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CAN WE IMPROVE PHARMACOKINETIC MODELLING BY CORRELATION WITH CLINICAL AND BIOCHEMICAL PARAMETERS? A TRIAL WITH DOXYCYCLINE

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Summary

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Doxycycline is a tetracycline antibiotic registered for treatment of infectious diseases in animal species of veterinary interest but its pharmacokinetics is not yet well studied in small ruminants. Therefore the aim of the current investigation was to evaluate its oral pharmacokinetics in correlation with clinical and biochemical parameters in sheep with different stage of digestive system development. The pharmacokinetic parameters were correlated with blood biochemical parameters which reflect the different extent of maturation of important organs such as liver, heart and kidney. One-compartmental model with absorption was fitted to the data and few nonlinear mixed-effects models were tested to evaluate the influence of age, body weight, AST and ALT on pharmacokinetics (Phoenix 8.1, Certara, USA). The model with covariates age, body weight, AST and ALT showed the best fit. The final population values for volume of distribution and absorption rate constant were 2.66 $L.kg^{-1}$ and 2.50 h^{-1}, respectively when all covariates were included in modelling. This model can be further developed and validated by collection of few blood samples from higher number of individual animals treated with doxycycline.

Key words: biochemical parameters, correlation, lamb, pharmacokinetics, sheep

INTRODUCTION

Large variations in the gastro-intestinal mucosa between mature ruminants and pre-ruminant suckling animals, determined by the way of feeding, lead to differences in the absorption of many antibacterial drugs after oral administration (Mzyk *et al.,* 2017). Nowadays prudent use of antibiotics requires sufficient knowledge on pharmacokinetics and source of variations in order to design a proper dosing regimen.

Doxycycline, a tetracycline antibiotic, is registered for many animal species because of its broad spectrum of activity against Gr+ and Gr- bacteria and protozoa, high efficacy and low toxicity. It has shown high activity against pathogens causing infections in small ruminants

(Woldehiwet, 2010). Although its advantages over the other tetracyclines, its pharmacokinetics was not well described in lactating sheep and their offspring. Doxycycline is available as oral drug dosage forms which requires sufficient knowledge on the effect of the rumen maturation on its absorption. Undeveloped gastrointestinal tract in suckling animals together with immature function of the liver as well as differences in muscle mass between sheep and lamb can contribute to significant inter-individual variations in doxycycline pharmacokinetics. The population approach allows precise calculation of individual pharmacokinetic parameters by considering factors such as age, body weight and specific markers for the maturation of the function of important organs (Nassar *et al.,* 2009). Therefore, the study aimed to apply a population approach for estimation of individual pharmacokinetic parameters and characterisation of the variables after oral doxycycline treatment of small ruminants.

MATERIAL AND METHODS

Animals and experimental design

Six clinically healthy adult female sheep and their one month old lambs, local Bulgarian breed, were included in the study. Body weight and age of sheep and lambs were 50.0 (45.1–56.4) kg and 4.4 (3.0–6.0) years and 14.2 (12.9–16.7) kg and 1.0 (1.0–1.3) month, respectively. The trial was conducted in the farm. Sheep were fed hay and concentrate. Lambs were still sucking pre-ruminant but also had free access to alfalfa hay. Water was available *ad libitum*. Three days prior to the experiment blood ALT and AST were assessed. Commercially available oral powder of doxycycline (HydroDoxx 500 mg/g, Huvepharma NV) was used. On the day of the trial a water solution of the antibiotic was prepared *ex tempore* and was administered at a dose rate of 10 mg/kg as a single oral dose. Blood samples were collected from *v. jugularis* at 0.5, 1, 2, 3, 4, 6, 8, 10, 12 and 14 h after drug administration.

Doxycycline analysis

A HPLC method with PDA detection (Thermo Fisher Scientific, USA), described by Lagzay *et al.* (2001) was used for analysis of plasma concentrations of doxycycline. Analyses were performed in the Central Scientific Laboratory at Trakia University.

Pharmacokinetic and statistical analysis

Pharmacokinetic analysis was performed by using population modelling with Phoenix software (Phoenix NLME version 8.1, Certara, USA). Population parameters were estimated using the first order conditional estimation-extended least squares (FOCE ELS) algorithm. Variances of pharmacokinetic parameters were also computed. The one-compartmental model with absorption was applied and it was parameterised in terms of absorption rate constant (Ka) and volume of distribution (V). Body weight, age, values of AST and ALT were defined as fixed effects for the population. Random effects (concentrations and pharmacokinetic parameters) were attributed to inter-individual variations.

