CLINICAL AND LABORATORY STANDARDS INSTITUTE®

CLSI Veterinary Antimicrobial Susceptibility Testing Subcommittee (VAST)

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

CLSI-VAST Generic Drug Working Group

June 14-15, 2019

CLINICAL AND LABORATORY STANDARDS INSTITUTE®

Generic Drug Working Group

Proposal

- To determine susceptibility testing breakpoints for levofloxacin in dogs

Proposed action

VET 08, Table 2

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	MIC Interpretive Criteria (µg/mL)			Comments
				S	I	R	
Fluoroquinolones							
Dogs							
B? or E?	Skin, soft tissue, Urine	Levofloxacin	Enterobacteriaceae	**≤0.5**	**1**	**≥2**	Levofloxacin breakpoints were determined from an examination of MIC distributions of isolates and PK-PD analysis of levofloxacin, after administration at a dose of 25 mg/kg oral, every 24 hours.
B? or E?	Skin, soft tissue, Urine	Levofloxacin	*Pseudomonas aeruginosa*	**≤1**	**2**	**≥4**	Levofloxacin breakpoints were determined from an examination of MIC distributions of isolates and PK-PD analysis of levofloxacin, after administration at a dose of 25 mg/kg oral, every 24 hours.

Current Breakpoint

CLSI Rationale Document

CLSI Rationale Document MR02

Fluoroquinolone Breakpoints for Enterobacteriaceae and *Pseudomonas aeruginosa.* February 2019.

Breakpoint revisions:

Revised CLSI Fluoroquinolone MIC Breakpoints (last reviewed, January 2018) to be published in M100S 29th Edition*)

Organism Group	Antimicrobial Agent	S	SDD	I	R
Enterobacteriaceae	Ciprofloxacin	≤0.25	–	0.5	≥1
	Levofloxacin	≤0.5	–	1	≥2
Pseudomonas aeruginosa	Ciprofloxacin	≤0.5	N/A	1	≥2
	Levofloxacin	≤1	N/A	2	≥4

*pending approval of disk diffusion breakpoints in June 2018

Replaces CLSI Fluoroquinolone MIC Breakpoints (last published in M100S 28th Edition)

Organism Group	Antimicrobial Agent	S	SDD	I	R
Enterobacteriaceae	Ciprofloxacin	≤1	–	2	≥4
	Levofloxacin	≤2	–	4	≥8
Pseudomonas aeruginosa	Ciprofloxacin	≤1	N/A	2	≥4
	Levofloxacin	≤2	N/A	4	≥8

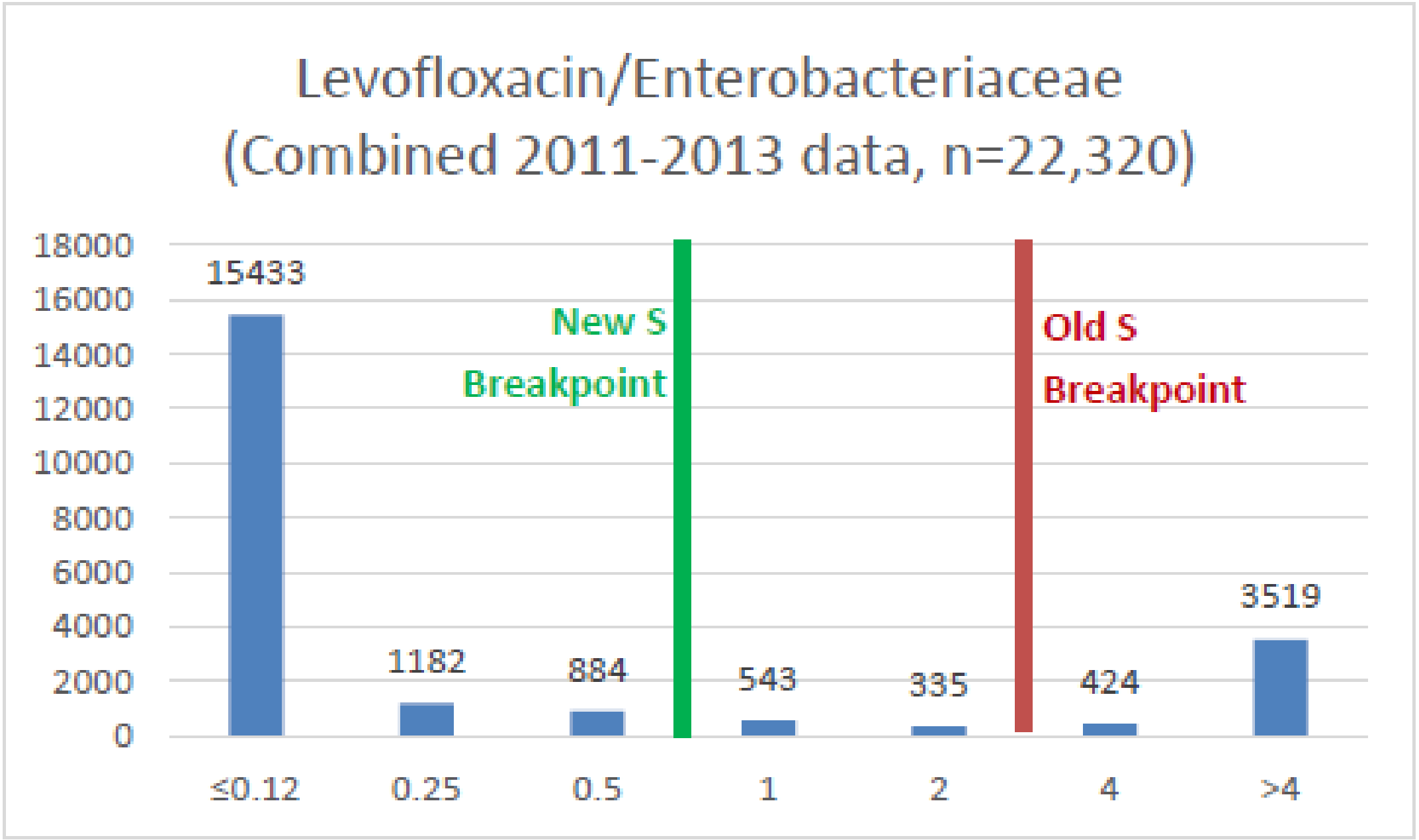


Figure 4.2. MIC distribution for Enterobacteriaceae and levofloxacin.

