
Results

Single dose experiments

For the oral and intravenous single dose experiments, pharmacokinetic parameters were calculated by use of one-compartmental, as well as non-compartmental analysis (Table 1). The average plasma concentrations after the administration of 10 mg/kg BW PO and IV are shown in Fig. 1. The maximum concentration level of voriconazole in the plasma was 3,317.87 μg/l at 2.15 h after oral bolus administration (Table 1).

The average plasma concentrations of the intravenous bolus administrations at 10, 5 and 2.5 mg/kg BW are shown in Fig. 2 (Table 1). One and a half hour after nebulization, a low C_max (407.27 ± 202.53 μg/l) and a fast decrease of the plasma concentration below LOQ was seen. The average lung concentration immediately after nebulization was 412.33 μg/l (±274.82) and 1 h after nebulization below LOQ for five pigeons, but 719.12 μg/l for one pigeon.

Multiple dose experiments

Twelve pigeons received voriconazole orally q24h during three days at a dose of 10 mg/kg BW. The observed average plasma concentrations and the predicted values at C_max and C_min during this multiple dose regimen are shown in Fig. 3. The average plasma concentration at C_max declined from 3,683.65 μg/l on the first day to 2,417.83 μg/l on the third day (= a 34% decrease). The average plasma concentration at C_min declined from 418.09 μg/l after the first day to 133.99 μg/l after the third day (= a 68% decrease). The observed values (except for the value at 2 h) were significantly (p < 0.05) lower than the predicted value. The average lung concentration at C_max (2 h after the final administration) was 2,391.68 μg/l (±350.27) and at C_min (24 h after the final administration) below LOQ for five pigeons and 799 μg/l for one.

Twelve pigeons received voriconazole orally q24h during 10 days at a dose of 20 mg/kg BW. The average plasma concentrations at C_max and C_min during this multiple dosage regimen are shown in Fig. 4. The average C_max declined from 8,621.31 μg/l on the first day to 5,352.18 μg/l on the fourth day ( = a 38% decrease). The remaining period of the experiment, C_max ranged between 6861.85 μg/l (day 8) and 9182.26 μg/l (day 9). During the whole experiment average C_min was below or equal to 154.24 μg/l (±198.53) except for 1,516.29 μg/l (±1,263.09) on the second day, 775.53 μg/l (±1,239.72) on day 5 and 725.90 μg/l (±1,426.64) on day 7. After the final drug administration blood was collected from six pigeons to determine AST, bile acids, creatine kinase and uric acid. The values obtained were within reference ranges [17,18].

In the multiple dose regimen 10 mg/kg BW PO q12h the average C_max was decreasing to 2,350.94 μg/l after the final administration (Fig. 5). In contrast with the predicted value the observed data did not show accumulation. There was a significant difference (p < 0.05) between the observed and predicted value at 26 h and from 50 h till the end of the experiment. The elimination half-life on the fourth day was only 1.6 h (PK functions for Microsoft Office Excel) in comparison with 10.3 h after a single dose of 10 mg/kg BW PO (Table 1). Eight hours after the final administration the plasma concentration was below LOQ for three out of four pigeons. Ten to 12 h after the final oral administration pigeons were euthanized and the lung concentration at that point was below LOQ for nine pigeons. The three remaining pigeons had an average lung concentration of 398.97 μg/l (±97.13). No clinical signs of side effects were seen except for one pigeon that was lying down frequently on the fourth day of the experiment. The AST, bile acids, creatine kinase and uric acid levels in the blood collected from pigeons prior to euthanasia were within reference ranges.

Fig. 1 Semi logarithmic plot of average plasma concentrations (±SD) versus time in racing pigeons (n = 24) given a single oral and intravenous dose of voriconazole (10 mg/kg BW) in a cross over design.

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randomly distributed binuclear (n = 7, 9, 10) oval cell proliferation of the limiting plate (=raw of hepatocytes immediately adjacent to the portal tract; n = 9), moderate (n = 3) to marked (n = 6) hepatocellular vacuolization, moderate portal heterophilic and lymphocytic infiltration (n = 12). Occasional randomly distributed binuclear (n = 8) and apoptotic (n = 9) hepatocytes and moderate congestion of the sinusoids (=slit like spaces containing a few blood cells and separating cords of hepatocytes) (n = 2) at H&E staining. In the Von Gieson staining, no increase of fibrous tissue was observed in any sample.