A few models were developed to examine potential relationship between clinical and biochemical characteristics of individual animals such as body weight, age, AST and ALT and exposure to doxycycline. First, a model without covariables was created and after that covariates were added one by one and the level of signifi-

cance was tested. The Akaike criterion and the -2(LogLikelihood) were used to compare the models by means of model comparer option of the specialised software. Results were considered as statistically significant at P value of 0.05.

RESULTS

Model 1 was without covariables and it was used as a root model (Table 1). Further, it was tested for the effect of body weight or age on the random variables. Significant improvement of the model 1 was not observed when the impact of covariate age on *Ka* and *V* and body weight on *V* was tested (P=0.09).

The root model 1 was tested with covariates age, body weight, AST and ALT. According to the lowest values of Akaike criterion and -2(LogLikelihood), Model 1 with all covariates indicated the best fit between predicted and observed concentrations (Table 1 and Fig. 1). Model comparer showed significant difference (P=0.006) between Model 1 with covariates age, weight, AST and ALT and the same model without covariates. There was also a significant difference between Model 1 with covariate age and the model with all covariates (P=0.017).

DISCUSSION

Population approach is applied in veterinary medicine to characterise the variability in a population and to correlate different clinical factors with the pharmacokinetic parameters and for precise calculation of dosing regimens (Martín-Jiménez & Riviere, 1998). The published studies in farm animals are still limited to doxycycline pharmacokinetics in pigs (del Castillo *et al.,* 2006). Another advantage of the population analysis is the possibility for calculation of individual pharmacokinetic parameters on the basis of sparse data, after receiving of limited number of samples from individual animal, usually in zoo animals (Sanchez *et al.,* 2019). Realising the power of this analysis and facing of the main challenge for the veterinary profession – prudent and precise use of antibiotics, the current study aimed to evaluate different models for better prediction of doxycycline pharmacokinetics in sheep and their offspring.

Intravenous administration of doxycycline in calves was not associated with biotransformation, because only parent drug has been detected (Riond *et al.,* 1989). Metabolites of the antibiotic were not detected with the applied HPLC method in our study. Doxycycline is eliminated mainly by excretion through the bile and by secretion through the intestinal wall (Giguere *et al.,* 2006). Impact of changes in the relative amount of body water and fat on pharmacokinetics with the maturation of the body has been reviewed by Lu & Rosenbaum (2014). According to the cited authors higher body water composition in pediatric patients can change the distribution of the hydrophilic and hydrophobic drugs. Therefore covariables such as age and body weight can be added in the pharmacokinetic modelling. Young immature animals have higher water content in the body and less muscle mass than the adults, which can have significant impact on the volume of distribution of the drugs. Therefore model 1 was tested for the correlation between body weight and volume of distribution where body weight was added as fixed effect for V but insignificant improvement (P=0.09) of the model was observed.

Table 1. Population pharmacokinetic parameters of doxycycline administered orally at a dose rate of 10 mg/kg to healthy sheep (n=6) and lambs (n=6), and population pharmacokinetic models

Pharmacokinetic variable	Population parameter	Statistical criteria		Model without or with covariates
		-2(LL)	AIC	
Model 1, without covariates				
tvK_a (hr^{-1})	0.22	127.72	137.72	$Ka_i = 0.22*\exp(\eta Ka)$
$tvV(L*kg^{-1})$	3.24			$V_i = 3.24*\exp(\eta V)$
Model 1, covariate body weight (wt)				
$tvK_a(hr^{-1})$	0.22	124.84	136.84	$Ka_i = 0.22*\exp(\eta Ka)$
$tvV(L*kg^{-1})$	1.62			$V_i = 1.62*wt^{0.033}*\exp(\eta V)$
Model 1, covariate age				
$tvK_a(hr^{-1})$	0.22	121.87	135.87	$Ka_i = 0.22*age^{0.056}*\exp(\eta Ka)$
$tvV(L*kg^{-1})$	3.45			$V_i = 3.45*age^{0.140}*\exp(\eta V)$
Model 1, covariates age, body weight, AST, ALT				
$tvK_a(hr^{-1})$	2.50	108.05	132.05	$Ka_i = 2.50*age^{0.060}*AST^{-0.868}*ALT^{-0.198}*\exp(\eta Ka)$
$tvV(L*kg^{-1})$	2.66			$V_i = 2.66*age^{0.466}*wt^{-1.624}*AST^{1.418}*ALT^{0.585}*\exp(\eta V)$

Ka_i – absorption rate constant for individual subject; tvKa – population value of the absorption rate constant; V_i – volume of distribution for individual subject; tvV – population value of the volume of distribution; η - random effects (eta) for each individual; wt – body weight.