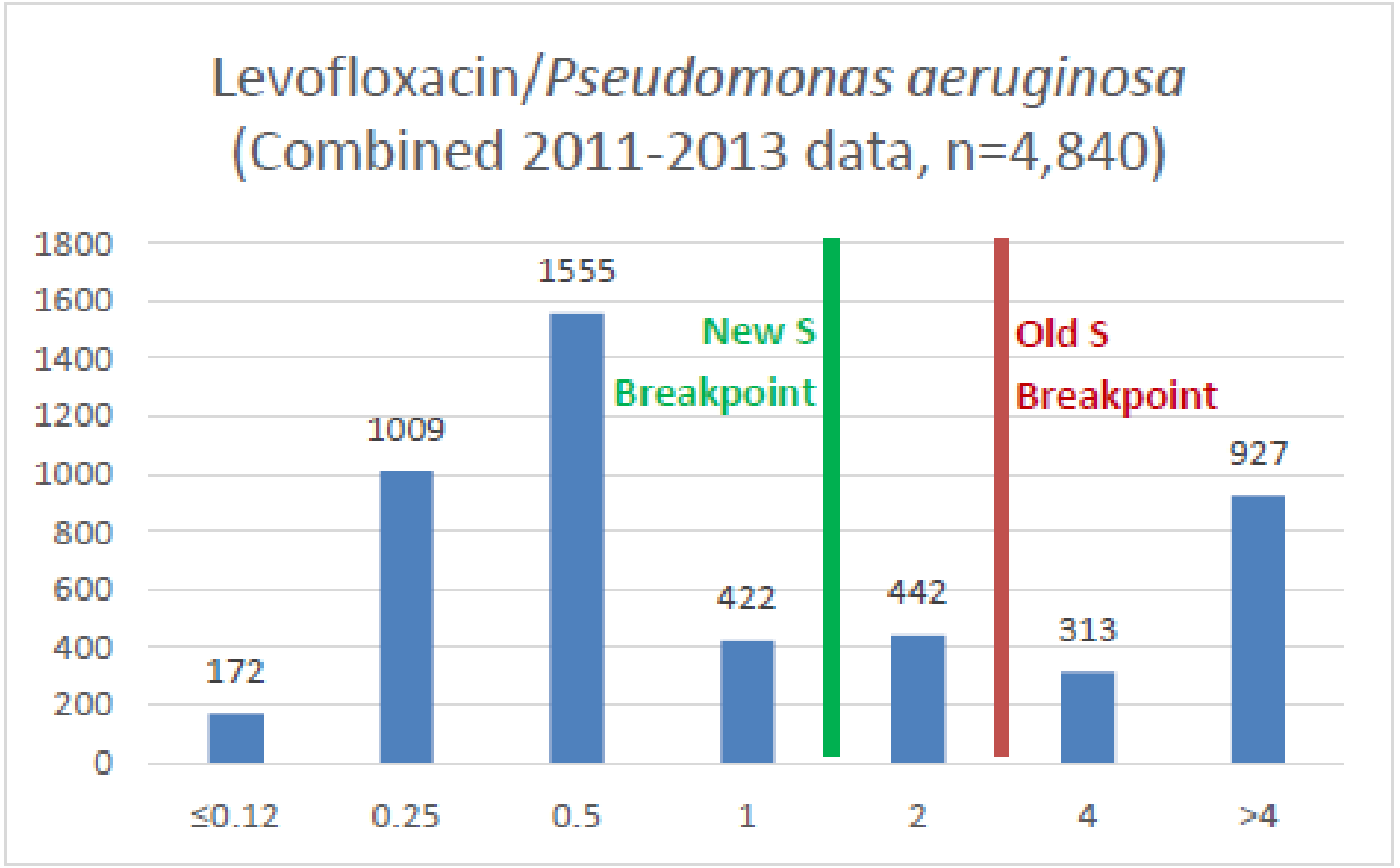
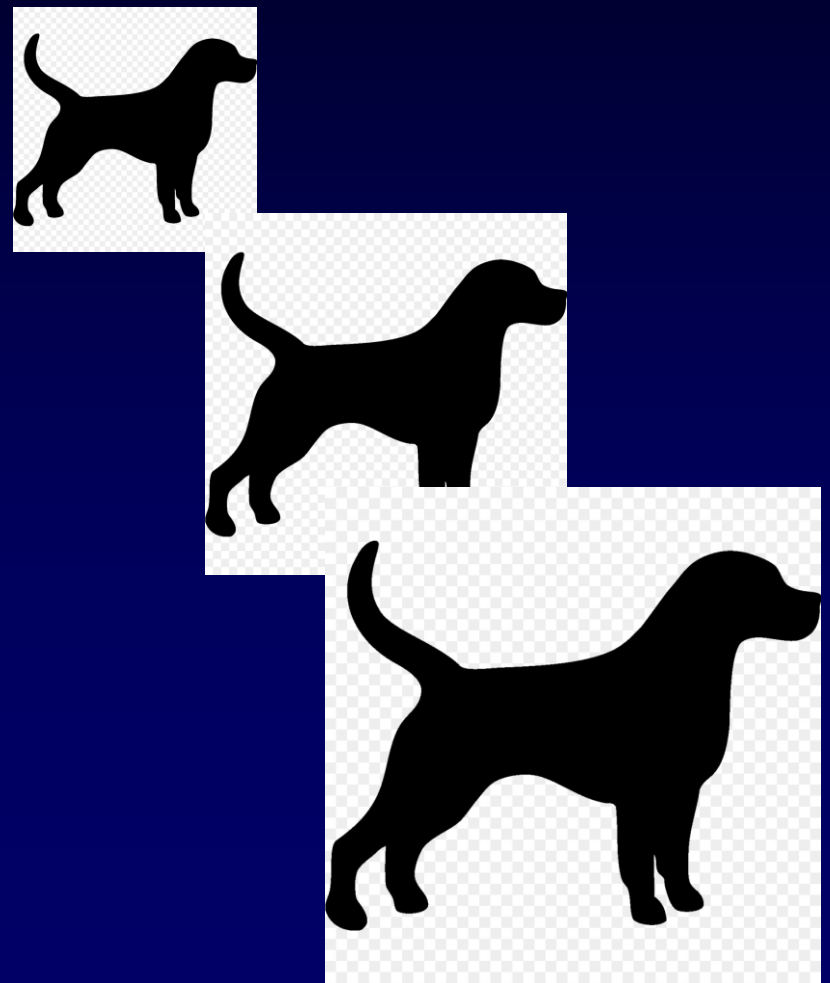


