Bulgarian Journal of Veterinary Medicine, 2022, **25**, No 1, 1–20
ISSN 1311-1477; DOI: 10.15547/bjvm.2321

Review

DOXYCYCLINE PHARMACOKINETICS IN MAMMALIAN SPECIES OF VETERINARY INTEREST – AN OVERVIEW

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Summary

Mileva, R. & A. Milanova, 2022. Doxycycline pharmacokinetics in mammalian species of veterinary interest – an overview. *Bulg. J. Vet. Med.,* **25**, No 1, 1–20.

Doxycycline is a broad-spectrum tetracycline antibiotic widely used in veterinary medicine. The current review aims to summarise the available data about pharmacokinetics in mammalian species of veterinary interest and to indicate the basic strategies for refining dosage regimens in order to use this antibiotic reasonably. Additionally, the available data about population pharmacokinetics are reviewed as this approach exhibits a number of benefits in terms of determination of drug pharmacokinetics, prediction of drug disposition and interpretation of the variations in the pharmacokinetic parameters. Further research with animal species of veterinary interest and pathogens causing diseases in animals is needed to clarify the pharmacokinetics and pharmacodynamics of doxycycline.

Key words: doxycycline, pharmacokinetics, population approach

DOXYCYCLINE IN VETERINARY PRACTICE

Tetracyclines are the most extensively used antibiotic group in veterinary practice (del Castillo, 2013). Discovered more than 70 years ago, by virtue of broad spectrum activity, pharmacokinetic features and possibility for incorporation in various drug formulations, they are still commonly used in almost all animal species of veterinary interest.

Doxycycline is a tetracycline antibiotic, a semi-synthetic derivative of oxytetracycline (Brunton *et al.*, 2006). It possesses the group-specific 4-ring nucleus, with a hydroxyl group attached to C5 and a methyl group: to C6. The OH group at C5 contributes to the antimicrobial activity of the molecule (Kogawa & Salgado, 2012). No halogen atoms are present in doxycycline molecule.

Being a second-generation tetracycline, doxycycline is characterised with higher lipophilicity compared to first-generation tetracyclines e.g. tetracycline, chlortetracycline and oxytetracycline (del Castillo, 2013). This contributes substantially for its better penetration through

biomembranes. Doxycycline salts are water-soluble, but solutions are more stable at an acid pH (Marx *et al.*, 2014). In alkaline medium, they precipitate. Compared to older members of the group, doxycycline has a lower affinity to metal ions (Yang *et al.*, 2015), yet it is able to form chelate complexes with them (Smith & Cook, 2004).

In the practice, the antibiotic is used under the form of doxycycline hyclate or monohydrate. In the crystal structure of hyclate salt, one molecule of doxycycline is bound to one molecule HCl, 0.5 C_2H_5OH and 0.5 H_2O under the form of doxycycline hydrochloride hemiethanolate hemihydrate (Mitić *et al.*, 2008). The solubility of hyclate in water is much better compared to that of monohydrate and this is the main reason for its more frequent use in the composition of various veterinary drug formulations (Mitić *et al.*, 2008). The preferred route of administration of doxycycline is the oral one. There are numerous drug forms, applied mainly with feed and drinking water in line with modern trends in antibacterial therapy of livestock (Anonymous, 2015a). The most commonly used doses in domestic animal species are summarised in Table 1. Doxycycline is not intended for use in lactating cattle and layer hens (Anonymous, 2015b) as no maximum residue limits (MRL) are available. There are drug forms registered for use in small ruminants in Europe, but they are not applied in animals whose milk is intended for human consumption (Anonymous, 2010). In horses, it is also used off-label (Winther *et al.*, 2011). The drug is prohibited in Europe for use as a growth promoter (Anonymous, 2016).

Doxycycline has a wide therapeutic index and is tolerated relatively well by most animal species. Local irritation is possible. Therapeutic doses in dogs caused anorexia, vomiting, diarrhoea and increased activity of alanine aminotransferase (ALT) and alkaline phosphatase (AP) in some animals (Schulz *et al.*, 2011). In cats treated with doxycycline oesophageal inflammation and strictures were reported (German *et al.*, 2005). The lethal dose (LD_{50}) after a single oral application to rats was 1893.03±286.20 mg/kg (Tkachenko *et al.*, 2015). Signs of intoxication were observed in calves after application of doses 3- to 10-fold higher that therapeutic ones (Brihoum *et al.*, 2010). Depression, lack of appetite, salivation, dysphagia, arrhythmia and pulmonary distress were reported. Possible side effects from doxycycline application are dysbacteriosis (Boynton *et al.*, 2017), photosensibilisation (Goetze *et al.*, 2017), foetal harm (Rebuelto & Loza, 2010). A specific feature of the entire group is allergy triggering (Riviere & Papich, 2009). Another disadvantage of tetracyclines due to their bacteriostatic effect is the more prolonged treatment compared to bactericidal antibiotics (del Castillo, 2013).