Administering 20 mg/kg BW twice a day resulted in voriconazole plasma concentrations ranging from 5,847.03 µg/l (± 3,122.58) and 15,876.47 µg/l (± 7,987.79) during the whole experiment (Fig. 6). In general the standard deviation became larger as the experiment proceeded. On the third day one pigeon regurgitated when handled and on the fourth day eight of the 12 pigeons had one of the following symptoms: ruffled feathers as a general sign of illness (n = 4), lying down (n = 3), closing the eyes (n = 2), retching (n = 1), stretching the neck (n = 1) or having a swollen crop (n = 1). Four hours after the final administration one pigeon died. Prior to euthanasia blood was collected from all pigeons and the biochemical parameters AST, bile acids, creatine kinase and uric acid were elevated in 4, 1, 1 and 1 pigeon(s) respectively (Table 2). Pigeons were euthanized 10–12 h after the final oral administration and the lung concentration at that point was below LOQ for six pigeons and ranging from 249.79–17325 µg/l (4,965.38 ± 7,161.022 µg/l) for five pigeons. Data of one pigeon were absent. At necropsy all organs were macroscopically normal except the liver. The liver showed multiple white spots in five pigeons, was extremely swollen in three pigeons and showed multiple white spots and was swollen in four pigeons (one pigeon had abdominal fluid). Histopathology using the H&E staining revealed moderate (n = 9) to severe (n = 2) oval cell proliferation of the limiting plate, moderate (n = 5) to severe (n = 3) hepatocellular vacuolization, moderate portal heterophilic and lymphocytic infiltration (n = 10), occasional randomly distributed binuclear (n = 6) and apoptotic (n = 10) hepatocytes and moderate (n = 7) to marked (n = 2) dilated sinusoids. In two samples of this group numerous apoptotic hepatocytes as well as focal eosinophilic hepatocytes were found. No increase of fibrous tissue was observed in any sample in the Von Gieson staining.

**Discussion**

Nonlinearity of voriconazole is demonstrated in this study by the fact that the AUC triples from the dosage 2.5 to 5 mg/kg BW. This has also been described in humans, mice, rats, rabbits, guinea pigs, dogs and in African grey parrots [12,19,20]. Since voriconazole is eliminated predominantly after biotransformation, it is likely that saturation of metabolic clearance is the cause of the nonlinearity [20]. The AUC of 10 mg/kg BW is only 2.26 times the AUC of 5 mg/kg BW, which indicates that saturation of one or more enzymatic pathways occurs. Although also characteristic for nonlinearity, the semi-log plasma curves reveal no convex shape. The reason for this might be that the time points between 12 and 24 h post-administration are sparse.

In our experiment a bioavailability of 43.7% was found. In humans, the bioavailability was estimated to be greater than 90% and in chickens less than 20% [11,19]. However, it should be mentioned that the calculation of pharmacokinetic parameters (for example bioavailability) using classical compartmental or non-compartmental methods may be distorted for nonlinear drugs [21]. The difference in bioavailability between humans and birds is in accordance with findings with
other molecules [22] and can be influenced by presystemical metabolism and/or gastrointestinal motility.

A decreased elimination half-life and very low C_{min} concentrations were seen in multiple dose experiments in pigeons. These findings are probably due to an important induction of liver enzymes. These results are in contrast with humans, guinea pigs and rabbits, but in accordance with mice, rats, dogs and African grey parrots [12,19,20]. A dose-related increase in hepatic cytochrome P450 content in livers and an associated increase in relative liver weight in rats and dogs was consistent with auto induction of voriconazole metabolism, thereby leading to a significant increase in clearance upon multiple dosing [20].

In humans cytochrome P450 CYP2C19 plays a major role in the metabolization of voriconazole and exhibits genetic polymorphism dividing the population into poor and extensive metabolizers [23,24]. Genetic polymorphism in the hepatic cytochrome P450 families in pigeons may also exist but is not yet investigated.