Figure 4.4. MIC distribution for *Pseudomonas aeruginosa* and levofloxacin.

Formulations

Formulations and Administration

- 250- 500-, and 750 mg tablet (Levaquin ® and generic)



Levofloxacin Properties and Advantages

Levofloxacin Properties and Advantages

- Levofloxacin is the levorotatory isomer of ofloxacin
- Limited metabolism; not metabolized by CYP450 enzymes
- Almost entirely eliminated in the urine
- Tablets can be taken without regard for meals
- Considered a "3rd-generation Quinolone" with improved activity than 2nd-generation agents (enrofloxacin, etc.)
- No 3rd-generation quinolones approved for dogs in the U.S.

Levofloxacin Properties

- Spectrum includes gram-positive aerobic bacteria, some gram-negative aerobic bacteria, some anaerobic bacteria, and other organisms (e.g., Chlamydia, Mycoplasma, Mycobacterium) (Davis & Bryson, 1994; Eliopoulos, 1995, Hect & Wexler, 1996).
- More active in vitro against gram-positive bacteria, and anaerobes than some other fluoroquinolones (Eliopoulos, 1995, Hect & Wexler, 1996).

Pharmacokinetic Data for Levofloxacin in Dogs

Levofloxacin Pharmacokinetics in Dogs after Oral Administration										
	Dose	**n=**	**Cmax**	**Tmax**	**AUC**	**T-half**	**VD/F**	**CL/F**	**F**	**Reference**
	mg/kg		mg/L	hr	mg hr/L	hr	L/kg	L/kg/hr	%	
Mean	24	6	15.54	2.44	167.74	5.84	1.2	0.142	104	Madsen, et al 2019
Std Dev			3.7	1.18	36.68	1.17	0.288	0.007	30	
Mean	5.6	6	3.2	1.8	32.92	6	1.54	0.185	60.94	Landoni, et al. 2018
Std Dev	0.65		0.69	0.97	9.85	1.32	0.467	0.068	14.99	
Mean	37.5	6	21.8	1.2	172.6	4.92	1.54	0.217		Yin, et al. 2011
Std Dev			1.65	0.4	11.24	1.94		0.014		
Mean	22.37	6	13.51	1.81	124.42	5.59	1.43	0.18	82.47	
Std.Dev.	0.65		2.01	0.85	19.26	1.48	0.38	0.03	22.50	

Levofloxacin Pharmacokinetics in Dogs

Summary of all studies (18 observations; 3 data sets):

- Mean T½:
  5.59 hours (Std Dev 1.5)

- Mean Clearance (CL/F):
  0.181 L/kg/hr (Std Dev 0.049)