Table 1. Dosing regimen of doxycycline in different animal species

Animal species	Application route	Dose (mg/kg)	Dosing interval (h)	Reference
Cat	PO	5	12	Papich, 2013
Dog	PO	5	12	Papich, 2013
Horse	PO	10	12	del Castillo, 2013
Pig	PO	10	24	Prats *et al.,* 2005
Sheep	PO	20	24	Anonymous, 2010
Cattle (preruminant calves)	PO	5	12	Brihoum *et al.,* 2011

PHARMACODYNAMICS OF DOXYCYCLINE

Being a tetracycline, doxycycline possesses bacteriostatic activity (Riviere & Papich, 2009). It blocks microbial protein synthesis by binding to 30S ribosomal subunit (del Castillo, 2013). Its effect is time-dependent (Cunha, 2000).

Antimicrobial activity of doxycycline is broad. Its activity against intracellular pathogens e. g. *Anaplasma* spp. (Woldehiwet, 2010), *Ehrlichia* spp. (Branger *et al.*, 2004), *Rickettsia* spp. (Rolain *et al.*, 1998), *Chlamydia* spp. (Bommana & Polkinghorne, 2019) and some *Mycoplasma* spp. (Prats *et al.*, 2005) is valuable. *Coxiella burnetii* was also reported to be doxycycline-sensitive (Lever *et al.*, 2004). Table 2 presents more detailed data about the sensitivity of some microorganisms to doxycycline.

Doxycycline and minocycline, applied together with rifampin or streptomycin have a superior efficacy against *Brucella* spp., due to better intracellular penetration (del Castillo, 2013; Safi *et al.*, 2013). Such effect was present also after co-administration with pyrimethamine, demonstrated in the treatment of mice with experimental toxoplasmosis (del Castillo, 2013). Doxycycline is active also against the endosymbiont *Wolbachia*, present in blood nematodes *Dirofilaria immitis* preventing the development of larvae to adult parasites (Kramer *et al.*, 2007) and reducing microfilaraemia (Papich, 2017).

Apart its antibacterial and antiprotozoal activity, doxycycline acts also as immunomodulatory (Pradhan *et al.*, 2016), anti-inflammatory (Lai & Todd, 2006) and antineoplastic drug (Wang *et al.*, 2016). It has a chondroprotective effect due to inhibition of MMP-13 activity (Anonymous, 2018). During the last years, is recommended for treatment of osteoarthritis in horses (Maher *et al.*, 2014) and dogs (Nganvongpanit *et al.*, 2009). Doxycycline-induced irreversible inhibition of corneal MMP-2 benefits the healing of ocular surface diseases (Smith & Cook, 2004).

If tetracyclines are used reasonably, the resistance emerges slowly. The main mechanisms of development of antimicrobial resistance to tetracyclines are active efflux and ribosomal protection (Tejedor-Junco *et al.*, 2018). There are other mechanisms for onset of resistance including enzyme inactivation, ribosomal mutation and loss of porins from the cellular wall (del Castillo, 2013). Almost 50 genes encoding resistance to tetracyclines are discovered (del Castillo, 2013).

Vela *et al.* (2001) reported for reduced sensitivity to doxycycline of *L. monocytogenes* isolated from sheep (MIC=4 μg/mL). Resistant strains of *Salmonella indiana* and *Salmonella enteritidis* were detected by Lu *et al.* (2011). Naz *et al.* (2012) demonstrated that a small proportion of buffalo *Pasteurella multocida* isolates were resistant to doxycycline. Tejedor-Junco *et al.* (2018) established lack of sensitivity in *Staphylococcus aureus* and *Enterococcus spp.* after one-month treatment of dogs with doxycycline.

The antimicrobial spectrum of doxycycline allows its use in a number of diseases in domestic animals. Having in mind the possibility for emergence of resistance against it, although less frequently compared to other tetracyclines, principles for prudent use on the basis of pharmacodynamic and pharmacokinetic properties of the drug should be followed.

Table 2. Minimum inhibitory concentrations (MIC) of doxycycline against some bacterial pathogens

Microorganism	MIC (μg/mL)	Reference
Actinobacillus pleuropneumoniae	2.387	Prats *et al.* (2005)
Anaplasma phagocytophilum	0.125	Woldehiwet (2010)
Bordetella bronchiseptica	0.053	Prats *et al.* (2005)
Borrelia burgdorferi	0.25	Embers *et al.* (2013)
Chlamydia pecorum	0.008–0.031	Pudjatmoko *et al.* (1998)
Chlamydia psittaci	0.1	Butaye *et al.* (1997)
Chlamydia trachomatis	0.031	Pudjatmoko *et al.* (1998)
Clostridium spiroforme	16	Agnoletti *et al.* (2009)
Ehrlichia canis	0.03	Branger *et al.* (2004)
Ehrlichia chaffeensis	0.03	Branger *et al.* (2004)
Escherichia coli	1–4	Moskowitz *et al.* (2004)
Listeria monocytogenes	0.12	Vela *et al.* (2001)
Mycoplasma hyopneumoniae	0.2	Prats *et al.* (2005)
Mycoplasma gallisepticum	0.2	Takahashi and Yoshida (1989)
Pasteurella multocida	0.517	Prats *et al.* (2005)
Rhodococcus equi	≤0.25	Bryant *et al.*, 2000
Salmonella Group C1	2	Bryant *et al.* (2000)
Staphylococcus aureus	≤0.25	Bryant *et al.* (2000)
Streptococcus equi	≤0.12	Bryant *et al.* (2000)
Streptococcus pneumoniae	0.25	Dallas *et al.* (2013)
Streptococcus pneumoniae	<0.4	Aronson (1980)
Streptococcus zooepidermicus	≤1	Bryant *et al.* (2000)

