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Pharmacokinetic-pharmacodynamic integration and modelling of oxytetracycline for the calf pathogens *Mannheimia haemolytica* and *Pasteurella multocida*

Short running title: Oxytetracycline and calf pathogens

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ABSTRACT

A calf tissue cage model was used to study the pharmacokinetics (PK) and pharmacodynamics (PD) of oxytetracycline in serum, inflamed (exudate) and non-inflamed (transudate) tissue cage fluids. After intramuscular administration, the PK was characterised by a long mean residence time of 28.3h. Based on Minimum Inhibitory Concentrations (MICs) for six isolates each of *Mannheimia haemolytica* and *Pasteurella multocida*, measured in serum, integration of *in vivo* PK and *in vitro* PD data established area under serum concentration-time curve ($AUC_{0-\infty}$)/MIC ratios of 30.0 and 24.3h for *M.haemolytica* and *P.multocida*, respectively. Corresponding $AUC_{0-\infty}$/MIC ratios based on MICs in broth were 656 and 745h, respectively. PK-PD modelling of *in vitro* bacterial time-kill curves for oxytetracycline in serum established mean AUC_{0-24h}/MIC ratios for $3log_{10}$ decrease in bacterial count of 27.5h (*M.haemolytica*) and 60.9h (*P.multocida*). Monte Carlo simulations predicted target attainment rate (TAR) dosages. Based on the potency of oxytetracycline in serum, the predicted 50% TAR single doses required to achieve a bacteriostatic action covering 48h periods were 197mg/kg (*M.haemolytica*) and 314mg/kg (*P.multocida*) respectively, against susceptible populations. Dosages based on the potency of oxytetracycline in broth were 25- and 27-fold lower (7.8 and 11.5mg/kg) for *M.haemolytica* and *P.multocida,* respectively.

Key words: Oxytetracycline, calf, pharmacokinetics, pharmacodynamics, *M.haemolytica, P.multocida*

46 INTRODUCTION

47

48 The spectrum of activity of oxytetracycline includes two major bacterial species causing
49 bovine pneumonia, *Mannheimia haemolytica* and *Pasteurella multocida* (Nouws *et al.*, 1985;
50 Nouws *et al.*, 1985; Nouws *et al.*, 1990). Oxytetracycline remains in extensive use for the
51 treatment of calf pneumonia as it possesses the advantage of availability in both low (5-
52 10%w/v) and high (20-30%w/v) strength injectable products. The latter provide high dose
53 (20-30mg/kg) long acting formulations; single dose therapy may be clinically effective when
54 these formulations are administered intramuscularly. These depot formulations provide
55 sustained absorption from the intramuscular injection site, leading to flip-flop
56 pharmacokinetics (PK) (Nouws & Vree, 1983; Toutain & Raynaud, 1983; Nouws et al.,
57 1990).
58 Dosages for oxytetracycline were set many years ago and it may now be appropriate to re-
59 evaluate them in light of currently accepted PK/pharmacodynamic (PD) concepts.
60 Scientifically, the soundest approach to prediction of dosage for antimicrobial drugs (AMDs)
61 is to link PK parameters and variables with an appropriate PD index of potency and efficacy,
62 applying the universal equation for systemically acting drugs:

63 $$Dose = \frac{Cl \times AUC}{F} \quad (1)$$

64 Where Dose is the computed dose, Cl=body clearance, F=bioavailability and AUC=area
65 under plasma/serum concentration-time curve (Toutain & Bousquet-Melou, 2004). For those
66 AMDs for which the PK/PD index that best predicts efficacy is $AUC_{0\text{-}24h}$/MIC, such as
67 oxytetracycline in the present investigation (see Results and Discussion), this equation was
68 adapted by Aliabadi & Lees (2001; 2002) and Toutain & Lees to:

69 $$Dose_{(per\ day)} = \frac{Cl \times \frac{AUC_{(0-24h)}}{MIC_e} \times MIC_{distribution}}{f_u \times F} \quad (2)$$

where Cl=body clearance per h, $AUC_{0\text{-}24h}/MIC_e$ (in h) = *in vitro* ratio of experimentally determined area under the serum or broth concentration-time curve over 24h to the Minimum Inhibitory Concentration (MIC_e) of the tested experimental isolates for a target end-point (bacteriostatic or bactericidal effect), $MIC_{distribution}$=distribution of MICs of oxytetracycline from an epidemiological literature survey, f_u (from 0 to 1)=fraction of drug not bound to serum protein and F=bioavailability (from 0 to 1). MIC distributions for *P.multocida* (498 strains) and *M.haemolytica* (481 strains) were obtained from infected cattle; MICs were measured at the Iowa state Veterinary Diagnostic Laboratory Data from 2000, 2001, 2002 and 2003 (http://vads.vetmed.vt.edu/index.cfm). From this, it is clear that selection of an optimal dose depends on: (1) assessment of both PK (Cl, F, $f_{u,}$) and PD (MIC) properties; and (2) determination of an appropriate breakpoint value of the $AUC_{0\text{-}24h}$/MIC ratio for bacteriostatic or bactericidal effect.

The internationally accepted European Union Committee on Antimicrobial Testing (EUCAST) and the Clinical Laboratory Standards Institute (CLSI, 2004; CLSI, 2008) methods for MIC determinations are based on the use, almost universally, of non-biological growth media, such as Mueller Hinton Broth (MHB) (Papich, 2013; Papich, 2014). Whilst such media are specifically formulated to provide optimal i*n vitro* growth conditions, they differ in composition from body fluids. For example, most broths contain small amounts of protein including negligible amounts of albumin, whereas treatment of disease *in vivo* depends on drug concentration in the biological fluid of the biophase. Concentration in the latter is driven by the plasma concentration of free drug. As the protein bound fraction is microbiologically inactive, it is common to link the free rather than total serum concentration with an *in vitro* MIC (or MBC) value (f_u in equation 2). A potential problem with this approach is the assumption that the differences in MIC determined in broth, serum and the local biophase milieu are attributable solely to drug protein binding in the latter two fluids. It

is potentially flawed *additionally*, because artificial broths are quantitatively dissimilar to biological fluids in most chemical constituents (not only albumin, to which most drugs bind to some degree) and also in the absence of proteins such as serum complement, which may impact on drug potency. Therefore, bacterial growth and AMD action may commonly differ in differing growth matrices.

For the foregoing reasons, experiments in our laboratory have routinely compared MIC and MBC for calf pathogens in broth and biological fluids (serum, transudate and inflammatory exudate) obtained from calves, to provide more biologically relevant growth matrices and to identify any possible matrix effect (Aliabadi & Lees, 2002; Aliabadi *et al.*, 2003; Sidhu *et al.*, 2010; Brentnall *et al.*, 2012). The latter group reported that protein concentrations in exudate (44.7 g/L) and transudate (40.7 g/L) were lower than in calf serum (61.9 g/L). For example, for tulathromycin and the bovine pneumonia pathogens, *M.haemolytica* and *P.multocida,* serum:broth MIC ratios were of the order of 1:50, despite some 40% binding to serum protein (Illambas *et al.*, 2009). In stark contrast, for a single strain of *M.haemolytica,* oxytetracycline MICs (μg/mL) were higher in serum (14.8) exudate (12.8) and transudate (11.2) than in MHB (0.5) (Brentnall et al., 2012). These marked differences between artificial broth and biological fluids are both drug and microbial species dependent and cannot be explained by binding to plasma protein.

Determination of PD properties of oxytetracycline in biological matrices is therefore a pre-requisite for the use of PK-PD integration and modelling approaches to dose determination, aimed at eradication of bacteria and/or minimising opportunities for the emergence of antimicrobial resistance (Lees *et al.*, 2004; Martinez & Silley, 2010; Mouton *et al.*, 2011; Papich, 2014). For other drugs, smaller broth serum differences in potency have been reported, but it should be noted that a difference in MIC, between serum and broth, generally regarded as small in microbiological terms, could readily lead, when the objective is

prediction of dosage for bacteriological cure in diseased animals, to significant over or under estimation of dose required.