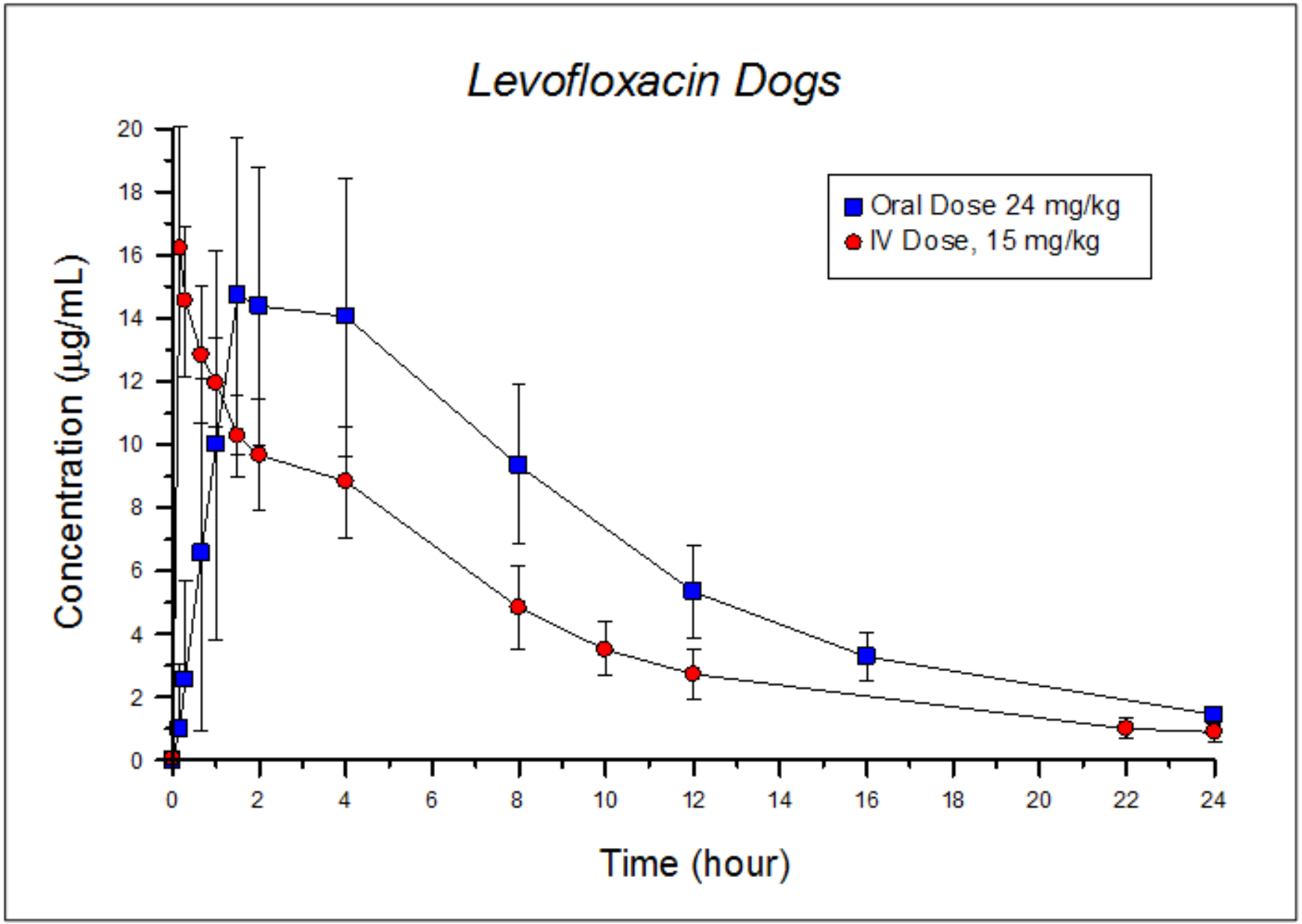


Figure 1: Plasma concentrations of levofloxacin after administration to 6 dogs (graph generated from data presented in Madsen, et al. 2019).

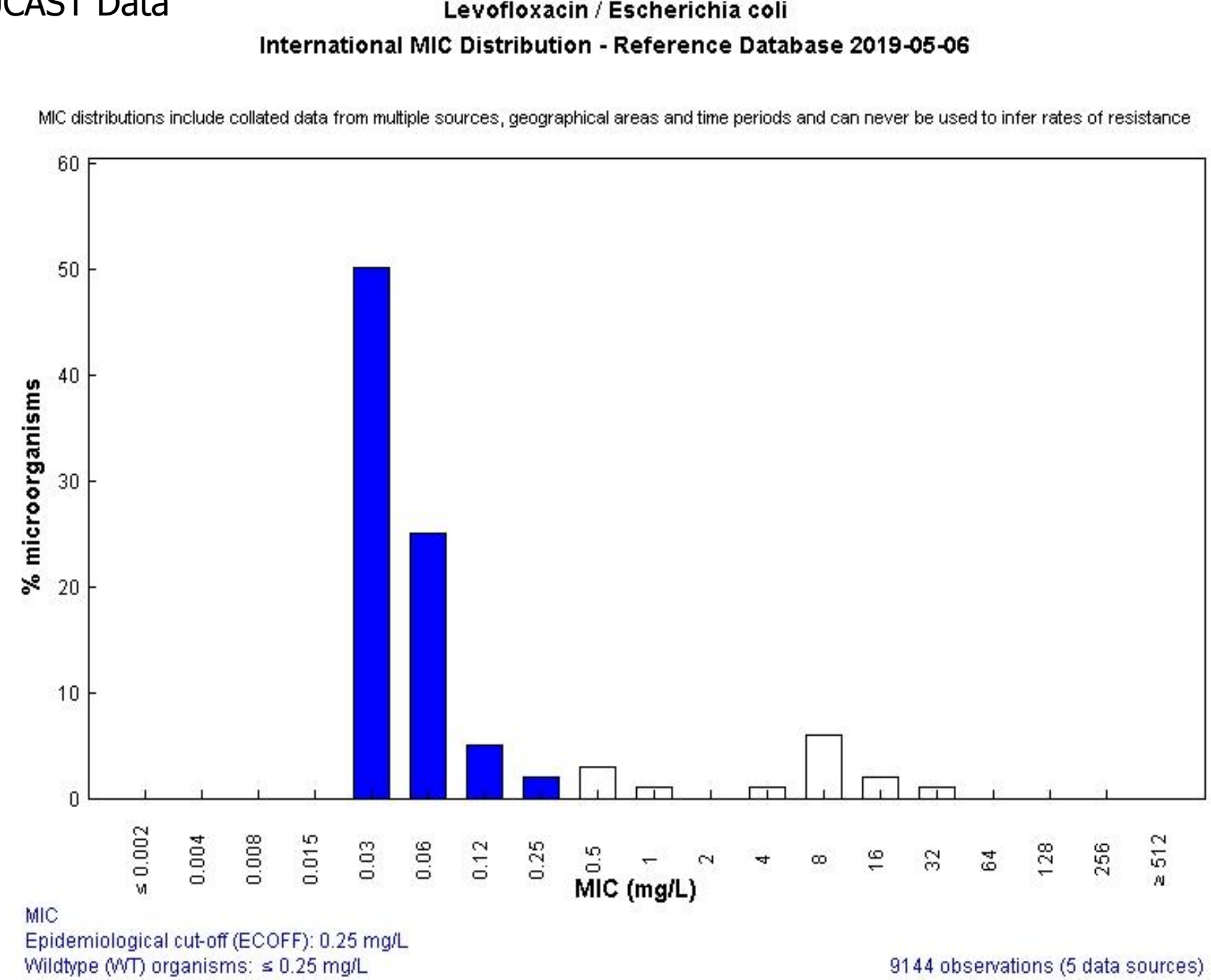
Levofloxacin: Protein Binding

10 µg/mL		5 µg/mL		1 µg/mL	
Replicate	% Binding	Replicate	% Binding	Replicate	% Binding
1	22.8	1	23.6	1	27.0
2	17.1	2	23.6	2	26.9
3	19.9	3	23.4	3	29.9
Mean	19.9	Mean	23.5	Mean	27.9
Std.Dev.	2.8	Std.Dev.	0.1	Std.Dev.	1.7