PHARMACOKINETICS OF DOXYCYCLINE

The systemic behaviour of doxycycline after intravenous administration is most accurately characterised with the three-compartment model in calves (Meijer *et al.*, 1993), and with two-compartment model in goats (Abd El-Ati *et al.*, 2004), pigs (del Castillo *et al.*, 2006), cats and dogs (Riond *et al.*, 1990). In sheep injected intravenously with doxycycline, the three-compartment model turned out to be more appropriate for some animals (n=6), whereas the two-compartment one: in the other subjects (n=5) (Castro *et al.*, 2009). After oral application, doxycycline pharmacokinetics is characterised with the two-compartment model in sheep (Castro *et al.*, 2009), calves (Meijer *et al.*, 1993) and pigs (del Castillo *et al.*, 2006). According to Davis *et al.* (2006) the one-compartment model is the best to explain the behaviour of orally applied doxycycline in horses. Vargas *et al.* (2008) reported that the two-compartment model described the best its pharmacokinetics in goats following intramuscular administration.

Absorption

In most animal species, oral absorption of doxycycline is not significantly influenced by feed intake. In horses, feed considerably slows down the absorption (T_{max} 4 h) and results in almost two-fold lower plasma concentrations (C_{max} 0.43 μg/mL) vs animals treated before feeding: C_{max} 0.97 μg/mL and T_{max} 0.75 h (Davis *et al.*, 2006). The dose used in this study was 20 mg/kg. Prats *et al.* (2003) found out that

in pigs treated with 10 mg/kg doxycycline with feed, C_{max} was higher (1.7±0.6 µg/mL) and T_{max} was lower (6±2.8 h) compared to application of the same dose with drinking water (C_{max} 1.4±1.1 µg/mL and T_{max} 12.2±6.5 h).

According to Davis *et al.* (2006), the time to attain maximum plasma concentrations is relatively short in horses – from 1.54±1.3 h (after single oral dose of 20 mg/kg) to 1.63±1.36 h (after repeated treatment with 20 mg/kg). After oral administration of 20 mg/kg to fattening pigs, Gutiérrez *et al.* (2014) reported T_{max} of 2.38±0.04 h. Rabbits treated with 20 mg/kg exhibited an average T_{max} of 3.00±0.00 h (Fu *et al.,* 2011). The same pharmacokinetic parameter in calves was 3.48±0.63 h after application at 10 mg/kg with milk replacer (Meijer *et al.*, 1993). In sheep it was 3.60±3.35 h after oral doxycycline dose of 20 mg/kg (Castro *et al.*, 2009). In dogs, Gutiérrez *et al.* (2012) reported that T_{max} was 3.88±0.4 h after oral treatment with doxycycline at 10 mg/kg. C_{max} was attained more slowly after oral application of 5 mg/kg in cats: 4.33 ± 3.20 h (Hartmann *et al.*, 2008).

Biological absorption half-life after oral application of the antibiotic ($t_{1/2k01}$) was relatively short in horses: 0.18±0.18 h (Davis *et al.*, 2006). Higher values were found out in rabbits – 0.79±0.63 h (Fu *et al.*, 2011) and fattening pigs – 1.06±0.06 h (Gutiérrez *et al.*, 2014). In sheep, Castro *et al.* (2009) reported $t_{1/2k01}$ of 36.28±14.57 h.

Similar data about AUC and MRT are shown in Tables 3 and 4. AUC data are influenced by the experimental design. In more prolonged collection of samples, the terminal stage of doxycycline elimination is characterised, resulting in higher calculated values of the parameter. On the basis of AUC data after extra-venous and intravenous application, doxycycline bioavailability is calculated depending on the route of application and animal species. Oral bioavailability (F) depends substantially on the animal species. In orally treated sheep at a dose of 20 mg/kg bioavailability was relatively low: 35.77± 10.20% (Castro *et al.*, 2009). In preruminant calves, Meijer *et al.* (1993) observed average F value of 69±12% after single doxycycline application (10 mg/kg). In adult horses, Winther *et al.* (2011) administered a dose of 10 mg/kg and found out low bioavailability of 6% (after topdressing application) and 17% (intragastric application). Doxycycline bioavailability in horses was only 2.7% after administration of 20 mg/kg doxycycline tablets (Davis *et al.,* 2006). In pigs, Gutiérrez *et al.* (2014) observed a bioavailability of 7.8% after a dose of 20 mg/kg, whereas Baert *et al.* (2000) applied 10.5 mg/kg and found out a F of 21.2±7.5%. High values of 74.88% were measured in dogs following a dose of 10 mg/kg (Gutiérrez *et al.*, 2012).

Various mean residence time (MRT) values have been reported, which could be species-related, but more probably, differences were due to various methods of antibiotic concentrations analysis and blood samples' collection schedules.

