EUCAST European Committee on Antimicrobial Susceptibility Testing

Rationale for EUCAST clinical breakpoints

Agent	**Oxytetracycline (OXY) in cattle (VetCAST) for intramuscular (long acting) and intravenous administrations**

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Foreword

EUCAST
The European Committee on Antimicrobial Susceptibility Testing (EUCAST) is organised by the European Society for Clinical Microbiology and Infectious Diseases (ESCMID), the European Centre for Disease Prevention and Control (ECDC), and the active national antimicrobial breakpoint committees in Europe. EUCAST was established by ESCMID in 1997, was restructured in 2001-2002 and has been in operation in its current form since 2002. The current remit of EUCAST is to harmonise clinical breakpoints for existing drugs in Europe, to determine clinical breakpoints for new drugs, to set epidemiological (microbiological) breakpoints, to revise breakpoints as required, to harmonise methodology for antimicrobial susceptibility testing, to develop a website with MIC and zone diameter distributions of antimicrobial agents for a wide range of organisms and to liaise with European governmental agencies and European networks involved with antimicrobial resistance and resistance surveillance.

Information on EUCAST and EUCAST breakpoints is available on the EUCAST website at http://www.EUCAST.org.

EUCAST rationale documents
EUCAST rationale documents summarise the information on which the EUCAST clinical breakpoints are based.

Availability of EUCAST documents
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This rationale document should be cited as: “European Committee on Antimicrobial Susceptibility Testing. Oxytetracycline: Rationale for the clinical breakpoints, version 1.0, 2026. http://www.eucast.org.”

1. Introduction

- Oxytetracycline (OXY) is a naturally-occurring, bacteriostatic tetracycline antibiotic with a broad spectrum of activity against many aerobic and anaerobic Gram-positive and Gram-negative bacteria (Chopra, I., & Roberts, M. 2001).

- OXY is extensively used in cattle farming for the treatment and management of infections such as pneumonia, bacterial enteritis, and metritis, associated with *Mannheimia haemolytica*, *Pasteurella multocida*, *Mycoplasma bovis*, and *Histophilus somni*. It is most commonly prescribed for bovine respiratory disease (BRD) (Riviere, J.E. & Papich, M. 2018).

- In cattle, resistance to tetracyclines as a class occurs, though it remains moderate and stable over recent years (Lachowicz-Wolak et al., 2023). MIC50 and MIC90 for oxytetracycline against key bovine pathogens collected in the EU typically range from 0.25–0.5 µg/mL and 1–4 µg/mL for *Mannheimia haemolytica* and *Pasteurella multocida*, with minimal resistance observed in *Histophilus somni* isolates (Lachowicz-Wolak et al., 2023; de Jong et al., 2023). The most frequent resistance mechanisms observed are active efflux pumps and ribosomal protection proteins (RPPs). Other identified mechanisms include enzymatic degradation, decreased drug permeability, and target mutation.

- Due to its low oral bioavailability, OXY is primarily administered parenterally in both adult cattle and calves. Intravenous (iv) formulations typically use the hydrochloride salt, whereas intramuscular (im) formulations are available as short-acting (10%) and long-acting (20% or 30%) preparations. In clinical practice, long-acting formulations are preferred due to their convenience, usually requiring a single im dose of 20 mg/kg, with some products recommending a repeat dose after 48 to 72 hours (European Medicines Agency, n.d.). The im bioavailability of OXY is 78%.

- To establish the pharmacokinetic-pharmacodynamic (PK/PD) cut-off, this rationale focuses on the commonly administered dose of 20 mg/kg, with a claimed duration of efficacy of 48 hours.

References	• Chopra, I., & Roberts, M. 2001. *Microbiology and Molecular Biology Reviews*, 65(2), 232-260. • Riviere, J. E., & Papich, M. G. (Eds.). (2018). *Veterinary Pharmacology and Therapeutics* (10th ed.). Wiley-Blackwell. • Lachowicz-Wolak, A., et al. (2023). Antimicrobial resistance of bacterial pathogens isolated from dairy calves with respiratory disease in Poland. *Animals*, 13(3), 522. • de Jong, A., et al. (2023). Monitoring of antimicrobial susceptibility of veterinary pathogens isolated from diseased cattle across Europe: VetPath survey results. *Veterinary Microbiology*, 285, 109830. • European Medicines Agency. (n.d.). *Union Product Database (UPD)*. Retrieved from https://www.hma.eu/veterinary-medicines/cmdv/union-product-database-upd.html

2. Dosage

Minimum OXY daily dose: 5 mg/kg/d BW

Standard OXY daily dose: 10 mg/kg/d BW, iv or im
Standard OXY long-acting dose: single im 20 mg/kg/BW. In some SmPCs, the maximum dosing scheme allows for certain EU-approved long-acting im VMPs the possibility of redosing after 48 hours if clinical signs have not resolved.