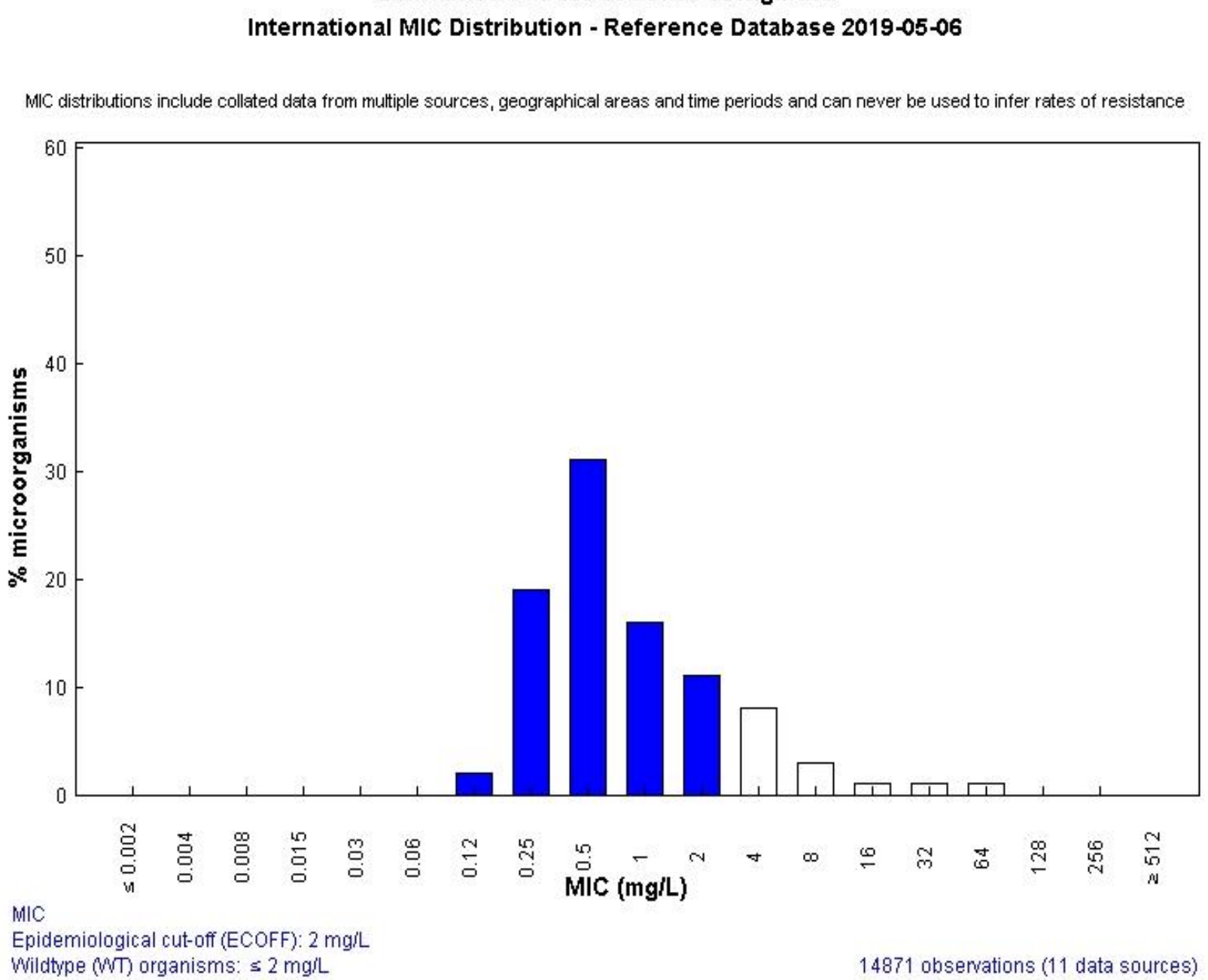
For Monte Carlo Simulation: Unbound fraction *(fu)* 0.76 +/- 0.12