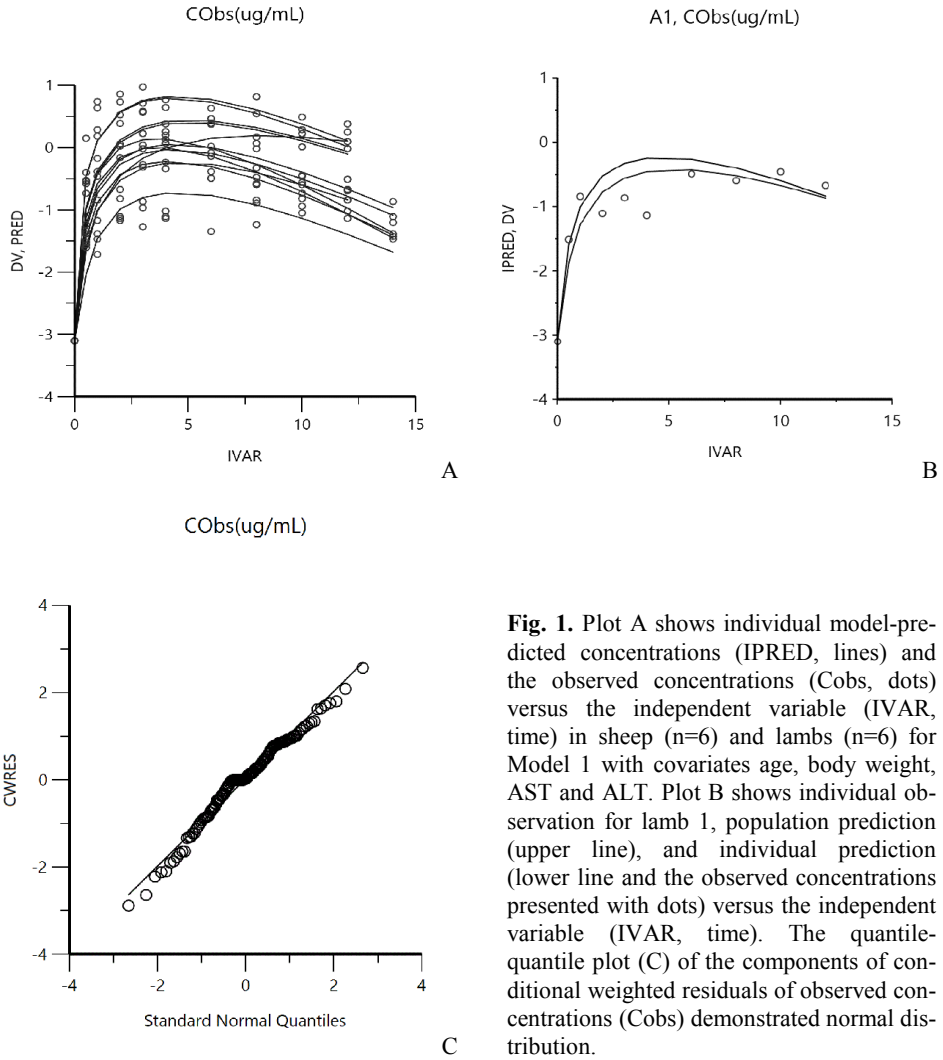

Fig. 1. Plot A shows individual model-predicted concentrations (IPRED, lines) and the observed concentrations (Cobs, dots) versus the independent variable (IVAR, time) in sheep (n=6) and lambs (n=6) for Model 1 with covariates age, body weight, AST and ALT. Plot B shows individual observation for lamb 1, population prediction (upper line), and individual prediction (lower line and the observed concentrations presented with dots) versus the independent variable (IVAR, time). The quantile-quantile plot (C) of the components of conditional weighted residuals of observed concentrations (Cobs) demonstrated normal distribution.

As a next step, more reliable biochemical parameters were tested for the correlation with pharmacokinetics of doxycycline. AST is found in liver but also in the skeletal muscle, in the kidneys and in the cardiac muscle (Giannini *et al.,* 2005). Therefore its values in sheep and lambs were correlated with volume of distribution. ALT is more informative for liver function and it was used as a marker for it in many clinical cases (Kozat & Denizhan, 2010).