Three integrated PK-PD surrogates for clinical efficacy; maximum serum concentration (C_{max})/MIC, time of serum concentration exceeding MIC (T>MIC) as a percentage of the inter-dose interval, and area under curve (AUC)/MIC, the ratio of the area under the plasma/serum concentration-time curve to MIC (in steady-state conditions) have been widely used (Craig, 1998; Schentag, 2000; Frimodt-Moller, 2002; Lees & Shojaee Aliabadi, 2002; Mouton *et al.*, 2002; Toutain *et al.*, 2002; Toutain & Lees, 2004; Martinez & Silley, 2010; Mouton et al., 2011; Martinez *et al.*, 2012; Papich, 2014). This study focusses on AUC/MIC, as oxytetracycline has a long terminal half-life and it was shown that this index is the most appropriate for any AMD having a long terminal half-life (Nielsen & Friberg, 2013).

The objectives of this investigation were: (1) to establish the serum concentration-time profile and to derive PK data for oxytetracycline in 10 healthy calves after intramuscular administration at the dose rate of 20mg/kg; (2) to determine the rate and extent of oxytetracycline penetration into and elimination from carrageenan-inflamed (exudate) and non-inflamed (transudate) fluids in a tissue cage model; (3) to integrate these *in vivo* PK findings with *in vitro* PD (MIC) data for oxytetracycline against *M.haemolytica* and *P.multocida*; (4) to model *in vitro* time-kill profiles of oxytetracycline against six isolates each of *M.haemolytica* and *P.multocida* in both serum and MHB, in order to generate AUC/MIC breakpoints for each organism to achieve bacteriostatic and bactericidal levels of growth inhibition; (5) to use the derived PK and PD data, with epidemiological MIC distributions, to calculate, using Monte Carlo simulations, dosages of oxytetracycline for both an empirical (probabilist) therapeutic response i.e. taking into account the entire MIC distribution but also considering only susceptible subpopulations of *P.multocida* and *M. haemolytica.* Such dual simulations are necessary to investigate the clinical value of an

antimicrobial sensitivity test (AST) and also to determine its appropriate numerical value. Simulations were undertaken for: (a) each bacterial species; (b) two levels of growth inhibition (bacteriostatic and bactericidal); and (c) both a single dose (efficacious over the subsequent 48h) and a maintenance dose administered every 48h under steady-state conditions for 50 and 90% Target Attainment Rates (TARs).

MATERIALS AND METHODS

Animals and surgical procedures

An *in vivo* study was conducted in 10 healthy female Aberdeen Angus calves. Weights were in the range 145-204kg (mean=179kg, S.D.=16.7) and ages ranged from 79-131 days (mean =108, S.D.=15 days). Tissue cages were implanted subcutaneously in the paralumbar fossa, as previously described (Sidhu *et al.*, 2003). Oxytetracycline hydrochloride (Alamycin LA, Norbrook Laboratories Ltd., Newry, Co. Down, N. Ireland) was injected intramuscularly into gluteal muscles (two equal volumes into right and left muscles) at a dose rate of 20mg/kg at zero time. Also at zero time, 0.5mL of 1%w/v sterile lambda carrageenan solution in saline (Viscarin, Marine Colloids, Springfield, U.S.A.) was injected into a single tissue cage. This was used to harvest inflammatory exudate. A second, unstimulated cage was used to collect non-inflammatory extracellular fluid (transudate). The study was approved by the Royal Veterinary College Ethics Committee.

Sampling procedures

Blood samples (10mL) were collected, protected from light, from a jugular vein, into vacutainers (Becton, Dickinson and Company, Oxford, Oxon, U.K.) without anticoagulant, prior to and at times of 15, 30 and 45min and 1, 1.5, 2, 4, 6, 8, 10, 12, 24, 32, 48, 72, 96 and 120h after injection of oxytetracycline. Exudate and transudate samples (1.5mL) were

collected, protected from light, before and at pre-determined times of 2, 4, 6, 8, 10, 12, 24, 32, 72, 96 and 120h. All samples were centrifuged to remove cells at 2,000g for 10min at 4°C and supernatants were stored at -70°C until analysed for oxytetracycline.

Analysis of oxytetracycline

A high pressure liquid chromatography (HPLC) method with ultraviolet detection was used for analysis of oxytetracycline concentrations in serum, exudate and transudate (Brentnall et al., 2012). All reagents were HPLC grade and obtained from Sigma-Aldrich Chemicals (Poole, Dorset, UK). Chromatographic data were analysed using Chromeleon^{TM} Version 6.80 (Dionex Corporation) and concentrations of oxytetracycline were calculated using peak area ratios. Standards were prepared by spiking blank serum, exudate and transudate with oxytetracycline, using eight concentrations over the range 0.1 to 25µg/mL (serum) and 0.1 to 5µg/mL (exudate and transudate). They were run with every assay to evaluate linearity and reproducibility. For linearity r^2 was >0.98. The lower limit of quantification (LLOQ) for oxytetracycline in all three fluids was 0.1µg/mL. The LLOQ had a coefficient of variation of less than 20% and all other standards were less than 15% of nominal concentration. The intra- and inter-assay percentage inaccuracies were 3.50% and 9.57%, respectively, at a concentration of 10µg/mL, 1.43% and 4.78%, respectively, at a concentration of 5µg/mL and 2.06% and 10.6%, respectively, at a concentration of 0.1µg/mL.

Pharmacokinetic analyses

Oxytetracycline concentration-time data in serum, exudate and transudate in individual calves were analysed using the WinNonlin® regression programme (version 5.2, Pharsight Corporation, Mountain View, California, USA). Data for each fluid were submitted to non-compartmental analysis using the statistical moment approach described by (Yamaoka *et al.*,

1978). The linear trapezoidal rule was used to calculate AUC values and area under the first moment curve (AUMC). The mean residence time (MRT) was determined as AUMC/AUC.

PK-PD integration

The PK-PD surrogates C_{max}/MIC, $AUC_{0\text{-}24h}$/MIC (first 24h after dosing) and $AUC_{0\text{-}\infty}$/MIC were calculated for each fluid (serum, exudate and transudate) harvested in the tissue cage study from 10 calves. Results were expressed as ratios of geometric mean C_{max}, $AUC_{0\text{-}24h}$ and $AUC_{0\text{-}\infty}$ for individual calves (n=10) and geometric mean MIC (n=6 for each bacterial species). Geometric means were selected for measurements which are lognormally distributed. In addition, the ratios of average serum concentration (C_{av})/MIC, for four consecutive 24h periods after administration of oxytetracycline, were calculated.

PK-PD modelling of in vitro time-kill data

For six isolates each of *M.haemolytica* and *P.multocida* growth inhibition curves over 24h were determined in two matrices, MHB and calf serum, as previously described (Lees et al., 2015). Ratios of $AUC_{0\text{-}24h}$/MIC were calculated for each of the six isolates of the two organisms at each of the five oxytetracycline concentrations tested (from 0.25 to 4xMIC multiples). $AUC_{0\text{-}24h}$ values were computed in terms of MIC multiples (*vide infra*). The data were modelled to the sigmoidal E_{max} equation (Equation 3) using the non-linear regression WinNonlin® programme:

$$E = E_0 + \frac{E_{max} \times X^N}{EC_{50}^N + X^N}. \quad (3)$$

where E_0 is the bacterial growth after 24h incubation in the absence of oxytetracycline (control samples), expressed as $\log_{10}$cfu/mL subtracted from the initial inoculum $\log_{10}$ cfu/mL; E_{max} is the maximum antimicrobial growth inhibition determined as the change in $\log_{10}$cfu/mL after 24h incubation with oxytetracycline; EC_{50} is the $AUC_{0\text{-}24h}$/MIC value