After intramuscular application of doxycycline at 5 mg/kg in non-lactating goats (Abd El-Ati *et al.*, 2004) an almost complete absorption was reported, F= 99.40±5.38%. After rectal administration of suppository with 10 mg/kg, Christ *et al.* (2020) observed a bioavailability of 50% (51.43±4.50% for coconut oil suppositories and 49.13±14.69% for polyethylene glycol suppositories).

Although doxycycline interaction with metal ions, antacids and bismuth is at a lesser extent, their co-application could result in formation of chelates and therefo-

Table 3. Area under the curve (AUC) values after various routes of doxycycline adminsitration in mammals

Animal species	Dose (mg/kg)	$AUC_{0-\infty}$ (µg∗h/mL)			Reference
		I.V.	P.O.	I.M.	
Cat	5		33.37±7.22, AUC_{0-24}		Hartmann *et al.* (2008)
Dog	10	97.34±7.45	72.89 ± 6.23		Gutiérrez *et al.*(2012)
	20		24.18 ± 2.47		Arciniegas Ruiz *et al.* (2015)
Swine	10.5	64.24 ± 12.79	13.79 ± 6.06		Baert *et al.* (2000)
Horse	3	14.05 ± 2.33			Winther *et al.* (2010)
	10 (intragastric)		8.11 ± 2.33		
	10 (feed)		2.83 ± 0.64		
	20		13.35 ± 2.71		Davis *et al.* (2006)
Cattle	5	30.32 ± 3.8	42.86 ± 5.87		Meijer *et al.* (1993)
	10	21.46 ± 2.71		21.16 ± 2.01	Vargas-Estrada *et al.* (2008)
Sheep	20	128.56± 28.87	46.12 ± 12.60		Castro *et al.* (2009)
			44.57 ± 11.01*		Castro *et al.* (2009)
	20			65.67±9.88	Castro Robles *et al.* (2012)
Goat	5	6.92 ± 0.33		6.89 ± 0.33	Abd El-Aty *et al.* (2004)

* Noncompartmental analysis.

Table 4. Mean residence time (MRT) values after various routes of doxycycline adminsitration in mammals

Animal species	Dose (mg/kg)	MRT (h)			Reference
		I.V.	P.O.	I.M.	
Cat	5		8.92 ± 2.28		Hartmann *et al.* (2008)
Dog	5	15.12+3.39			Wilson *et al.* (1988)
Swine	10.5	5.16 ± 0.89	7.36 ± 2.68		Baert *et al.* (2000)
Horse	3	4.85 ± 0.64			Winther *et al.* (2010)
	10 (intragastric)		12.88 ± 1.90		
	10 (feed)		12.83 ± 3.70		
Cattle	10	12.48 ± 1.99		12.00 ± 1.62	Vargas-Estrada *et al.* (2008)
Sheep	20	11.18 ± 3.152	36.73 ± 13.86		Castro *et al.* (2009)
	20			91.10 ± 40.78	Castro Robles *et al.* (2012)
Goat	5	2.12 ± 0.12		2.80 ± 0.11	Abd El-Aty *et al.* (2004)

re, in decreased bioavailability (Riviere & Papich, 2009; del Castillo, 2013).

Distribution

Compared to other tetracyclines, doxycycline binds to blood proteins at the highest extent (del Castillo, 2013), followed in descending order by minocycline, chlortetracycline, tetracycline and oxytetracycline (lowest extent of protein binding). Protein binding percentages for the different animal species are listed in Table 5.

Despite its high protein binding, doxycycline is outlined with good tissue distribution. It is due to its higher lipophilicity: 5-10-fold higher compared to tetracycline, oxytetracycline and chlortetracycline (Riviere & Papich, 2009). That is why doxycycline's tissue distribution is better than that of other tetracyclines (including inside cells) and the volume of distribution is higher. Due to the possibility for precise calculation of this parameter only after intravenous administration, this overview presents data obtained after i.v. doxycycline application. In ruminants, doxycycline steady-state volume of distribution (V_{ss}) after intravenous administration was relatively high: 1.76±0.31 L/kg in sheep (Castro *et al.*, 2009), and 1.31±0.11 L/kg in calves with developed forestomachs (Riond *et al.*, 1989). In preruminant calves, V_{ss} was 1.81±0.24 L/kg (Riond *et al.*, 1989). In dogs, Wilson *et al.* (1988) also reported a high V_{ss} of doxycycline 1.47± 0.24 L/kg. In pigs it averaged 0.89±0.16 L/kg (Baert *et al.*, 2000). According to Riond *et al.* (1989), volume of distribution (V_{darea}) of ruminant calves (1.38±0.15 L/kg) differed from that in preruminant calves (1.89±0.25 L/kg). V_{darea} of doxycycline after i.v. application in pigs was 1.06±0.22 L/kg (Baert *et al.*, 2000).

Doxycycline passes through the blood-milk barrier. The ratio of milk to blood serum concentrations in ruminants after intravenous administration of 20 mg/kg doxycycline demonstrated rapid and substantial transfer of the antibiotic in milk (Ziv & Sulman, 1974). Measurable concentrations appear within 30 minutes and with time, they become equal to those in blood (AUC_{milk}/AUC_{serum}=1.05±0.44), and when a pseudoequilibrium is attained, even exceed them (AUC_{milk}/AUC_{serum}= 1.53±0.36). Binding to milk proteins is 36±8% (Ziv & Sulman, 1974). Shortcomings of data from this study are general presentation and lack of information about different ruminant species (lactating cows and sheep). Freeman *et al.* (2013) reported tear doxycycline concentrations approximately equal to 10% of those in plasma after oral administration of 10 and 20 mg/kg doxycycline in elephant seals.