Available formulations:

- Injection (short-acting): 100 mg/ml oxytetracycline *as hydrochloride*
- Injection (long-acting): 200 mg/ml oxytetracycline *as hydrochloride*
- Injection (long-acting): 200 mg/ml oxytetracycline *as dihydrate*
- Injection (long-acting): 300 mg/ml oxytetracycline *as dihydrate*

3. MIC distributions and epidemiological cutoff values

MIC distributions and epidemiological cutoff values for oxytetracycline and tetracycline can be found at https://mic.eucast.org/

4. Clinical breakpoints

Exitsting breakpoints:
Assuming that TET can predict results for OXYTET, the CLSI-VAST CBP proposed for *Pasteurella multocida*, *Mannheimia haemolytica* and *Histophilus somni* is ≤2, 4 and >4 for S. I and R, respectively. This CBP was derived from PK data of **oxytetracycline administered at 20 mg/kg IM, once**. The CLSI-VAST emphasizes that this breakpoint is applicable only for the injectable formulations.

Reference: CLSI. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 7th ed. CLSI supplement VET01S. Clinical and Laboratory Standard Institute; 2024

5. Pharmacokinetics after intravenous (iv) and intramuscular (im) administration of 20mg/kg (Winter *et al*. 2024)		
	Typical values for calves (< 6 months)	Typical values for adult cattle
C_{max} (mg/L) (NCA) (im)	4.7	7.6
T_{max} (h) (NCA) (im)	3.7	4.5
$AUC_{0-\infty}$ (mg.h/L) (NLMEM) (im)	148	254
$AUC_{0-\infty}$ (mg.h/L) (NLMEM) (iv)	190	326
Bioavailability (%) (NLMEM) (im)	78%	
Total body clearance (mL/kg/h) (Cl) (NLMEM)	107*	63*
Steady state volume of distribution (mL/kg) (V_{ss}) (NLMEM)	3631	
Mean Residence Time (h) (NLMEM)	33	57
Fraction unbound (%) (literature)	50 %**	
Comments	Parameters and variables generated from a meta-analysis of OXY pharmacokinetic data from 69 cattle, including 1,730 plasma concentration samples from animals administered 20 mg/kg intramuscularly and/or 20 or 40 mg/kg intravenously. These included calves (*n* = 28, aged < 6 months) and adult cattle (*n* = 41), dairy (*n* = 24) and beef (*n* = 45) breeds and male (*n* = 30) and female (*n* = 39) animals. 14 animals had infections, of which 8 were calves in a model of severe pneumonia. Animals were euthanised after 48 h and upon post-mortem analysis 6 had metritis associated with *Trueperella pyogenes*. Eight OXY products were used: one for iv administration, the remainder for im administration of long-acting (LA) formulations administered at the licensed dose of 20 mg/kg. In three studies, OXY was administered im once; in three studies, two OXY products were administered im with wash-out periods of 7, 10 or 33 days; and in two studies, OXY was first administered iv then im. Data were analysed using either a Non-Compartmental Analysis (NCA) or a Non-Linear Mixed Effect Model (NLMEM) (Winter *et a*l, 2024). *OXY clearance is dependent on renal excretion, which is higher in calves. **OXY plasma protein binding estimates ranged from 31.6 to 71.7% in 5 studies, using equilibrium dialysis method (Ziv & Sulman, 1972 and Pilloud, 1973) and ultrafiltration method (Ziv *et al.,* 1972, Nouws *et al*.,1985 and Lees *et al*. 2016). On average, oxytetracycline's plasma protein binding was estimated to be 50%.	