220 providing 50% of the maximum antibacterial effect; X is the predictive variable (expressed as
221 $AUC_{0\text{-}24h}$/MIC) and N is the Hill coefficient, which describes the slope of the $AUC_{0\text{-}24h}$/MIC-
222 effect curve. Bacteriostatic (E=0, no change form initial inoculum count), bactericidal (E=-3,
223 a $3\log_{10}$ reduction from initial inoculum count) and E=-4, a $4\log_{10}$ reduction from initial
224 inoculum count $AUC_{0\text{-}24h}$/MIC values, were determined for each isolate of each organism in
225 MHB and serum. E=-4, a $4\log_{10}$ reduction in count, represents a 10,000-fold decrease from a
226 starting count of 10^7 cfu/mL to a count of 10^3 cfu/mL; therefore it does not indicate virtual
227 eradication.
228 The $AUC_{0\text{-}24h}$/MIC values are proportionality factors between the MIC of the test pathogen
229 (i.e. AUC/MIC_e in equation 2) and the average MHB or serum oxytetracycline concentration
230 required to achieve each level of growth inhibition. From the $AUC_{0\text{-}24h}$/MIC values, the
231 average concentrations corresponding to the three levels of kill over 24h were calculated and
232 expressed as multiples of MIC by dividing each value of $AUC_{0\text{-}24h}$/MIC by 24h (Toutain *et*
233 *al.*, 2007).
234
235 *Dosage prediction using Monte Carlo simulations*
236 *General principles*
237 Equation 1 (see Introduction) is the general equation used to determine dosage for
238 systemically acting drugs. For those AMDs, for which the PK-PD index that best predicts
239 efficacy is $AUC_{0\text{-}24h}$/MIC, such as oxytetracycline in the present investigation, this equation
240 was adapted to Equation 2 (see Introduction).
241 *Dosage determination using a steady state approach (48h dosing interval)*
242 In Equation 2, the term $AUC_{0\text{-}24h}$/MIC (h) is the experimentally determined PK-PD index to
243 be achieved, expressed as the ratio of area under the serum concentration-time curve over 24h
244 to MIC, obtained using a test pathogen for a given bacteriological effect (bacteriostatic,

bactericidal or $4\log_{10}$ reduction in count). For greater clarity, we replaced the $AUC_{0\text{-}24h}$/MIC ratio in h, by a more readily understood dimensionless equivalent PD factor: κ_{PD}, (Toutain et al., 2007). κ_{PD} is obtained by dividing $AUC_{0\text{-}24h}$/MIC in h by 24h and this requires, for consistency, serum clearance to be expressed per day (Cl_{day}) where Cl_{day}=24h x Cl expressed per h as for equation 2, when the computed dose is a daily dose. Hence, κ_{PD} represents the scaling factor by which the clinical MIC (or any MIC from the MIC distribution) should be multiplied to obtain the appropriate serum concentration to be achieved for a given PD effect (bacteriostatic, bactericidal or $4\log_{10}$ reduction in count). When κ_{PD} is substituted in Equation 2, it yields:

$$Dose_{(maintenance\ per\ day)} = \frac{Cl_{day}}{F} \times K_{PD} \times \frac{MIC_{distribution.}}{f_u} \quad (4)$$

where $Dose_{(maintenance\ per\ day)}$ is a daily maintenance dose in steady-state equilibrium conditions. The expression can be extended to time intervals longer than 24h (Toutain et al., 2007). For the long-acting formulation of oxytetracycline used in this study, with a recommended interval of 48h between two doses at steady-state, Cl_{day} is substituted in equation 5 by Cl_{48h} (where Cl_{48h} = 48h x Cl expressed per h as for equation 2).

$$Dose_{(maintenance\ per\ 48h)} = Cl_{(48h)} \times \frac{K_{PD} \times MIC_{distribution}}{F \times f_u} \quad (5)$$

Dosage determination for a single dose (active over the first 48h period)

It is relevant, for a long-acting formulation, to estimate the *single* dose required to achieve bacteriostatic, bactericidal and $4\log_{10}$ reductions in count over the first dosage interval (in this case 48h) *i.e.* before reaching steady-state conditions, if achieved. This first dose is a loading dose, whilst the dose computed by equation 5 is a maintenance dose. The ratio between the loading dose and the maintenance dose is equal, by definition, to the accumulation ratio and for the present formulation is indicated by equation 6 (Toutain & Bousquet-Mélou, 2004):

$$R = \frac{AUC_{(loading\ dose)}}{AUC_{(maintenance\ dose)}} \quad (6)$$

Assuming that administration of the dose *n+1* occurs at a time after which the distribution of the previous dose *n* is complete (pseudo-steady state) the accumulation ratio can be simplified as per equation 7:

$$R = \frac{1}{1 - \exp(-K_{10} \times \tau)} \quad (7)$$

with k_{10} expressed in h^{-1} and τ is the dosing interval in h. Therefore, R is dimensionless. For further explanation see Lees et al. (Lees *et al.*, 2015). Combining equations 6 and 7 and assuming PK linearity (clearance identical with two dose levels), the loading dose for 48h effect for the i^{th} calf $Dose_{i(\text{loading dose})}$ is calculated from equation 8:

$$\text{Dose}_{(\text{loading dose 48h})} = \frac{1}{1 - \exp(-K_{10} \times \tau)} \times \text{Dose}_{(\text{maintenance per 48h})}$$

i.e.

$$\text{Dose}_{(\text{loading dose 48h})} = \frac{1}{1 - \exp(-K_{10} \times 48)} \times Cl_{(48h)} \times \frac{K_{PD} \times MIC_{distribution}}{F \times f_u} \quad (8)$$

Monte Carlo simulation for the two approaches to dose estimation:

Dosages were computed using Monte Carlo simulations in Oracle Crystal Ball (Oracle Corporation, Redwood Shores, CA, USA). The maintenance dose (per 48h) was calculated using equation (5) and the loading dose (for 48h interval) was calculated using equation 8. Loading and maintenance doses were determined to achieve bacteriostatic and bactericidal responses. The probabilistic approach took into account the different distribution of variables embedded in Equations 5 and 8. The average point estimate of the serum κ_{PD} was calculated from the data obtained with four isolates of each species, but variability in κ_{PD} was not included in the Monte Carlo simulation, as the number of isolates was small and inter-isolate variability was low. The distribution of individual plasma clearances within the sample population (10 calves in the present study) was included for calculation of the maintenance

293 dose (Equation 5). The observed statistical distribution of products of individual serum
294 clearance by individual accumulation ratio for a 48h dosing interval (determined by
295 individual k_{10} values) was incorporated for calculation of the loading dose (Equation 8).
296 The distribution of field MIC values for *M.haemolytica* and *P.multocida* (considered
297 separately) were included in the simulation. $MIC_{distribution}$ is the MIC for *M.haemolytica* (481
298 isolates) and *P.multocida* (498 isolates); these were published online by the Iowa State
299 Veterinary Diagnostic Laboratory data (2000-2003) and are represented in Fig. 2a and 2b.
300 These distributions reflect the current U.S.A. situation and prompted us to determine the
301 corresponding susceptible wild-type population; the latter is expected to be the same
302 throughout the world, see Discussion). This wild-type distribution was statistically
303 determined by calculation of the 99.9% wild-type cut-off values plotted in Fig. 2c and 2d
304 (Turnidge *et al.*, 2006). Only the MIC distribution of wild type bacteria was included in the
305 simulation, corrected by the experimentally determined value of f_u to allow for
306 oxytetracycline protein binding in serum, as the reported MIC literature values were
307 determined in broth. A further correction factor was applied to account for MIC differences
308 between broth and serum for both species. The probabilities of distribution for each dosage
309 estimation were run for 50,000 simulated trials.
310 *Figure 1*
311 *Statistical analyses*
312 PK variables are presented as geometric, harmonic or arithmetic means and SD. MIC and
313 MBC data are presented as geometric means and SD. Differences in MIC and MBC values
314 between MHB and serum were compared with the paired t-test or the non-parametric
315 Wilcoxon test, depending on whether the data passed a normality test. Mean differences in
316 $AUC_{0\text{-}24h}$/MIC ratios determined in MHB compared with those determined in serum for
317 bacteriostatic, bactericidal and $4log_{10}$ reductions in count were compared by ANOVA.