Table 5. Blood protein binding of doxycycline in animals

Animal species	Dose (mg/kg)	Protein binding (%)	Reference
Cat	5	98.35 ±0.24 (SEM)	Riond *et al.* (1990)
Dog	5	91.40 ± 0.93 (SEM)	Riond *et al.* (1990)
Swine	20	93.1 ± 0.2	Riond and Riviere (1990)
Horse	20	81.76 ± 2.43	Davis *et al.* (2006)
Cattle	20	92.3 ± 0.8 (SEM)	Riond *et al.* (1989)
Sheep	20	90.2 ± 2.4 (SD)	Ziv and Sulman (1972)
Goat	5	32.8	Abd el-Aty *et al.* (2004)

SEM – standard error of the mean, SD – standard deviation.

After oral treatment, Davis *et al.* (2006) detected doxycycline concentrations in the anterior eye chamber of horses, equal to 10% of plasma levels. Detectable concentrations close to method's sensitivity threshold were found out in feline tears after oral application of the antibiotic at a dose of 5 mg/kg (Hartmann *et al.*, 2008). According to Collins *et al.* (2016) there is no correlation between blood serum and tear levels of doxycycline. The cause for the good penetration ability of doxycycline is its pKa value and high lipophilicity. The latter explains the achievement of high efficient concentrations in various tissues, in which tetracyclines from previous generations were not usually found at a significant extent (del Castillo, 2013).

Metabolism and excretion

No data are available for bioconversion of doxycycline and it is eliminated unchanged. In pigs, cats and dogs, it is not transformed (Riond & Riviere, 1990). Doxycycline is distinguished from the other tetracyclines by its high rate of elimination through secretion through the intestinal wall. It is characterised with enterohepatic cycling (Riviere & Papich, 2009). A very small part of administered dose is eliminated with urine (Brunton *et al.*, 2006). The higher values of doxycycline total body clearance compared to those of older members of the group could be attributed to the higher extent of binding to blood proteins and better tissue distribution (Brunton *et al.*, 2006). Del Castillo *et al.* (2006) assumed that during the night, elimination of doxycycline in pigs was delayed. They affirm that urine pH and daily activity of this species favourised retention of urine during the night, so that a part of the antibiotic reenters the circulation through lymphatic vessels. A small share of doxycycline dose, applied intravenously (0.026±0.0085%) is eliminated with milk in ruminants (Ziv & Sulman, 1974).

Total body clearance (Cl_B) values after intravenous administration of 5 mg/kg doxycycline in dogs (100.8±26.4 – 103.2± 10.2 mL/kg/h) and cats (65.4±12.6 mL/kg/h) differ insignificantly (Wilson *et al.*, 1988; Riond *et al.*, 1990). Similar Cl_B values (100.2±10.8 mL/kg/h) were found out in pigs treated intravenously with 20 mg/kg doxycycline (Riond & Riviere, 1990). The same authors reported considerable breed-related differences in Cl_B values between Angus calves (64.2±3.6 mL/kg/h) and Holstein calves (132±12.6 mL/kg/h) treated intravenously with 20 mg/kg doxycycline (Riond & Riviere, 1989). Total body clearance values in sheep injected i.v. with 20 mg/kg doxycycline were 162.48±34.98 mL/kg/h (Castro *et al.*, 2009). In goats treated intravenously with 5 mg/kg, the highest Cl_B values were demonstrated: from 414.6±25.8 mL/kg/h to 710.2±4 mL/kg/h (Jha *et al.*, 1989; Abd El-Aty *et al.*, 2004). The accurate comparison of doxycycline clearance among animal species is not possible without calculation of extraction ratio values.

Elimination half-life ($t_{1/2el}$) also varies among species and studies, which could be attributed to experimental design (applied dose and duration of blood sampling) and analytical method sensitivity. Its values in intravenously injected dogs increased parallelly to the doses (Table 6). Oral treatment results in highest $t_{1/2el}$ in horses and ruminants, lower values in pigs and dogs and the lowest ones – in cats. This information should, however, be interpreted carefully due to reasons explained above.