Based on the published literature on age-related differences in these biochemical parameters in small ruminants, demonstrating immature function of the important organs in pre-ruminating animals (Abdel-Fattah *et al.,* 2013; da Cruz *et al.,* 2017), they were correlated with pharmacokinetic parameters in our investigation.

Therefore, a more advanced model based on Model 1 with addition of the individual values of covariables AST, ALT, body weight and age was tested and significant improvement of the model was observed. However limited number of animals were included in the current study and a large scale investigation is required to evaluate the reproducibility of these results. Further investigation should be performed with higher number of animals and a possibility for collection of few representative blood samples to minimise the stress and blood loss in very young lambs can be applied. Implication of population PK modelling in correlation with important biochemical markers and clinical characteristics can be used to improve the model for better prediction of pharmacokinetic parameters and to refine the treatment of sheep with doxycycline.

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REFERENCES

Abdel-Fattah, M. S., A. L. S. Hashem, Y. M. Shaker, A. M. Ellamei & H. Z. Amer, 2013. Effect of weaning age on productive performance and some plasma biochemical parameters of Barki lambs in Siwa Oasis, Egypt. *Global Veterinaria*, **10**, 189–202.

da Cruz, R. E. S., F. M. Rocha, C. V. B. Sena, P. G. Noleto, E. C. Guimarães, J. A. Galo & A. V. Mundim, 2017. Effects of age and sex on blood biochemistry of Dorper lambs. *Semina: Ciências Agrárias*, **38**, 3085–3094.

del Castillo, J. R., V. Laroute, P. Pommier, C. Zémirline, A. Keïta, D. Concordet & P. L. Toutain, 2006. Interindividual variability in plasma concentrations after systemic exposure of swine to dietary doxycycline supplied with and without paracetamol: A population pharmacokinetic approach. *Journal of Animal Science*, **84**, 3155–3166.

Giannini, E. G., R. Testa & V. Savarino, 2005. Liver enzyme alteration: A guide for clinicians. *Canadian Medical Association Journal*, **172**, 367–379.

Giguère, S., J. F. Prescott, J. D. Baggot, R. D. Walker & P. M. Dowling, 2006. Antimicrobial Therapy in Veterinary Medicine, Fourth Edition, Blackwell Publishing.

Kozat, S. & V. Denizhan, 2010. Glucose, lipid, and lipoprotein levels in sheep naturally infected with *Fasciola hepatica*. *Journal of Parasitology*, **96**, 657–659.

Laczay, P., G. Semjén, J. Lehel & G. Nagy, 2001. Pharmacokinetics and bioavailability of doxycycline in fasted and nonfasted broiler chickens. *Acta Veterinaria Hungarica*, **49**, 31–37.

Lu, H. Z. & S. E. Rosenbaum, 2014. Developmental pharmacokinetics in pediatric populations. *The Journal of Pediatric Pharmacology and Therapeutics*, **19**, 262–276 .

Martín-Jiménez, T. & J. E. Riviere, 1998. Population pharmacokinetics in veterinary medicine: potential use for therapeutic drug monitoring and prediction of tissue residues. *Journal of Veterinary Pharmacology and Therapeutics*, **21**, 167–189.

Mzyk, D. A., R. Gehring, L. A. Tell, T. W. Vickroy, J. E. Riviere, G. Ragan, R. E. Baynes & G. W. Smith, 2017. Considerations for extralabel drug use in calves. *Journal of the American Veterinary Medical Association*, **250**, 1275–1282.

Nassar, A. F., P. F. Hollenberg & J. Scatina, 2009. Drug Metabolism Handbook: Concepts and Applications, John Wiley & Sons, Inc., Hoboken, New Jersey.

Riond, J. L., K. Tyczkowska & J. E. Riviere, 1989. Pharmacokinetics and metabolic inertness of doxycycline in calves with mature or immature rumen function. *American Journal of Veterinary Research*, **50**, 1329–1333.

Sánchez, C. R., S. Zalesak, A. A. Pich & M. G. Papich, 2019. Population pharmacoki-

netics of itraconazole solution after a single oral administration in captive lesser flamingos (*Phoeniconaias minor*). *Journal of Veterinary Pharmacology and Therapeutics*, **42**, 1–6.

Woldehiwet, Z., 2010. In-vitro studies on the susceptibility of ovine strains of *Anaplasma phagocytophilum* to antimicrobial agents and to immune serum. *Journal of Comparative Pathology*, **143**, 94–100.

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