318

319 RESULTS

320 *Pharmacokinetics*

321 The mean (±SEM) concentrations of oxytetracycline in calf fluids after intramuscular
322 administration at a dose rate of 20mg/kg are presented in Fig. 2. PK variables are presented in
323 Table 1. In 6 of 10 calves the serum concentration-time profile was characterised by two
324 peaks, the first occurring within 1h and the second between 1.5 and 4h.
325 Oxytetracycline penetration into exudate and transudate was quantitatively similar. Exudate
326 and transudate C_{max} were significantly lower than peak serum concentration ($P<0.01$).
327 However, from 32 to 120h oxytetracycline concentrations in tissue cage fluids were greater
328 than those in serum (Fig.1). Numerically lower $AUC_{0\text{-last}}$ values were obtained in exudate and
329 transudate, 125µg.h/mL and 105µg.h/mL, respectively, compared to 153µg.h/mL in serum,
330 but these differences were not statistically significant ($P>0.05$). For all three fluids, the
331 percentage of $AUC_{0\text{-}\infty}$ occurring after the last sampling time (120h) was <12%. Mean
332 residence times were similar in exudate and transudate and both were significantly longer
333 ($P<0.01$) than MRT in serum (Table1).

334 *Table 1*

335 *Fig.2*

336 *PK-PD integration*

337 PK-PD integration established the surrogates, C_{max}/MIC, T>MIC, $AUC_{0\text{-}24h}$/MIC (first 24h)
338 and $AUC_{0\text{-}\infty}$/MIC, derived from *in vivo* oxytetracycline serum concentrations in the PK study
339 and *in vitro* MICs of the test organisms measured in both MHB and serum. Data are
340 presented in Appendix 1.
341 Average oxytetracycline concentrations (C_{ave}) in serum in the PK study, over four successive
342 24h time periods, from 0-24 to 72-96h, were determined. Based on MHB MICs, C_{ave}/MIC

ratios exceeded 1.5:1 up to 72-96h, whereas based on serum MICs the ratios were less than 1:1 for all four time intervals (Table 2). Ratios of oxytetracycline C_{ave} in exudate and transudate relative to mean MICs for *M.haemolytica* and *P.multocida* over each of the five successive time periods, from 0-24 to 96-120h, were greater than 1:1 for all periods based on MHB MICs but less than 0.4:1 for all periods based on serum MICs (data not shown).

Table 2

PK-PD modelling and dosage determination

Time-kill curves for oxytetracycline for six isolates each of *M.haemolytica* and *P.multocida* were determined in MHB and calf serum (data reported in Lees et al., 2016a). The killing patterns were judged to be co-dependent. Values of $AUC_{0\text{-}24h}$/MIC producing three levels of bacterial kill [bacteriostatic, $3\log_{10}$ reduction (bactericidal) and $4\log_{10}$ reduction from initial inoculum count] were determined for both MHB and serum (Tables 3 and 4). For *M.haemolytica* 3 or $4\log_{10}$ reductions in count were not obtained for all isolates (Table 3). Mean $AUC_{0\text{-}24h}$/MIC serum values (with $AUC_{0\text{-}24h}$ expressed in terms of multiple of MIC for a given matrix and the MIC of the test bacteria for the same matrix) producing bacteriostatic and bactericidal responses for *M.haemolytica* were 19.1 and 27.5h, respectively, corresponding to average concentrations over 24h incubation (κ_{PD} values) of 0.79 and 1.15 multiples of MIC for the given matrix (Toutain et al., 2007). Corresponding $AUC_{0\text{-}24h}$/MIC and κ_{PD} values using MHB as growth medium were 25.2 and 46.0h and 1.05 and 1.92, respectively. For both matrices and both pathogens, bacteriostatic and bactericidal effects were obtained with concentrations of the same order of magnitude and observed differences are likely due to the limited precision of the killing curve measurements.

Tables 3 and 4

Predicted doses for both single dose administration and dosing at steady state are presented in Table 5. For single administration (duration of action of 48h), the Monte Carlo derived doses

368 for TARs of 50 and 90% providing a bacteriostatic action against *M.haemolytica* were 197
369 and 283 mg/kg, respectively, based on serum MICs of the sensitive population (Table 5).
370 However, based on broth MICs, corresponding values were much lower, 7.81 and 11.24
371 mg/kg (Appendix 2). Higher dosages were required for TARs to provide a bactericidal level;
372 for MICs determined in serum 50 and 90% TARs were 314 and 452 mg/kg, respectively.
373 For *P.multocida* and a bacteriostatic action with single dose administration and a duration of
374 action of 48h, 50 and 90% TARs were 314 and 682 mg/kg, based on serum MICs (Table 5).
375 However, based on broth MICs corresponding values were much lower, 11.5 and 24.9 mg/kg
376 (Appendix 2). As for *M.haemolytica,* for a bactericidal action, higher doses were predicted.
377 As expected from the accumulation ratio over a dosing interval of 48h (approximately 1.5-
378 1.6), the predicted alternate day doses, at steady state, were lower than those calculated for
379 the single dose approach. Thus, based on serum MICs and a bacteriostatic action, TARs of 50
380 and 90% were 125 and 141 mg/kg for *M.haemolytica* and 200 and 424 mg/kg for *P.multocida*
381 (Table 5). Much lower doses were predicted for alternate day administration at steady state
382 based on broth MICs. Predicted doses were 4.97 and 5.58 mg/kg for *M.haemolytica* for 50
383 and 90% TARs for bacteriostasis. Corresponding predicted doses were 7.28 and 15.4 mg/kg
384 for *P.multocida* (Appendix 2).
385 *Table 5*
386
387 DISCUSSION
388 *Pharmacokinetics*
389 Tissue cages comprise hollow perforated devices, which become surrounded by and partially
390 infiltrated with granulation tissue, when implanted subcutaneously (Higgins *et al.*, 1984; Lees
391 *et al.*, 1987). When using tissue cages to study the extravascular distribution of drugs, it is
392 important to recognise that the time courses of penetration into and removal from tissue cage

393 fluid are model (shape) dependent. Thus, solute (including drug) penetration and elimination
394 rates vary with each drug/solute, tissue cage age, size, location and geometry, most notably
395 with surface area:volume ratio of the cage.
396 Intracaveal injection of the mild irritant carrageenan provides an ethical means of generating
397 and readily sampling inflammatory exudate (Lees et al., 1987; Sidhu et al., 2003). The tissue
398 cage model therefore provides a mean of studying a possible matrix effect when investigating
399 *ex vivo* PD of AMDs not only in serum (which is not the ultimate site of AMD action) but
400 also in matrices that better reflect composition of the AMD biophase for extracellular
401 pathogens namely exudate (in the presence of inflammation as appropriate for curative
402 treatment) and transudate (in the absence of inflammation as appropriate for prophylaxis and
403 for metaphylaxis) (Aliabadi et al., 2003; Sidhu et al., 2010; Brentnall et al., 2012). The tissue
404 cage model thus facilitates comparison of PD data with findings generated in non-biological
405 growth matrices, such as MHB.
406 The serum concentration-time profile of oxytetracycline, using a high strength depot
407 formulation, was similar to those reported in earlier studies with the same dose rate of 20
408 mg/kg administered intramuscularly (Nouws & Vree, 1983; Toutain & Raynaud, 1983;
409 Davey *et al.*, 1985; El Korchi *et al.*, 2001; Mestorino *et al.*, 2007; Brentnall et al., 2012).
410 Toutain and Raynaud (1983) reported that oxytetracycline absorption occurred in two phases;
411 the first was rapid and the second slower phase led to a flip-flop PK profile. The findings in
412 this study, likewise, indicated rapid initial absorption and, in most animals, two early
413 concentration peaks. It is very likely that, as in previous studies, the PK profile was flip-flop,
414 with slow passage of the drug into solution at the injection site (Nouws et al., 1990). Thus, in
415 the previous studies and the present investigation, the terminal half-life, representing a slow
416 absorption phase, was prolonged, ranging from 21.7h (Brentnall et al., 2012) to 30.1h (this
417 investigation).