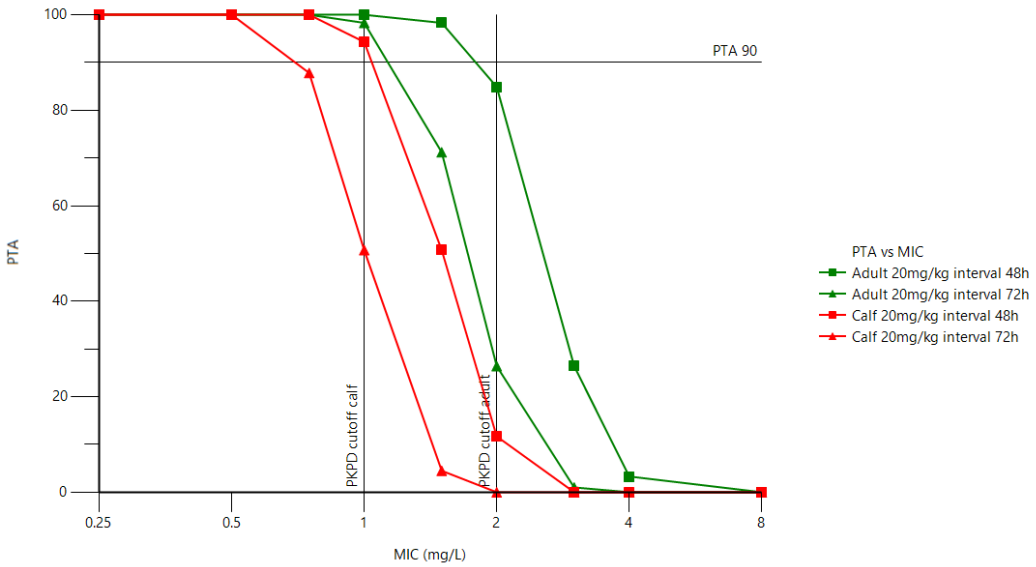
References	• Winter, E. A., *et al*. (2024). *Frontiers in Microbiology*, 14, 1498219 • Ziv, G., & Sulman, F. G. (1972). *Antimicrobial Agents and Chemotherapy*, 2(3), 206–213. • Pilloud, M. (1973). *Research in Veterinary Science*, 15(2), 224-230. • Nouws, J. F., *et al*. (1985a). *Veterinary Quarterly*, 7, 306–314 • Lees, P., *et al*. (2016). *Veterinary Journal*, 218, 60–64

6. Pharmacodynamics

In vitro PD (Lees *et al*., 2018)				
	Mannheimia haemolytica (mean, n=6, Mueller-Hinton Broth (MHB))	*Mannheimia haemolytica* (mean, n=6, serum)	*Pasteurella multocida* (mean Mueller-Hinton Broth, n=6 (MHB))	*Pasteurella multocida* (mean, n=6, serum)
*f*AUC$_{0\text{-}24h}$/MIC for bacteriostasis (h)	25.2	19.1	19.2	28.0
In vitro PD (Brentnall *et al.,* 2013)				
	Mannheimia haemolytica (mean, cation-adjusted Mueller Hinton Broth (CAMHB))	*Mannheimia haemolytica* (mean, serum)		
*f*AUC$_{0\text{-}24h}$/MIC for bacteriostasis (h)	42.0	23.2		
Clinical Data	Not available			
Comments	For OXY, total drug exposure over time relative to the MIC correlates most strongly with antibacterial efficacy, making *f*AUC/MIC the pharmacokinetic-pharmacodynamic index of choice (Nielsen & Friberg, 2013). The pharmacodynamic target (PDT) for this index over a 24-hour dosing interval is set at 24h to achieve an average plasma concentration equal to or greater than the MIC. For longer dosing intervals, the claimed duration of efficacy is assumed to be longer too. The administered dose was therefore divided by the dosing interval in days to test if the target exposure is achieved.			
References	• Lees, P., et al. (2018). *Journal of Veterinary Pharmacology and Therapeutics*, 41(1), 28–38. • Brentnall, C., et al. (2013). *Research in Veterinary Science*, 94(3), 687–694. • Nielsen, E. I., & Friberg, L. E. (2013). *Pharmacological Reviews*, 65(3), 1053–1090.			

7. Monte Carlo simulations and PK/PD cutoff

The figure below displays Probability of target attainment (PTA%) for MICs of 0.25, 0.50, 0.75, 1.0, 1.5, 2.0,3.0, 4.0 and 8.0 mg/L for calves and adult cattle when OXY was administered intramuscularly as a single dose of 20 mg/kg BW. The PDT was adjusted for a claimed duration of efficacy 48 hours (square symbols) or 72 hours (triangle symbols).



- The PK/PD index for OXY is the ratio ***f*AUC/MIC** with *f*, the unbound fraction (fu) equal to 0.5 (See Section 5). The **PD target to achieve is 24 h per day,** in steady-state conditions, for 90% of animals. For a claimed duration of efficacy (dosing interval) of 48 hours, the dose was divided by 2, and for a claimed duration of efficacy of 72 hours, the dose was divided by 3 (See Section 6).
- The final NLME model (section 5) was used for Monte Carlo Simulations of *f*AUC/MIC for a dose of 20 mg/kg for adult cattle (green curves) and calves (i.e. taking into account the covariate age on clearance, red curves) and MICs of 0.25, 0.50, 1.0, 2.0, 4.0 and 8.0 mg/L. Additional points between conventional dilutions were added in the simulation (0.5,1.5 and 3 mg/L).