MIC Data for Levofloxacin

Levofloxacin MIC: EUCAST Data

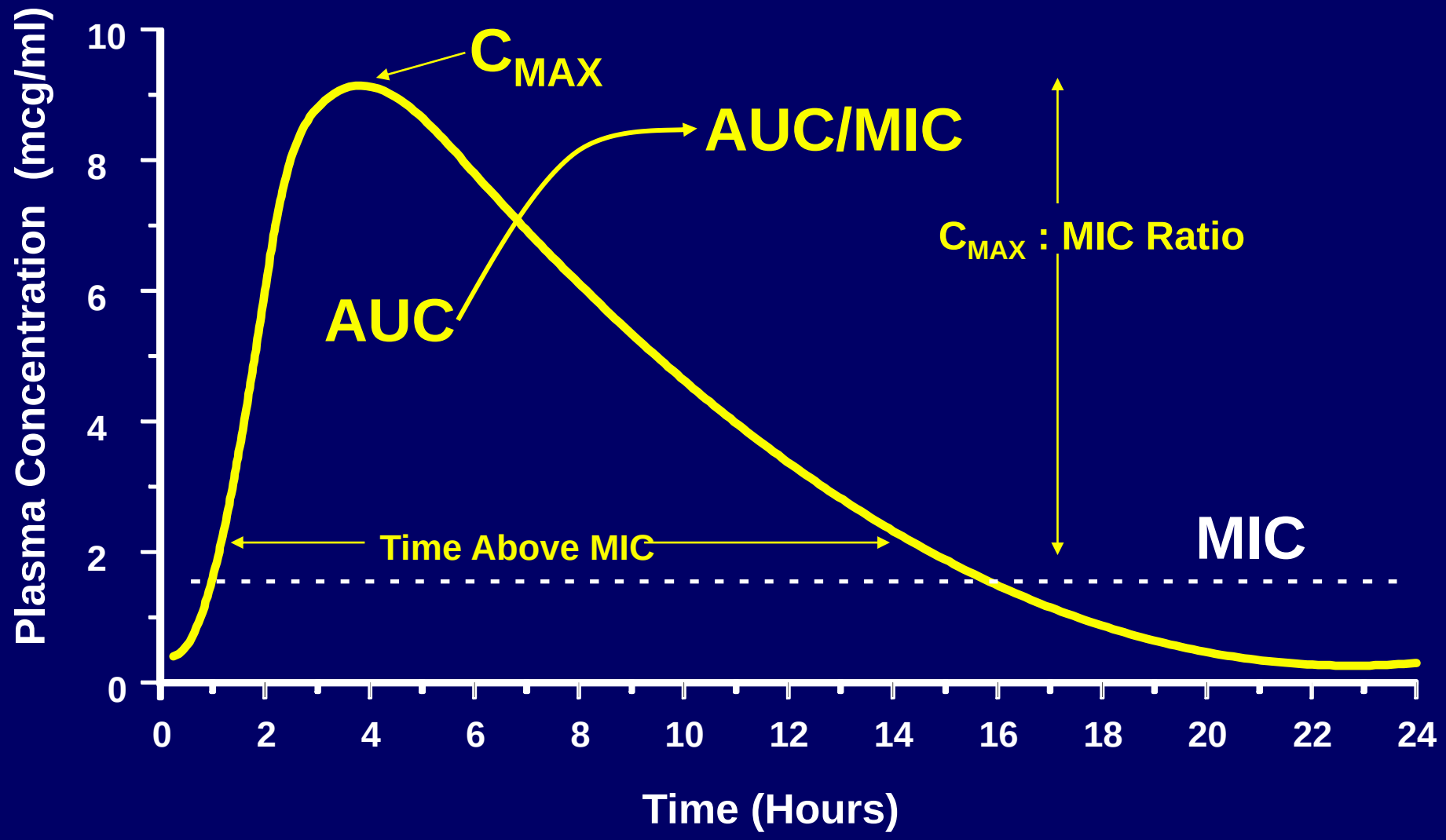


Levofloxacin MIC: EUCAST Data



PK-PD for Fluoroquinolones

Pharmacokinetic-Pharmacodynamic (PK-PD) Analysis



CLSI Rationale Document

CLSI Rationale Document MR02

Fluoroquinolone Breakpoints for Enterobacteriaceae and *Pseudomonas aeruginosa.* February 2019.

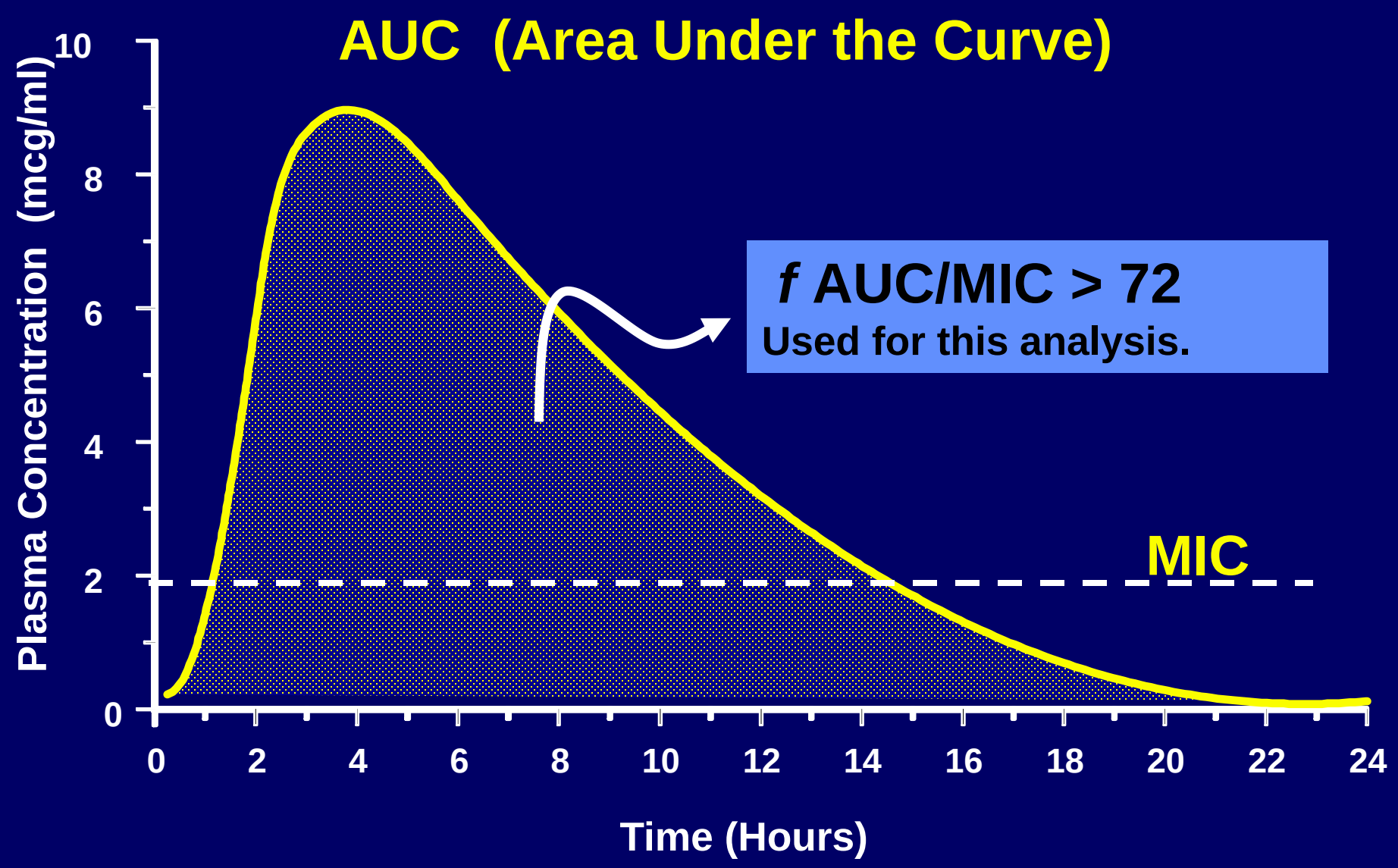
Table 5.1: Summary of non-clinical and clinical free-drug AUC:MIC ratio targets for efficacy