418

419 *PK-PD integration*

420 The underlying cause(s) of marked serum/MHB differences in potency of oxytetracycline, as
421 reflected in MICs, have not been established. Approximately two-fold higher MICs in serum
422 compared to MHB would be anticipated from the binding of oxytetracycline to serum
423 proteins, which was shown to be 53% of total concentration in calves (Lees et al., 2016). This
424 is well short of the approximately 25-fold differences in MIC obtained experimentally (Lees
425 et al., 2016). Serum/MHB MIC (µg/mL) ratios were 6.75/0.25 (*P.multocida*) and 5.46/0.22
426 (*M.haemolytica*).
427 Mean serum MIC of *M.haemolytica* in this study was 5.46µg/mL. Esaki et al. (2005) reported
428 MIC_{50} and MIC_{90} values, in broth, of 0.25 and 32µg/mL for oxytetracycline against 27
429 bovine strains of *M.haemolytica*. If MICs of these strains in serum had been 25 times greater
430 than in artificial growth media (as for the six strains used in this investigation), the
431 corresponding predicted MICs would be 6.3µg/mL (MIC_{50}) and 800 µg/mL (MIC_{90}).
432 Similarly, in the data from Iowa State University, broth MICs were ≥8µg/mL for 50% of
433 *M.haemolytica* and 38% of *P.multocida* isolates; applying the 25-fold broth/serum scaling
434 factor equates to >200µg/mL for a significant proportion of field isolates.
435
436 The most appropriate PK/PD index to correlate with clinical efficacy depends on AMD
437 terminal half-life; when this is relatively long, as for oxytetracycline in this study, AUC/MIC
438 ratio is the index of choice (Nielsen & Friberg, 2013). From the present data, the predicted
439 clinical efficacy of oxytetracycline *in vivo* would be at most slight, insofar as it depends on
440 both serum MIC and a direct inhibitory action on cell division. This conclusion was
441 confirmed in a previous study by *ex vivo* findings; time-kill curves obtained with near

maximum oxytetracycline concentrations in serum produced little or no growth inhibition of *M.haemolytica* and *P.multocida* isolates (Lees et al., 2016).

PK/PD modelling

For both *M.haemolytica* and *P.multocida* a bacteriostatic action was achieved with $AUC_{0\text{-}24h}$/MIC values in the range 19.1 to 28.0h in both MHB and serum. Breakpoint $AUC_{0\text{-}24h}/MIC_e$ values for a bactericidal action were 46.1h (MHB) and 27.5h (serum) for *M.haemolytica* and 25.8h (MHB) and 60.9h (serum) for *P.multocida*. Also of potential clinical significance is the inter-isolate within-species variability in breakpoint values, which was greater for *M.haemolytica* than *P.multocida*. However, these differences, for a small number of isolates, remain to be confirmed with more isolates in future studies and were not taken into account in our Mont Carlo simulations.

Dosage prediction

Predicted (TAR) doses for oxytetracycline were calculated using scientific literature values for oxytetracycline MIC distributions together with data from this study for PK variables (Cl/F and f_u) and PK-PD breakpoints ($AUC_{0\text{-}24h}/MIC_e$). Fifty and 90% TAR dosages were calculated for steady state and for single doses with a duration of action of 48h in both cases. All doses based on oxytetracycline MICs in serum were some 25-fold greater than doses based on MICs measured by the CLSI method in broth. For example, for single dosing and a period of 48h the 90% TAR dosages for a bactericidal action (serum first, broth second) were 452 and 17.9 mg/kg (*M.haemolytica*) and 1,523 and 55.6 mg/kg (*P.multocida*).

Despite these considerations, it should be noted that oxytetracycline is usually classified as a bacteriostat and it is therefore assumed that efficacy will generally require the support of the body's natural defence mechanisms. Moreover, the challenge presented to the killing action

of oxytetracycline in our time-kill experiments, with a starting inoculum count of the order of 10^7cfu/mL, may be described as heavy, in comparison with bacterial load in clinical subjects with natural infection. It is also approximately 100-fold higher than the inoculum count recommended for AMD PD studies by CLSI, the higher count being deliberately selected to represent a heavy load in this study. In those cases where infection is mild and treated early, when biophase bacterial counts would be predicted to be low, as discussed by Mouton et al. (2011), Martinez et al. (2012) and Papich (2013; Papich, 2014) lower doses of oxytetracycline are likely to suffice. Nevertheless, the calculated doses based on serum data were considerably higher than the recommended dose rate of 20 mg/kg oxytetracycline. These high dosages for both 50 and 90% TARs were calculated using the oxytetracycline epidemiological MIC distributions for *P.multocida* and *M.haemolytica* measured from 2000 to 2003 and published on the Veterinary Antimicrobial Decision Support Website (http://vads.vetmed.vt.edu/index.cfm). Distributions were bimodal, with 39-50% of isolates having broth MICs of 8µg/mL or greater and 48-55% with MICs of 1µg/mL or less. This suggests that the wild-type populations for *P.multocida* and *M.haemolytica* are characterised by a MIC of approximately 1µg/mL or less. In this regard, the MICs of the related drug, tetracycline, are of interest. Isolates obtained from four USA and one Canadian regions, yearly over a 10 year period, had similar bimodal distributions for *P.multocida* and *M.haemolytica,* with MICs of the order of ≤1.0µg/mL for approximately 50% of isolates and ≥8.0 µg/mL for some 30-50% of isolates (Portis *et al.*, 2012).

These data suggest that epidemiological information obtained for tetracycline might also be relevant for oxytetracycline. In this regard, de Jong et al. (de Jong *et al.*, 2014) reported for EU tetracycline isolates essentially unimodal distributions for *P.multocida* and *M.haemolytica* of bovine origin; 94 and 84 % of isolates, respectively, had MICs of 2µg/mL or less, which is consistent with a wild type distribution for *P.multocida* and *M.haemolytica* .

This could be explained by the fact that these authors collected samples from diseased or recently deceased calves not exposed to AMD treatment for at least 15 days prior to sampling i.e. having not been subjected to any selective pressure with an enrichment of less susceptible pathogens to oxytetracycline. We are not aware of any recent data of EU origin for oxytetracycline against these species but EUCAST provides a cut-off value for *M.haemolytica* for tetracycline of 2µg/mL and the EUCAST distribution for oxytetracycline for *P.multocida* also suggests a cut-off of 2µg/mL. The MIC distribution of field strains represents isolates that might be submitted to the laboratory in cases of failure with first intention treatment and for this reason the Monte Carlo simulations were performed using only the wild type sub-population. It should be noted that Epidemiological Cut Off values are useful tools for epidemiologists but clinicians require clinical breakpoints.

Clinical efficacy of oxytetracycline

In early field studies, usually with small animal numbers, oxytetracycline was reported as effective for metaphylaxis and therapy in cases of calf pneumonia, as assessed by resolution or improvement of clinical signs (Laven & Andrews, 1991; Morck *et al.*, 1993; Deleforge *et al.*, 1994; Musser *et al.*, 1996). On the other hand, O'Connor et al. (O'Connor *et al.*, 2013) used a mixed treatment comparison meta-analysis to compare the efficacy of 12 AMD treatments versus a non-active control for bovine respiratory disease in beef cattle. They concluded that oxytetracycline had the lowest ranking (11.24 with a credibility interval of 9-13) close to the ranking of the non-active control (12.52 with a credibility interval of 11-13). They also drew attention to the lack of recent data for oxytetracycline.

These clinical findings and the present data focus consideration on possible mechanisms of action of oxytetracycline, in addition to its direct growth inhibiting action, as discussed previously (Brentnall et al., 2012; Lees *et al.*, 2015). Drugs of the tetracycline group have

been shown to possess anti-inflammatory and host immune modulating actions, as well as reducing pathogen ability to attach to host cells Furthermore, in limited support of the 20mg/kg dose of oxytetracycline, in a *M.haemolytica*-induced model of calf pneumonia, the bronchial secretion count of *M.haemolytica* was reduced from 4.10^6 to 1.10^3 cfu/mL at 48h and rectal temperature rise was decreased by 0.5°C, compared to nil treatment. However, oxytetracycline did not reduce the bacterial count in lung tissue.

In summary, it is concluded, that oxytetracycline doses for a direct killing action, based on PK/PD relationships and using serum MIC data, are not achievable in clinical use. Moreover, it is unlikely that Antimicrobial Sensitivity Testing for this drug, against the calf pneumonia pathogens, *M.haemolytica* and *P.multocida,* can be used to predict clinical efficacy.

Conflict of interest statement

The authors have no conflicts of interest.