In humans voriconazole is generally well tolerated (maintenance dose for patients \( \geq 40 \text{ kg} = 200 \text{ mg q12h (} \leq 5 \text{ mg/kg BW BID})\) [25]. The most commonly reported adverse effects are transient visual disturbances, liver function abnormalities and dermatological reactions [26]. Chickens which were treated with 10 mg/kg BW PO for 30 days showed neither clinical signs of side effects nor relevant changes of clinical liver and kidney chemistries which would indicate organ damage [11]. A number of African grey parrots \((n > 70)\) with proven or suspected aspergillosis have already been successfully treated with voriconazole at 10 mg/kg BW PO at least up to 30 days without any side effects [8]. In a recent study, African grey parrots receiving 18 mg/kg BW q12h for nine days showed polyuria that was more prevalent and severe than in the control parrots. This polyuria might be due to renal dysfunction and stress [12]. Twenty falcons suffering from aspergillosis have been treated with 12.5 mg/kg BW PO q12h for three days (loading dose), then q24h for an additional 18 to 87 days \((n = 14)\) and with 12.5 mg/kg BW PO q12h for 44 to 100 days \((n = 6)\). Flicking meat \((n = 2)\), vomiting \((n = 2)\) and anorexia \((n = 2)\) were considered adverse reactions to treatment with voriconazole. One bird presented with hepatomegaly and high liver enzyme and bile acid concentrations after one week of treatment and was considered to be hepatic failure [9]. On the other hand, in falcons receiving voriconazole at 12.5 mg/kg BW PO q12h for 14 days AST and creatine kinase were within normal ranges [10]. In this study important indications for toxicity of voriconazole were seen in the pigeons that received the dose regimen of 20 mg/kg BW q12h. One pigeon died, eight pigeons showed adverse effects and in five pigeons the biochemical parameters AST, bile acids, creatine kinase and/or uric acid were elevated. Although AST is not specific for liver disease, this parameter is considered the most relevant for the diagnosis of hepatic disease in birds, together with bile acids. However, any soft tissue damage can result in an elevation of the plasma AST and normal AST does not necessarily indicate a normal liver function. Because creatine kinase is much more specific for soft tissue trauma (particularly muscle) and absent in liver tissue, normal creatine kinase levels suggest AST elevation due to liver damage. Acute insult of the liver may not be manifested by elevation of plasma bile acids and may only show AST elevation.

Fig. 4 Linear plot of average plasma concentrations (at C_{max} and C_{min}) versus time in racing pigeons given a repeated dose regimen (20 mg/kg BW PO q24h) for ten days.

Fig. 5 Linear plot of average (observed and predicted) plasma concentrations (at C_{max} and C_{min}) versus time in racing pigeons given a repeated dose regimen (10 mg/kg BW PO q12h) for four days.
Only one pigeon revealed additional elevated creatine kinase (mild) and bile acids (pronounced) levels. In this pigeon it is possible that liver and soft tissue damage co-occurred. The elevated AST concentrations in this study are consistent with the liver histopathology and confirm the possible hepatotoxicity of the voriconazole treatment. Two pigeons revealed more severe microscopic lesions in comparison with the others. The oval cell proliferation seen at the limiting plate might be a sign of regeneration as a result of hepatocellular degeneration. Indeed occasional hepatocellular single cell necrosis was found. This could indicate a possible hepatic injury due to voriconazole. The lack of increase of fibrous tissue on the Von Gieson staining excludes the presence of a chronic process. To exclude a clinical outbreak of herpesvirosis or ornithosis in the multiple dose regimens 10 and 20 mg/kg BW q12h, the livers were screened for these diseases using polymerase chain reaction [Bactotype® PCR amplification Kit Chlamydia sp., Labor Diagnostik, Leipzig; 27]. All livers were negative for herpesvirosis, as well as for ornithosis. Mild hepatic abnormalities without AST elevation were seen in the multiple dose regimen of 10 mg/kg BW q12h. Further toxicological investigations with control pigeons are necessary to reveal the true impact of voriconazole on the liver of pigeons. Histopathological changes may have occurred in non-hepatic tissues, but were not assessed. Therefore, it can not be excluded that the death and clinical signs in the dose regimen 20 mg/kg BW q12h may have been due to unobserved changes in other tissues. It is likely that toxicity is species specific, as well as dose dependent and should be studied in different avian species before long-term use of voriconazole in non-yet-studied birds.

Findings of literature data are scarce but we may assume that antimycotics have a rather time-dependent activity because of their action and necessity for long-term therapy. Theuretzbacher et al. [26] reported that voriconazole shows time-dependent fungistatic activity against Candida species and time-dependent slow

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**Table 2** Biochemical blood parameters (AST, bile acids, creatine kinase and uric acid) of pigeons after oral multiple dose regimens 10 and 20 mg/kg BW of voriconazole q12h for four days.