Organism	Non-clinical free-drug AUC:MIC ratio targets			Clinical free-drug AUC:MIC ratio targets
	Net bacterial stasis	1-$\log_{10}$ CFU reduction from baseline	2-$\log_{10}$ CFU reduction from baseline	
Enterobacteriaceae	35.6	67.4	140.0	72.0
P. aeruginosa	34.8	47.3	65.4	72.0

USCAST Recommendations for Fluoroquinolones (2015)

Table 3-36. Summary of non-clinical and clinical free-drug AUC:MIC ratio targets for efficacy

Organism	Non-clinical free drug AUC:MIC ratio targets			Clinical free-drug AUC:MIC ratio targets
	Net bacterial stasis	1-$\log_{10}$ CFU reduction from baseline	2-$\log_{10}$ CFU reduction from baseline	
Enterobacteriaceae	35.6	67.4	140	72.0
P. aeruginosa	34.8	47.3	65.4	72.0
S. aureus	35.8	68.7	187	-
S. pneumoniae	13.1	21.0	34.2	33.8


AUC (Area Under the Curve)
Plasma Concentration (mcg/ml)
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
18
20
22
24
Time (Hours)
f AUC/MIC > 72
Used for this analysis.
MIC

Determination of AUC / MIC

$$\text{Dose} = \frac{\text{CL} \cdot \text{AUC/MIC} \cdot \text{MIC}}{\text{fu} \cdot \text{F} \cdot 24\ \text{hr}}$$

$$\text{AUC/MIC} = \frac{\textit{fu} \cdot \text{F} \cdot 24\ \text{hr} \cdot \text{Dose}}{\text{CL} \cdot \text{MIC}}$$