The application of therapeutic doses of doxycycline in patients with renal failure

Table 6. Elimination half-life ($t_{1/2el}$) values of doxycycline in mammals ater different routes of administration

Animal species	Dose (mg/kg)	$t_{1/2el}$ (h)			Reference
		I.V.	P.O.	I.M.	
Cat	5	4.56 ± 0.68			Riond *et al.* (1990)
	5		4.24 ± 0.86		Hartmann *et al.* (2008)
Dog	0.1 mg/kg/h	4.56 ± 0.57			Bidgood & Papich (2003)
	5	6.99 ± 1.09			Riond *et al.* (1990)
	10	7.44 ± 0.06			Gutiérrez *et al.* (2012)
	20		7.54 ± 0.17		Arciniegas Ruiz *et al.* (2015)
Swine	10		7.2 ± 2.42		Prats *et al.* (2005)
	10.5	4.2	2.9		Baert *et al.* (2000)
Horse	3	2.98 ± 0.17			Winther *et al.* (2010)
	10 (intragastric)		13.80 ± 1.68		
	10 (feed)		14.23 ± 5.14		
	20		11.8 ± 3.51		Davis *et al.* (2006)
Cattle	5	9.5 ±3.0	12.6 ± 5.0		Meijer *et al.* (1993)
	10	5.80 ± 0.66		9.56 ± 1.84	Vargas-Estrada *et al.* (2008)
	20	14.9±0.9 (ruminant)			Riond *et al.* (1989)
		9.9±0.6 (pre-ruminant)			
Sheep	20	7.03±1.13 - 12.11±2.06, 2-3 compartmental model			Castro *et al.* (2009)
Goat	5	4.62 ± 0.11		3.65 ± 0.12	Abd El-Aty *et al.* (2004)

did not result in a significant cumulation and hence, it is one of the safest antibiotics for use in conditions accompanied with renal damage (Brunton *et al.*, 2006). Liver parasitic diseases had a slight effect on doxycycline elimination in men (Holmes & Charles, 2009).

Pharmacokinetics of long acting formulations of doxycycline

Various modified release drug forms have been tested in horses, dogs and rabbits in order to achieve maintenance of doxycycline concentrations, higher than minimum effective ones during the entire dosing interval or after a single administration. The use of such drug forms is rational due to the time-dependent antibacterial effect of the antibiotic. Orally applied doxycycline hyclate poloxamer at 10 mg/kg in horses resulted in high AUC values (17±2.2 µg.h/mL) vs doxycycline hyclate (3.1±0.2 µg.h/mL), and a bioavailability of 548%. An almost twice longer $t_{1/2el}$ of doxycycline hyclate-poloxamer was reported in comparison to doxycycline hyclate: 4.9±1 h and 2.8±0.9 h respectively (Zozaya *et al.*, 2013). The differences were due to the flip-flop kinetics of doxycycline. The authors demonstrated higher PK-PD values for the poloxamer drug form – a prerequisite for better effect in the treatment of bacterial diseases. A lower F value (70.43%) was found out after subcutaneous injection of 20 mg/kg doxycycline hyclate poloxamer to newborn piglets. Yet, in this study, the bioavailability was 10-fold higher than that obtained from oral use of doxycycline hyclate, which also resulted in higher PK-PD values and suggested a better antimicrobial effect (Gutiérrez *et al.*, 2014).

The oral application of long-acting (LA) formulations of doxycycline hyclate in dogs at a dose of 20 mg/kg improved bioavailability, maximum plasma concentrations and mean retention time depending on constituents ratio (Ruiz *et al.*, 2015). This study demonstrated the possibility for use of LA formulation in dogs to provide effective concentrations over 48 hours, an interval twice longer than that obtained with conventional drug forms (Ruiz *et al.*, 2015). Arcinegas *et al.* (2019) have tested excipients at a various ratios for a LA formulation intended for oral application in dogs. A statistically significant 2-fold increase of $t_{1/2el}$ from 7.54±0.17 h to 17.36±0.4 h and increase in C_{max} from 2.6±0.28 µg/mL to 4.11± 0.21 µg/mL was achieved. A substantial increase was noted for AUC values: from 24.18±2.5 to 112.7±4.4 µg.h/mL, that correlated to calculation of PK-PD indices suggesting a better efficacy for treatment of infections caused by sensitive pathogens (Arcinegas *et al.*, 2019). Better pharmacokinetic features were established in rabbits for microencapsulated suspension compared to doxycycline hyclate solution (20 mg/kg) (Fu *et al.*, 2011). A relative bioavailability of 289.4%, statistically significantly longer elimination half-life (from 2.19±0.38 h to 9±1.6 h), and flip-flop effect specific delayed absorption (k_{abs} from 1.26±0.71 L/h to 0.20±0.11 L/h) were found out (Fu *et al.*, 2011). Similar trends were observed after subcutaneous application of a LA formulation in goats (Vargas *et al.*, 2008) – absolute bioavailability of 545%, longer elimination half-life from 4.11±0.46 h to 40.92±4.25 h and delayed absorption (absorption half-life from 0.22±0.66 h to 4.99±0.35 h). Data for LA formulations indicate their advantage over conventional forms by providing higher PK-PD values presuming a better efficacy in the treatment of bacterial and protozoan infections.

Doxycycline pharmacokinetics is well studied in horses, large ruminants and pigs. Data about the behaviour of the antibiotic are mainly available after intravenous and oral administration. Its high bioavailability, good distribution, lack of biotransformation and primary elimination through the liver make it applicable in the therapy of a number of diseases in livestock and pets.