<table>
<thead>
<tr>
<th>Biochemical parameter (unit)</th>
<th>10 mg/kg BW q12h</th>
<th>20 mg/kg BW q12h</th>
<th>Reference value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST (IU/l) mean (range)</td>
<td>91.6 (43–188)</td>
<td>364 (88–2000)</td>
<td>17–191&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>stdev&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.1</td>
<td>552.7</td>
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</tr>
<tr>
<td>Bile acids (µmol/l) mean (range)</td>
<td>35.7 (&lt;35–43)</td>
<td>49.6 (&lt;35–2000)</td>
<td>22–60&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>stdev&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3</td>
<td>47.4</td>
<td></td>
</tr>
<tr>
<td>Creatine kinase (IU/l) mean (range)</td>
<td>217 (96–363)</td>
<td>248.8 (89–712)</td>
<td>166–629&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>stdev&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85.9</td>
<td>190.2</td>
<td></td>
</tr>
<tr>
<td>Uric acid (mg/dl) mean (range)</td>
<td>7.04 (4.3–10.0)</td>
<td>7.6 (4.1–16.1)</td>
<td>3.2–11.2&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>stdev&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05</td>
<td>3.57</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>stdev = standard deviation.
<sup>b</sup>Reference values according to Vereecken et al. [17].
<sup>c</sup>Reference value according to Pollock et al. [18].

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**Fig. 6** Linear plot of average plasma concentrations (at C<sub>max</sub> and C<sub>min</sub>) versus time in racing pigeons given a repeated dose regimen (20 mg/kg BW PO q12h) for four days.

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fungicidal activity against Aspergillus species. Therefore maximizing the duration of exposure of a fungus to the drug would optimize its activity [26]. The MIC₉₀ of A. fumigatus is 0.5 mg/l and 0.38 mg/l for humans and falcons, respectively [5,28].

Administration of 20 mg/kg BW twice a day produced a plasma concentration continuously above the MIC during the tested four days. In this schedule however, liver toxicity (mortality, elevated AST levels and abnormal livers) was present. Administering 10 mg/kg BW twice a day resulted in a plasma concentration continuously above the MIC the first two days. The fourth day however, the plasma concentration was already below the MIC after 6 h. Oral treatment once daily at a dose of 10 or 20 mg/kg BW resulted in a Cₘₐₓ (2 h after administration) higher and a Cₘᵢₙ (24 h after administration) lower than the MIC as a multiple dose regimen for 14 days in falcons (12.5 mg/kg BW PO q12h) [10].

Taking the MIC of 0.5 mg/l [5] into account, nebulization of the intravenous formulation of voriconazole is not sufficient. These low plasma and lung concentrations after nebulization could be due to the moderately lipophilic nature of the molecule voriconazole by which the particles of the nebulization itself may be too large to penetrate the respiratory system. A second reason could be that the absorption via the respiratory system may be too minimal.

Multiple dosage treatment schedules can usually be predicted from single dose data, particularly if the compound shows linear pharmacokinetics. However, voriconazole shows nonlinear pharmacokinetics in all species investigated thus far including pigeons. This is important because drug plasma concentrations and elimination half-life will vary with different doses. Auto induction after repeated dosing in some species further complicates dosage extrapolation. Consequently, it is not possible to predict pharmacokinetics across species. Therefore, designing an effective and safe treatment schedule with voriconazole requires both single and multiple dose testing in each species.

In conclusion our study suggests that the oral dose schedules of 10 mg/kg BW twice a day and 20 mg/kg BW once a day could be appropriate in treating pigeons with aspergillosis. Because of the auto induction, a balance between the time the plasma concentration is above the MIC and the relative absence of liver toxicity must be sought.

Acknowledgements

We thank Pasquale Wassink and Karl Jonckheere for the excellent technical assistance. Voriconazole pure substance and hydroxy-itraconazole were a kind gift from Pfizer Global Pharmaceuticals (Ixelles, Belgium) and Janssen Animal Health (Beerse, Belgium), respectively. This work was supported by the Institute for the Promotion of Innovation by Science and Technology in Flanders (IWT Vlaanderen), Brussels, Belgium.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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This paper was first published online on iFirst on 4 August 2008.