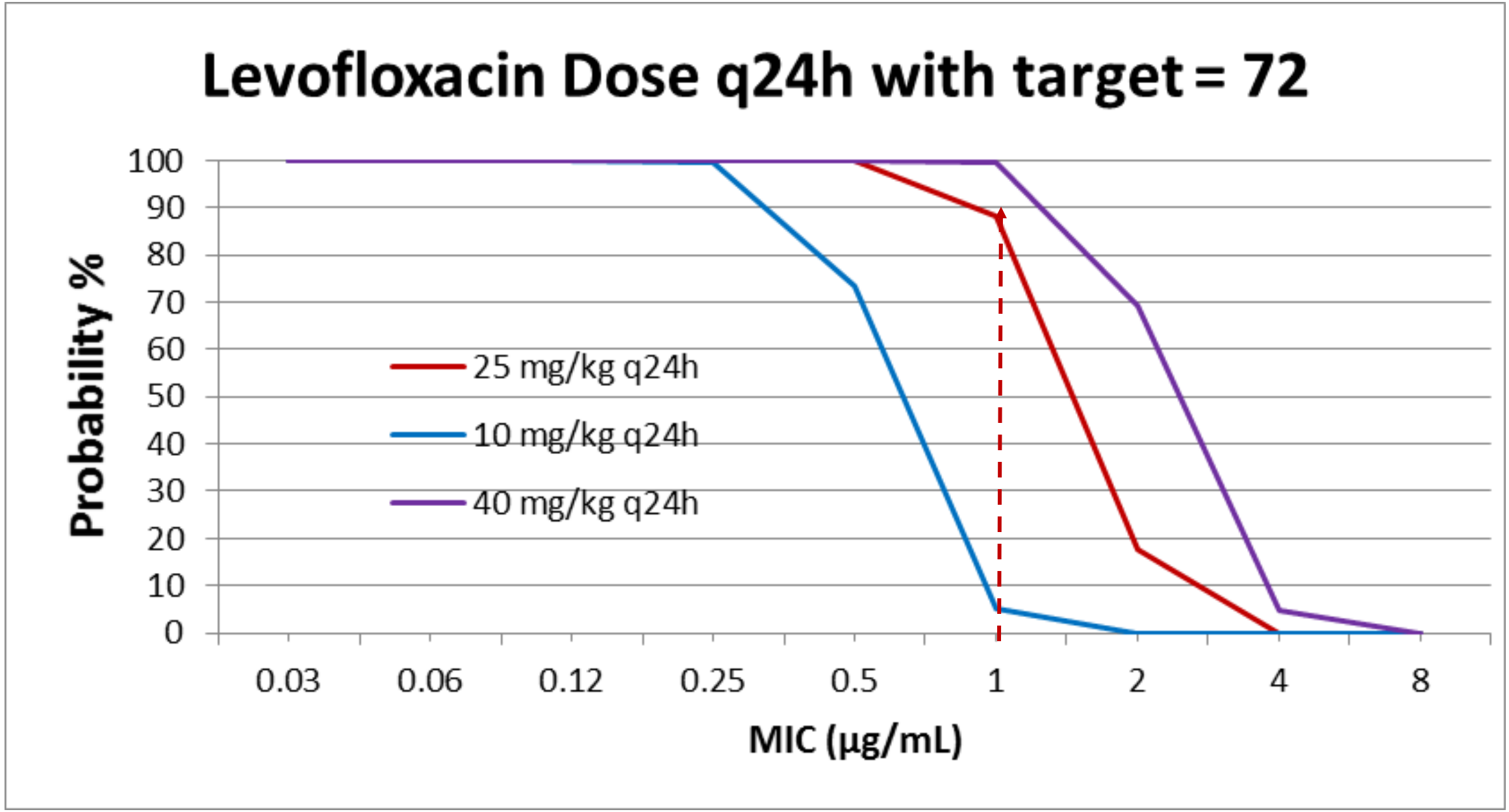
Monte Carlo Simulations

- AUC / MIC calculated
- Crystal Ball software (Oracle)
- 1,000 random trials simulated
- Input
  - MIC: 0.03 → 8 µg/mL
  - Oral dose: 25 mg/kg once every 24 hours
  - CL/F (mean & variance)
  - Protein binding (*fu = 0.76 +/- 0.12 for this analysis)*

Results
Levofloxacin in Dogs

Probability of Target Attainment (PTA) for levofloxacin administered to dogs

	MIC Values (µg/mL)								
Dose	0.03	0.06	0.12	0.25	0.5	1	2	4	8
10 mg/kg q24h	100	100	100	99.58	73.6	5.26	0	0	0
25 mg/kg q24h	100	100	100	100	100	88.35	17.57	0	0
40 mg/kg q24h	100	100	100	100	100	99.84	69.33	4.79	0


Levofloxacin Dose q24h with target = 72
Probability %
100
90
80
70
60
50
40
30
20
10
0
25 mg/kg q24h
10 mg/kg q24h
40 mg/kg q24h
0.03
0.06
0.12
0.25
0.5
1
2
4
8
MIC (µg/mL)

VET 02-A4 Guidelines

Table C10: Decision Table When Only 2 Cutoff Values Available (CO_{WT} and [CO_{PD} or CO_{CL}])

Abbreviations: CL, clinical cutoff value (CO_{CL}); PD, pharmacodynamic cutoff value (CO_{PD}); WT, wild type cutoff value (CO_{WT}).

Pseudomonas aeruginosa

Enterobacteriaceae

Ranking of Cutoffs	Suggested Breakpoint	Comments
WT > PD	PD	Could accept CO_{WT} as breakpoint if CO_{WT} only 1 dilution higher than CO_{PD}
PD > WT	PD	
WT = PD	WT = PD	
WT > CL	CL	Could accept CO_{WT} as breakpoint if CO_{WT} only if 1 dilution higher than CO_{CL}
CL > WT	CL	
WT= CL	WT= CL	

Conclusions

Conclusions

- High probability of target attainment (PTA) for free drug AUC/MIC > 72 can be achieved for a MIC value of 1 µg/mL or less, from administration of levofloxacin in dogs at an oral dose of 25 mg/kg every 24 hours.
- **Proposed breakpoint for Enterobacteriaceae**
- **S / I / R: 0.5 / 1 / 2 µg/mL**

- **Proposed breakpoint for *Pseudomonas aeruginosa***
- **S / I / R: 1 / 2 / 4 µg/mL**

Proposal from Generic Drug Working Group

Proposed action

VET 08, Table 2

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	MIC Interpretive Criteria (µg/mL)			Comments
				S	I	R	
Fluoroquinolones							
Dogs							
B or E?	Skin, soft tissue	Levofloxacin	*Pseudomonas aeruginosa*	**≤1**	**2**	**≥4**	Levofloxacin breakpoints were determined from an examination of MIC distributions of isolates and PK-PD analysis of levofloxacin, after administration at a dose of 25 mg/kg oral, every 24 hours.

Proposed action

VET 08, Table 2 for canine, tentatively for Test/Report Group B (TBD)

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	Disk Content	Interpretive Categories and Zone Diameter Breakpoints, nearest whole mm			Interpretive Categories and MIC Breakpoints, µg/mL			Comments
					S	I	R	S	I	R	
TBD	Skin and soft tissue	Levofloxacin	*Enterobacteriaceae*	5 µg	≥ 21	17–20	≤ 16	≤ 0.5	1	≥ 2	(XX) Levofloxacin breakpoints were determined from an examination of MIC distributions of isolates and PK-PD analysis of levofloxacin, after administration at a dose of 25 mg/kg oral, every 24 hours.
TBD	Skin and soft tissue	Levofloxacin	*Pseudomonas aeruginosa*	5 µg	≥ 22	15–21	≤ 14	≤1	2	≥4	(XX) Levofloxacin breakpoints were determined from an examination of MIC distributions of isolates and PK-PD analysis of levofloxacin, after administration at a dose of 25 mg/kg oral, every 24 hours.

Comparison to other Fluoroquinolones listed in Vet 08

Test/ Report Group	Body Site	Antimicrobial Agent	Organism	MIC Interpretive Criteria (µg/mL)			Comments
				S	I	R	
Fluoroquinolones							
Dogs							
A	Skin, soft tissue, respiratory, UTI	Enrofloxacin	*Enterobacteriaceae*	≤ 0.5	1-2	≥4	
A	Skin, soft tissue, UTI	Marbofloxacin	*Enterobacteriaceae*	≤ 1	2	≥4	
A	Skin, soft tissue, UTI	Orbifloxacin	*Enterobacteriaceae*	≤ 1	2-4	≥ 8	
A	Skin, soft tissue, UTI	Pradofloxacin	*Enterobacteriaceae*	≤ 0.25	0.5-1	≥ 2	
Dogs							
			Pseudomonas aeruginosa				no agents listed

Second Recommendation:

Levofloxacin (Dogs) in Table 1 Vet08:

- **Add Levofloxacin to Table 1 A**
- **Move to Group B or E? (secondary testing)**

Acknowledgements

Dr. Melanie Madsen

Dr. Kristen Messenger

(providing assistance with dog pharmacokinetic study)

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Thank you.

Any Questions?

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