Pharmacokinetic-pharmacodynamic (PK-PD) modelling for optimisation of treatment with doxycycline

As doxycycline is a bacteriostatic antibiotic, its efficacy is associated with dosage interval time, during which its concentration at the site of action is higher than MIC, T>MIC (Castro *et al.*, 2009). It is considered that the efficacy of antibiotic therapy with time-dependent antibiotics is directly related to sustaining desired concentration at T>MIC for at least 80% of the dosage interval. A number of publications outline the AUC/MIC index as the main predictor of effect from therapy with tetracyclines (Craig, 1998; Andes & Craig, 2002; Toutain *et al.*, 2002). This means that the aim is to maintain high average plasma levels throughout the dosage interval. More recent studies affirm that being a tetracycline, doxycycline belongs to the group of antibiotics with concentration-independent killing and prolonged persistent effect due to prevention of microbial regrowth at levels below MIC (Asín-Prieto *et al.*, 2015). Cited authors indicated that AUC/MIC values >25 correlate to achieving a desired efficacy in therapy with tetracyclines. One of the main factors in PK-PD modelling that should be considered for tetracyclines, is their high percentage of blood protein binding. *In vitro* PK-PD modelling of doxycycline against *Mycoplasma hyopneumoniae* showed that at $AUC_{24\ h}$/MIC 164 h and C_{max}/MIC 9.89, the microbial counts decreased to 1 $\log_{10}$CFU/mL (Zhang *et al.*, 2019). The results from modelling showed that a bactericidal effect against *M. hyopneumoniae* in pigs could be achieved with doses >10 mg/kg applied for 3 days (Zhang *et al.*, 2019). Additional studies for validation of PK-PD indices for microbial pathogens of veterinary relevance are needed also in clinical conditions, specific for farm animal practice. The proper use of antibiotics for group treatment of animals requires also good knowledge of possible variations in water and food intake, behavioural models and group hierarchy, as well as acquaintance with disease epidemiology. Population methods could be successfully used to depict variability in pharmacokinetics of many drugs, including antibiotics.

Population pharmacokinetics of doxycycline

Traditional pharmacokinetic analysis does not provide information allowing adequate description of interindividual pharmacokinetic variation within a population, its origins and related conclusions about the therapy (Riviere, 1999). The population approach permits largely to predict this variability by including various covariates in the analysis (Bon *et al.*, 2018). In veterinary medicine, these could be individual features (age, body weight, breed, sex, biochemical markers) or environmental factors (production system, group hierarchy). Consideration of these factors in pharmacokinetic model construction would lead to rather more consistent calculation of pharmacokinetic parameters. Using population methods, the relevant factors causing observed inter- and intra-individual differences could be outlined. Their application would contribute for

selection of the most appropriate route of drug administration. In available literature, there are data from population pharmacokinetic analysis in animals treated with various substances, mainly NSAIDs and antibiotics. Population-based analysis has made pharmacokinetic models in these studies much more optimised.

The strength of this analytical approach consists in the possibility for characterisation of differences in drug behaviour and drug effect by investigating the impact of variable e.g. clinical and blood laboratory parameters of the population (Bon *et al.*, 2018). It could be also useful in optimisation of dosage regimen, in analysis of incomplete data on antibiotic concentrations especially in animals presenting no opportunity for blood collection with sufficient amount or under intensive experimental designs (Sánchez *et al.*, 2019). Population-based analysis helps understanding the effects of factors such as age, sex, breed, disease etc. on drug behaviour and effects within a large animal cohort (Li *et al.*, 2014). It is applied for optimisation of the dose and clinical efficacy with reduction of risk from side effect. Pharmacokinetic parameters are calculated as a function from important features of the patient. They allow description of drug behaviour in animals, in which it differs substantially from that of most individuals. Therefore, variables that could alter considerably drug behaviour, are necessary. In human medicine, routinely used variables are the body weight, age, genetic factors, renal and liver function markers etc. (Dorajoo *et al.*, 2019). In veterinary practice, the hierarchy of pigs for orally administered drugs and licking by large ruminants for pour-on drug forms are other variables determining differences in drug kinetics (del Castillo *et al.*, 2006). Except for available studies with incomplete data sets in wild animals, population methods have been used for analysis of residues in the milk of ruminants (cows, goats) according to FDA regulations (Lin *et al.*, 2016), in single investigations on tobramycin pharmacokinetics in horses (Haritova *et al.*, 2012) and doxycycline in pigs (del Castillo *et al.*, 2006). Future investigations require detection of markers that would improve population modelling due to their important effect on doxycycline behaviour and finding the most adequate pharmacokinetic model. This would contribute to better knowledge of variation sources among the animals.

Population pharmacokinetic analysis is still of limited use in veterinary practice. Only one study in pigs has been published with respect to population pharmacokinetics of doxycycline. Population-based pharmacokinetic analysis of measured plasma doxycycline levels in treated pigs showed a statistically significant negative correlation between dose-normalised concentration (Cp) and body weight (del Castillo *et al.,* 2006). The authors affirmed that the effect of co-variates sex, group and treatment were insignificant. The disease also had a certain effect on Cp value: it was lower in healthy animals. No correlation has been found between AUC and C_{max} vs body weight from one hand, and vs body temperature, on the other. The negative correlation between T_{max} and body weight distinguished the significance of feed intake for attaining desired effective systemic concentrations. The clinical efficacy of antibiotics applied in-feed invariably depends on feed intake (del Castillo, 2006), thus requiring detailed familiarity with changes in animal behaviour, respectively water and feed intake in various diseases. Information received from population modelling could discriminate

clinical situations in which antibiotic application through water or feed would not result in the desired effect and instead, recommend parenteral treatment of animals with overt clinical signs.