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683

684

685 **Table 1.**

686 Pharmacokinetic parameters for oxytetracycline in serum, exudate and transudate (geometric

687 mean, unless stated, and SD, n=10)

Variable	Serum		Exudate		Transudate	
(units)	Mean	SD	Mean	SD	Mean	SD
C_{max} (µg/mL)	5.23	0.61	2.20	0.31	2.09	0.38
T_{max} (h)*	3.60	0.84	11.6	0.84	10.99	1.93
$T_{½}$ (h)**	30.10	10.23	31.4	5.47	34.75	8.65
$AUC_{0\text{-last}}$ (µg.h/mL)	153.2	17.22	125.2	21.22	105.24	16.05
$AUC_{0\text{-}\infty}$ (µg.h/mL)	163.9	16.05	138.3	22.98	118.8	16.55
$AUC_{0\text{-}24}$ (µg.h/mL)	86.98	10.69	40.7	5.12	36.71	7.01
$AUC_{0\text{-}48}$ (µg.h/mL)	121.90	16.32	78.4	10.78	68.86	10.51
$MRT_{(0\text{-last})}$ (h)*	28.31	2.12	42.3	2.39	40.7	1.16
Cl/F (mL/kg/h)	122.0	10.83	NA	-	NA	-

688 *Arithmetic mean **Harmonic mean

689 T_{max}: Time following dosing at which the maximum concentration (C_{max}) occurred.

690 $T_{½}$: Half-life

691 $AUC_{0\text{-last}}$: Area under the concentration-time graph from 0 to the last sample

692 $AUC_{0\text{-}\infty}$: Area under the concentration-time graph from 0 to infinity

693 $AUC_{0\text{-}24}$: Area under the concentration-time graph from 0 to 24h

694 $AUC_{0\text{-}48}$: Area under the concentration-time graph from 0 to 48h

695 MRT: Mean residence time

696 CI/F: Clearance scaled by bioavailability

697

698 **Table 2**

699 Average serum oxytetracycline concentration (C_{ave})/MIC ratios for four consecutive 24h

700 periods after oxytetracycline administration (n=10 calves)

		C_{ave}/MIC			
Time period after dosing (h)		0-24	24-48	48-72	72-96
P.multocida	Based on mean serum MIC (6.75µg/mL)	0.54	0.22	0.10	0.06
	Based on mean MHB MIC (0.25µg/mL)	14.6	5.88	2.71	1.52
M.haemolytica	Based on mean serum MIC (5.46µg/mL)	0.67	0.27	0.12	0.07
	Based on mean MHB MIC (0.22µg/mL)	16.6	6.68	3.08	1.73

701

Table 3

PK-PD modelling of in vitro time-kill data (mean and SD, n=6 unless stated) for three levels of growth inhibition of *M.haemolytica* by oxytetracycline in MHB and serum

Variable	MHB		Serum	
	Mean	SD	Mean	SD
Log E_{max} (cfu/mL)	-4.36	0.97	-5.08	3.88
Log E_0 (cfu/mL)	1.73	1.07	0.89	0.91
Log E_{max} – log E_0 (cfu/mL)	-6.10	0.70	-5.94	4.52
$AUC_{0\text{-}24h}$/MIC for bacteriostatic action (h)	25.2	15.19	19.1	18.30
$AUC_{0\text{-}24h}$/MIC for 3log_{10} count reduction (h)	46.0	22.76	27. 5*	15.95
$AUC_{0\text{-}24h}$/MIC for 4log_{10} count reduction (h)	71.3*	33.98	N.D.	-
N (slope)	7.95	6.05	8.17	7.50

*n=4; ND=not determined

707 **Table 4**

708 PK-PD modelling of *in vitro* time-kill data (mean and SD, n=6 unless stated) for three levels

709 of inhibition of *P.multocida* by oxytetracycline in MHB and serum

Measurement	MHB		Serum	
	Mean	SD	Mean	SD
Log E_{max} (cfu/mL)	-5.48	1.00	-4.35	1.98
Log E_0 (cfu/mL)	1.93	0.67	2.63	1.50
Log E_{max} – log E_0 (cfu/mL)	-7.41	0.69	-6.96	2.42
$AUC_{0\text{-}24h}$/MIC for bacteriostatic action (h)	19.2	11.53	28.0	3.43
$AUC_{0\text{-}24h}$/MIC for 3log_{10} count reduction (h)	25.8	10.75	60.9*	12.65
$AUC_{0\text{-}24h}$/MIC for 4log_{10} count reduction (h)	30.2	13.78	N.D.	
N (slope)	13.47	8.08	4.55	4.44

710 *n=5; ND=not determined

711

712

713 **Table 5.**

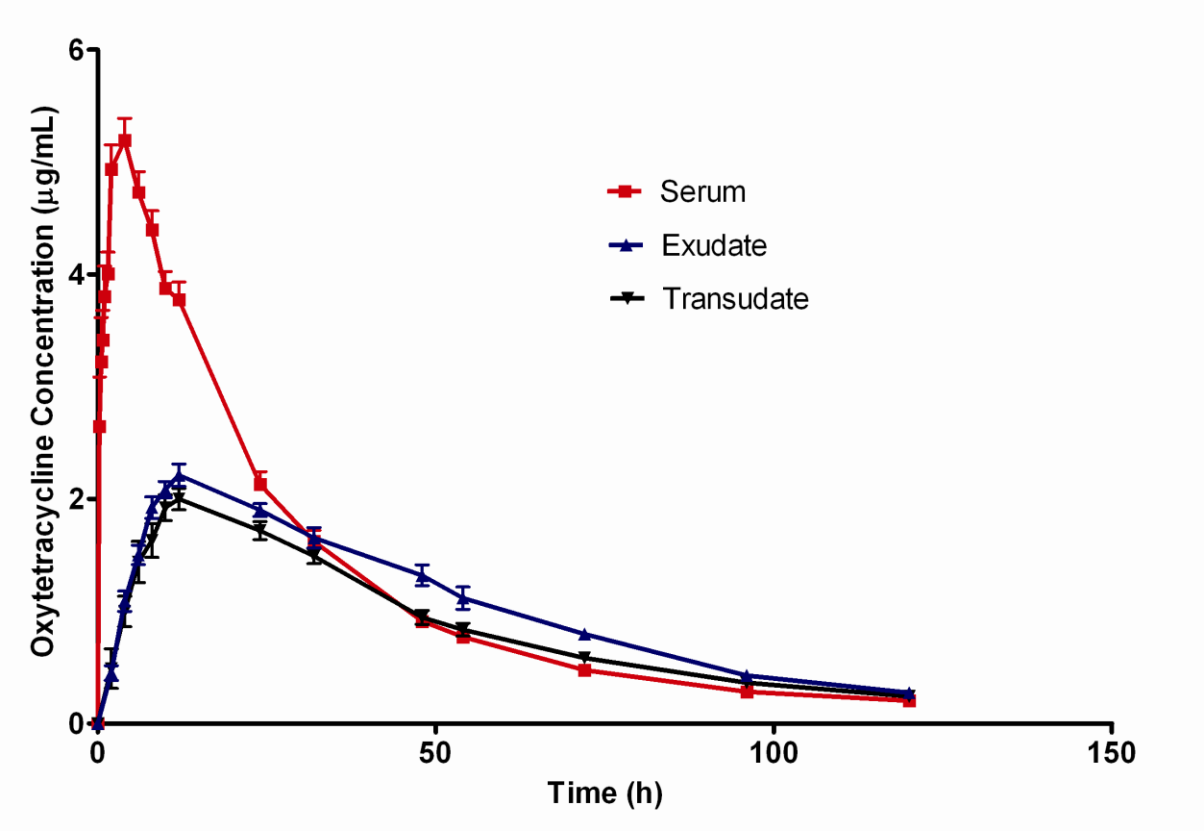
714 Predicted dosage (mg/kg) based on PK-PD modelling and Monte Carlo simulation of
715 oxytetracycline data in serum using either steady state or single dose (long duration of action)
716 for computation with application of serum:broth MIC ratio

Computed dose to guarantee average serum concentration of κ_{PD}-fold MIC for a duration of 48h:	Steady state approach		Single dose approach	
	TAR	TAR	TAR	TAR
Predicted doses for *P.multocida*	50%	90%	50%	90%
Bacteriostatic	199.5	423.6	313.7	682.3
Bactericidal	434.0	921.2	701.2	1523.2
	TAR	TAR	TAR	TAR
Predicted doses for *M.haemolytica*	50%	90%	50%	90%
Bacteriostatic	125.2	140.6	196.8	283.2
Bactericidal	180.2	202.6	313.5	451.6

717 TAR = target attainment rate (probability for the serum concentration to exceed the PD
718 endpoint for efficacy). Dosages were computed by Monte Carlo simulation using equations 5
719 and 8 for steady state and loading dose approaches, respectively, with: (1) Wild Type MIC
720 distributions ranging from 0.25 to 2µg/mL (n=498) for *P.multocida* and 0.25-1µg/mL
721 (n=481) for *M.haemolytica* determined by the Turnidge method; (2) average $AUC_{0-24h}/MIC_e)/24h = \kappa_{PD}$ calculated for experimentally obtained bacteriostatic or bactericidal
723 action (data from three or four strains) ; (3) individual animal clearance and elimination rate
724 constant (K_{10}) empirical distributions obtained for 10 healthy calves (present study)
725 receiving the dose recommended by the manufacturer (20mg/kg) ; (4) fu the average
726 oxytetracycline free fraction determined experimentally; and (5) the difference in MIC
727 broth:serum ratio of 27.4:1 for *P.multocida* and 25.2:1 for *M.haemolytica*.