In adult cattle, a dose of 20 mg/kg BW OXY administered im every 48 hours allowed nearly 90% of animals to achieve a fAUC/MIC ≥24h at a maximum MIC of 2 mg/L. The PK/PDcutoff is ~one dilution lower when OXY is given with a 72h dosing interval. In calves, due to higher drug clearance, the highest MIC at which 90% of animals achieved a fAUC/MIC ≥24h was 1 mg/L on the condition of redosing every 48 hours. With iv administration, a dose of 10 mg/kg/d exceeds the im PK/PD cutoffs.

8. Clinical data

- No clinical outcome data linked to pathogen susceptibility to OXY are available in cattle treated for BRD
- A pilot study in Sweden evaluated the efficacy of benzylpenicillin, oxytetracycline (20 mg/kg IM), and florfenicol in treating acute undifferentiated respiratory disease in calves. While oxytetracycline was found to be effective in mild cases, its applicability to more severely ill or stressed calves remains uncertain (Welling et al., 2020).
- A systematic review and meta-analysis examined various injectable antibiotics for bovine respiratory disease (BRD). The study included oxytetracycline at 20 mg/kg im or sc, administered as a single dose or repeated every 48-72 hours. Findings suggested that oxytetracycline was only marginally more effective than placebo (O'Connor et al., 2016). A second systematic review and network meta-analysis compared oxytetracycline (20 mg/kg im or sc) with other antibiotics in feedlot cattle for BRD treatment. The study concluded that oxytetracycline was less effective than macrolides or florfenicol, raising concerns about its efficacy in severe cases (O'Connor et al., 2019). The O'Connor meta-analyses collected studies mainly from North America where tetracycline was also largely used as a feed additive for various purposes at in feedlots (including the old label which was to increase Average Daily Gain in highly stressed calves). The antimicrobial pressure of this category of calves is very different from what we can see in cow-calves operations in Europe.

- Using pairwise comparisons instead of network analysis, the ENOVAT BRD systematic review on clinical efficacy (Scahill et al. 2026), indicates that the short-term therapeutic performance of different antimicrobials used for BRD treatment is "broadly comparable". Importantly, none of the BRD clinical experts involved in the review raised objections to this conclusion.
- A randomized field trial compared oxytetracycline (20 mg/kg im) with florfenicol (40 mg/kg im) for treating calf pneumonia. The study found that florfenicol led to better lung re-aeration (assessed by lung ultrasound) but that the probability of cure was not different between both antimicrobials (Jourquin et al., 2022, Jourquin et al., 2025).
- This study highlighted that minimum inhibitory concentrations (MICs) of oxytetracycline in serum were higher than in broth, which may contribute to reduced efficacy in clinical settings. This suggests that current dosing regimens may not be optimal, particularly for calves (Mead et al., 2019).

References	• Welling, V., et al. (2020). *Antibiotics, 9*(11), 736. • O'Connor, A. M., et al. (2016). *Prev. Vet. Med., 132*, 130–139. • O'Connor, A. M., et al. (2019). *Anim. Health Res. Rev., 20*, 163–181. • Scahill, et al. (2026). Open Research Europe, 2026, 6:41. • Jourquin, S., et al. (2022). *J. Vet. Intern. Med., 36*(2), 820–828. • Jourquin, S., et al. (2025). *J. Dairy Sci.., 108*(2), 1896-1913 • Mead, A., et al. (2019). *J. Vet. Pharmacol. Ther., 42*(1), 52–59.

9. Clinical breakpoints

PK/PD cut-off	In view of the lack of clinical data linked to pathogen susceptibility, the two CBPs proposed for OXY administered to cattle as an intramuscular injection were derived by considering only PK/PD cutoffs (see Section 7) and ECOFFs (see Section 3).				
Species-related breakpoints	**Organism group**		**MIC breakpoints (mg/L)**		**Notes**
			S ≤	R >	
	B. bronchiseptica	Calf (up to 6 months)	1	1	These breakpoints are based on the assumption that oxytetracycline MICs are the same as those of tetracycline for this species
		Adult	2	2	
	M. haemolytica / P. multocida	All	2	2	For calves (up to 6 months), the PTA could be lower than 90%, but the breakpoint was established at 2 mg/L (as for adult cattle) to avoid splitting the wild-type population
	E. coli	All	-	-	
Species without breakpoint					
Clinical qualifications	For formulations of OXY administered intravenously or long-acting intramuscularly to cattle				
Dosage linked to BP	im: 20 mg/kg BW of long-acting OXY, possibly redosed iv: 10 mg/kg/d BW of OXY administered daily.				
Additional comment	For long-acting im: if redosing is needed, it should be with an interval no longer than every 48h. For some products, a redosing interval of 72 h is mentioned in their SmPC, but in these cases, the clinical breakpoints will be inadequate.				