Population pharmacokinetic analysis has been used to describe the behaviour of orally applied doxycycline at either 10 mg/kg or 20 mg/kg in elephant seals (n=18), animals in which only few samples could be collected in order to reduce manipulation stress (Freeman *et al.*, 2013). This study has used naive-pooling approach to calculate main pharmacokinetic parameters on the basis of scarce data for doxycycline concentrations. Absorption rate constants (0.56–0.49 h^{-1} for doses of 10 and 20 mg/kg, respectively) and elimination rate constants (0.11–0.13 h^{-1}, for 10 and 20 mg/kg, respectively) in elephant seals were similar to those found out in other mammals e.g. sheep (Castro *et al.*, 2009). C_{max} of 1.5-2.2 µg/ml to 1.9–2.4 µg/mL, (for doses of 10 and 20 mg/kg) were attained for T_{max} 4.0–6.1 h and 2.3–5.8 h in elephant seals (Freeman *et al.*, 2013). The lack of possibility for intravenous administration of doxycycline due to ethical reasons does not permit the calculation of volume of distribution and total body clearance because these two parameters are calculated on the basis of bioavailability. V/F values ranging from 4.0 to 7.1 L/kg allowed concluding that doxycycline was distributed at a significant extent in the body of these mammals. An additional proof with this respect was the detection of measurable concentrations in tears after application of a dose of 20 mg/kg (Freeman *et al.*, 2013). The limitation of this study was the inability to calculate variability within the population due to the small number of samples, which is a frequent problem with wild animals, even in zoos.

Pharmacokinetics of doxycycline in sick animals

Data about the pharmacokinetics of doxycycline in sick animals are scarce. One study (Ole-Mapenay *et al.*, 1997) was focused on goats with pneumonia after experimental infection with *Pasteurella haemolytica*. The animals were treated intramuscularly with a depot doxycycline form at 20 mg/kg. No statistically significant differences were found out between C_{max} and T_{max} in healthy (3.87±0.52 µg/mL and 1.15±0.37 h, respectively) and diseased goats (5.56±0.58 µg/mL and 1.17±0.17 h). The only significant change was the prolonged $t_{1/2el}$: from 13.42±0.35 h in healthy to 37.43±0.29 h in diseased goats.

Intramuscular application of doxycycline in pigs with *Haemophilus parasuis* infection at a dose of 20 mg/kg did not result in altered pharmacokinetics of the drug (Zhang *et al.*, 2018). The C_{max} and AUC values were 4.53±0.46 µg/mL (healthy) and 55.51±5.72 µg.h/mL (diseased animals), and 4.31±0.42 µg/mL and 57.10±4.89 µg.h/mL for healthy and diseased pigs respectively. The values of k_{el} were almost the same (0.08±0.01 h^{-1} in healthy; 0.07±0.02 h^{-1} in diseased animals) (Zhang *et al.*, 2018). On the basis of classical PK/PD modelling, a daily dose of 18.17 mg/kg was validated with 90% efficiency at MIC_{90} of 1 µg/mL against *H. parasuis* (Zhang *et al.*, 2018).

Investigations in dogs with *Rikettsia rickettsii* infection treated orally with doxycycline hyclate at a dose of 5 mg/kg at 12-hour intervals showed significant variations in maximum drug concentrations: from 3.48±0.77 µg/mL to 17.14± 0.85 µg/mL (Breitschwerdt *et al.*, 1997; 1999). These concentrations were attained at T_{max}=1 h and persisted >1 µg/mL until the end of dosage interval, 12 h after application. The $t_{1/2el}$ values – 9.46±2.01 h

(Breitschwerdt *et al.*, 1997) were comparable to those reported in healthy dogs (Riond *et al.*, 1990). The pharmacokinetic analysis of oral doxycycline dose of 10 mg/kg in dogs with microfilariosis demonstrated $t_{1/2el}$ of 12.6±11 h and bioavailability of 61±8%; which are comparable to data in healthy subjects (Maaland *et al.*, 2013; Papich, 2017). Microfilariosis in dogs is associated also with infection with *Wolbachia, Rikettsiaceae*, which is controlled by tetracycline therapy. The population analysis of data using Monte Carlo simulation has shown that the oral dose of 5 mg/kg doxycycline at 12-hour intervals correlated with high efficacy against *Wolbachia* when AUC attained 40.6 µg.h/mL (Papich, 2017).

Data obtained from pharmacokinetic parameters of doxycycline in sick animals demonstrated that antibiotic pharmacokinetics was not significantly altered in disease states. The use of PK/PD modelling and population-based analysis provides objective information for adjustment of dosage regimens in animals with infections. The application of these approaches would lead to proper scheduling of the therapy with dosage regimens, correlating to the maximum efficacy.

The use of available methods for classical and population-based pharmacokinetic analysis to characterise the systemic behaviour of doxycycline would result in better understanding of factors influencing the pharmacokinetics of this drug with regard to its more accurate dosage regimen in veterinary practice.

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Paper received 10.12.2019; accepted for publication 20.01.2020

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