728 **Figure 1**: Mean ± SEM oxytetracycline concentration in serum, exudate and transudate of

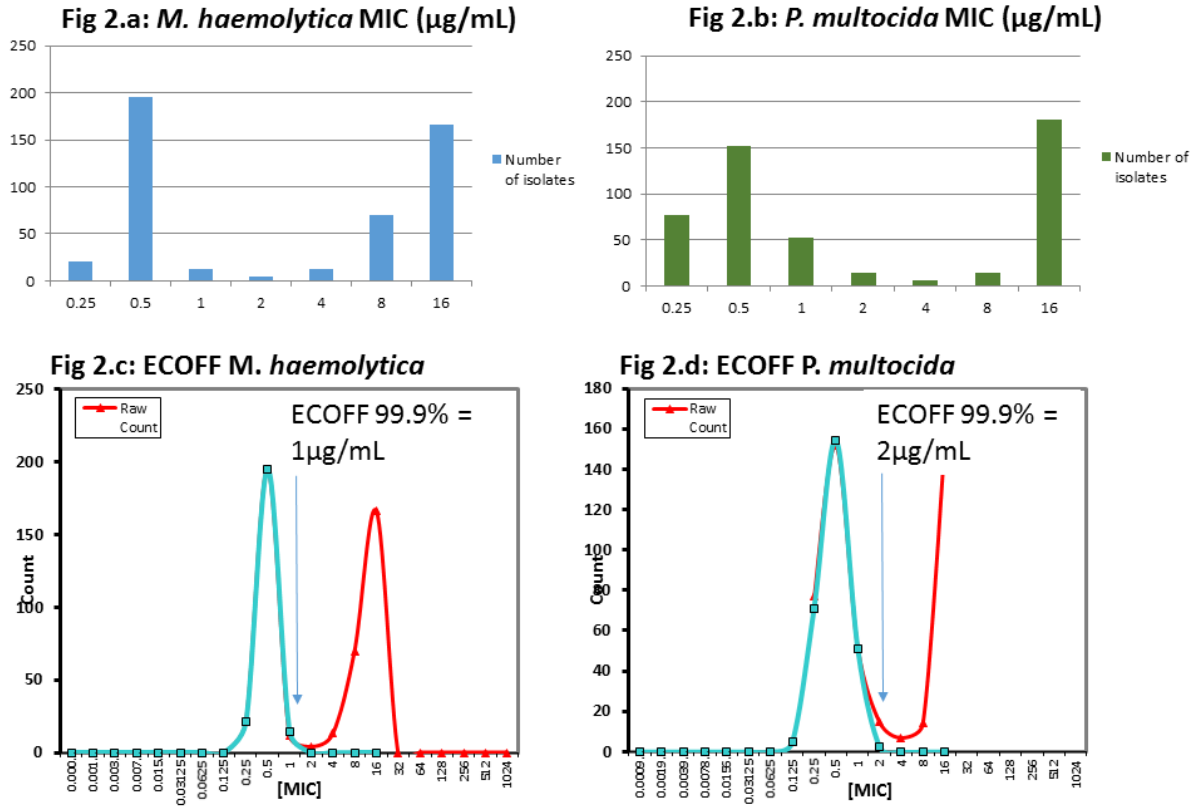
729 calves after intramuscular injection of oxytetracycline at a dose rate of 20mg/kg.



730

731

Figure 2: MIC distributions for *P.multocida* (498 strains, Fig 2.a) and *M.haemolytica* (481 strains, Fig 2.b). All specimens were collected from infected cattle and MIC measured at the Iowa state Veterinary Diagnostic Laboratory Data from 2000, 2001, 2002 and 2003 (http://vads.vetmed.vt.edu/index.cfm). The wild type populations were statistically determined according to Turnidge et al. (2006) to calculate the 99.9th percentile of the Epidemiological Cut-off (ECOFF). The WT distributions for *P.multocida* (Fig 2.c) and *M.haemolytica* (Fig 2.d) were fitted with a blue curve.



746 **Supplementary data**

747 **Appendix 1**

748 PK-PD integration for oxytetracycline in calf serum for *P.multocida* and *M.haemolytica*:

749 mean values (n=10 calves)

750

	P.multocida		*M.haemolytica*	
Variable (units)	Based on mean serum MIC (6.75 µg/mL)	Based on mean MHB MIC (0.25 µg/mL)	Based on mean serum MIC (5.46 µg/mL)	Based on mean MHB MIC (0.22 µg/mL)
C_{max}/MIC	0.77	20.92	0.96	23.77
AUC_{0-24h}/MIC (h)	12.97	350.1	16.03	397.8
$AUC_{0-\infty}$/MIC (h)	24.28	655.5	30.01	744.9
T>MIC (h)	0	104.4	1.14	110.5

751

752

753

754 **Appendix 2**

755 Predicted dosage (mg/kg) based on PK-PD modelling and Monte Carlo simulation of
756 oxytetracycline data in MHB using either steady state or single dose (long duration of action)
757 for computation without application of serum:broth MIC ratio

758

Computed dose to guarantee average serum concentration of κ_{PD}-fold MIC for a duration of 48h:	Steady state approach		Single dose approach	
	TAR	TAR	TAR	TAR
Predicted doses for *P.multocida*	50%	90%	50%	90%
Bacteriostatic	7.28	15.46	11.45	24.9
Bactericidal	15.84	33.62	25.59	55.59
	TAR	TAR	TAR	TAR
Predicted doses for *M.haemolytica*	50%	90%	50%	90%
Bacteriostatic	4.97	5.58	7.81	11.24
Bactericidal	7.15	8.04	12.44	17.92

759

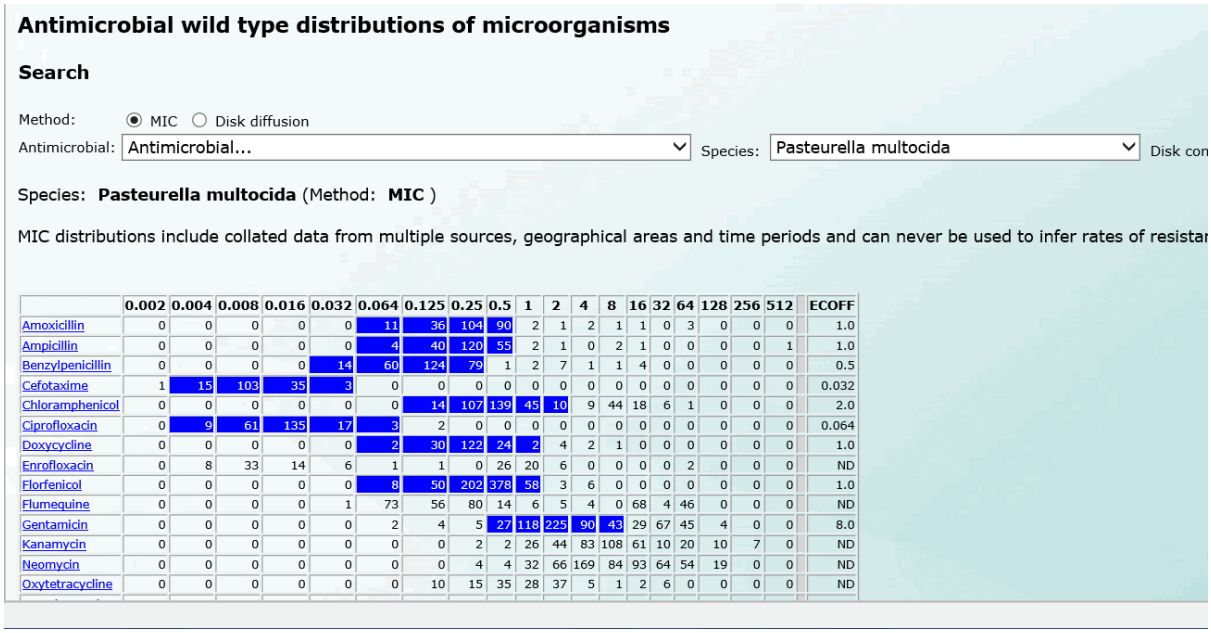
760 TAR = target attainment rate (probability for serum concentration to exceed the PD endpoint
761 for efficacy). Dosages were computed by Monte Carlo simulation using equations 5 and 8 for
762 steady state and loading dose approaches, respectively, with: (1) Wild Type MIC distributions
763 ranging from 0.25 to 2 µg/mL (n=498) for *P.multocida* and 0.25-1 µg/mL (n=481) for
764 *M.haemolytica* determined by the Turnidge (2006) method: (2) average $AUC_{0\text{-}24h}/MIC_e)/24h$
765 = κ_{PD} calculated for experimentally obtained bacteriostatic, bactericidal action (data from
766 three or four strains) ; (3) individual animal clearance and elimination rate constant (k_{10})
767 empirical distributions obtained for 10 healthy calves (present study) receiving the dose

768 recommended by the manufacturer (20 mg/kg) ; (4) f_u the average oxytetracycline free

769 fraction determined experimentally

770

771 To explore the EUCAST data for MH and PM base follow this link



Antimicrobial wild type distributions of microorganisms

Search

Method: (•) MIC ( ) Disk diffusion

Antimicrobial: Antimicrobial... Species: Pasteurella multocida Disk con

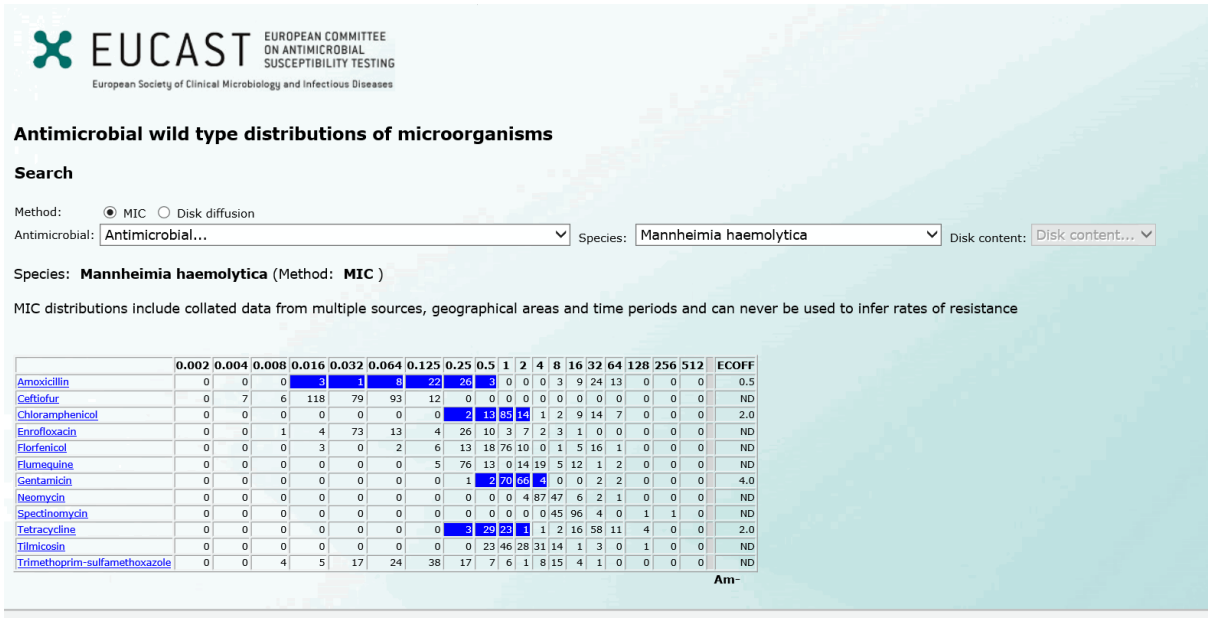
Species: **Pasteurella multocida** (Method: **MIC**)

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistar

	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
Amoxicillin	0	0	0	0	0	11	36	104	90	2	1	2	1	1	0	3	0	0	0	1.0
Ampicillin	0	0	0	0	0	4	40	120	55	2	1	0	2	1	0	0	0	0	1	1.0
Benzylpenicillin	0	0	0	0	14	60	124	79	1	2	7	1	1	4	0	0	0	0	0	0.5
Cefotaxime	1	15	103	35	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.032
Chloramphenicol	0	0	0	0	0	0	14	107	139	45	10	9	44	18	6	1	0	0	0	2.0
Ciprofloxacin	0	9	61	135	17	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0.064
Doxycycline	0	0	0	0	0	2	30	122	24	2	4	2	1	0	0	0	0	0	0	1.0
Enrofloxacin	0	8	33	14	6	1	1	0	26	20	6	0	0	0	0	2	0	0	0	ND
Florfenicol	0	0	0	0	0	8	50	202	378	58	3	6	0	0	0	0	0	0	0	1.0
Flumequine	0	0	0	0	1	73	56	80	14	6	5	4	0	68	4	46	0	0	0	ND
Gentamicin	0	0	0	0	0	2	4	5	27	118	225	90	43	29	67	45	4	0	0	8.0
Kanamycin	0	0	0	0	0	0	0	2	2	26	44	83	108	61	10	20	10	7	0	ND
Neomycin	0	0	0	0	0	0	0	4	4	32	66	169	84	93	64	54	19	0	0	ND
Oxytetracycline	0	0	0	0	0	0	10	15	35	28	37	5	1	2	6	0	0	0	0	ND

772

773



EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

Antimicrobial wild type distributions of microorganisms

Search

Method: (•) MIC ( ) Disk diffusion

Antimicrobial: Antimicrobial... Species: Mannheimia haemolytica Disk content: Disk content...

Species: **Mannheimia haemolytica** (Method: **MIC**)

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance

	0.002	0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	ECOFF
Amoxicillin	0	0	0	3	1	8	22	26	3	0	0	0	3	9	24	13	0	0	0	0.5
Ceftiofur	0	7	6	118	79	93	12	0	0	0	0	0	0	0	0	0	0	0	0	ND
Chloramphenicol	0	0	0	0	0	0	0	2	13	85	14	1	2	9	14	7	0	0	0	2.0
Enrofloxacin	0	0	1	4	73	13	4	26	10	3	7	2	3	1	0	0	0	0	0	ND
Florfenicol	0	0	0	3	0	2	6	13	18	76	10	0	1	5	16	1	0	0	0	ND
Flumequine	0	0	0	0	0	0	5	76	13	0	14	19	5	12	1	2	0	0	0	ND
Gentamicin	0	0	0	0	0	0	0	1	2	70	66	4	0	0	2	2	0	0	0	4.0
Neomycin	0	0	0	0	0	0	0	0	0	0	4	87	47	6	2	1	0	0	0	ND
Spectinomycin	0	0	0	0	0	0	0	0	0	0	0	0	45	96	4	0	1	1	0	ND
Tetracycline	0	0	0	0	0	0	0	3	29	23	1	1	2	16	58	11	4	0	0	2.0
Tilmicosin	0	0	0	0	0	0	0	0	23	46	28	31	14	1	3	0	1	0	0	ND
Trimethoprim-sulfamethoxazole	0	0	4	5	17	24	38	17	7	6	1	8	15	4	1	0	0	0	0	ND

Am-

774