

MODELLING THE IMPACT OF FECAL CIPROFLOXACIN CONCENTRATIONS ON FECAL COUNTS OF RESISTANT ENTEROBACTERIACEAE IN PIGLETS TREATED WITH CIPROFLOXACIN: TOWARDS NEW MEANS TO CONTROL THE SPREAD OF RESISTANCE ?

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BACKGROUND

- **Emergence of quinolone resistant bacteria in colonic flora during treatment**
 - Increasing therapeutic problem [1]
 - Colonic flora: reservoir of many of the *Enterobacteriaceae* (EB) species with clinical significance [2]
 - Association between oral administration of quinolone & emergence of quinolone resistant EB already showed: by analysing resistant EB as discrete outcome [3] or as continuous outcome using areas under the curve [4]
- **Modelling pharmacokinetic/pharmacodynamic (PK/PD) relationship for antibiotics**
 - Considering the whole time-course of both antibiotic concentrations and bacteria densities
 - Useful tool in antibiotic dosing strategies [5]
 - Increasingly performed for time-kill curves in *in vitro* studies [6,7,8] but still rare in *in vivo* studies
 - Population approach using nonlinear mixed effect models allow to analyse subjects with repeated PK/PD measures and to account for inter-subject variability

OBJECTIVES

To develop a PK/PD model to characterize the link between ciprofloxacin (CIP) concentrations and amounts of CIP resistant EB in feces of piglets treated with CIP

1. Modelling evolution of CIP fecal concentrations
2. Modelling evolution of CIP resistant bacteria counts

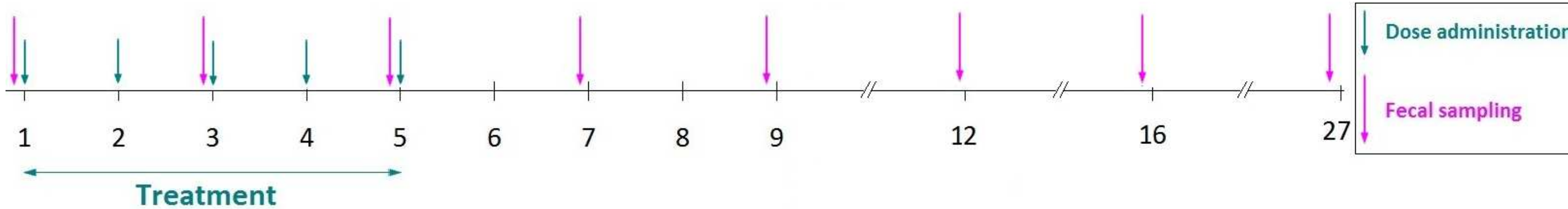
MATERIALS AND METHODS

• Study design

- **Prospective study in piglets**
 - * 4 weeks old, not received any antimicrobial since birth
- **Treatment & fecal sampling**
 - * 29 piglets randomly assigned to 3 groups treated with CIP or placebo (mineral water) by oral administration once a day from D1-D5

Treatment group	Number of piglets
Placebo	9
CIP 1.5mg/kg/day	10
CIP 15mg/kg/day	10

* Sampling schedule

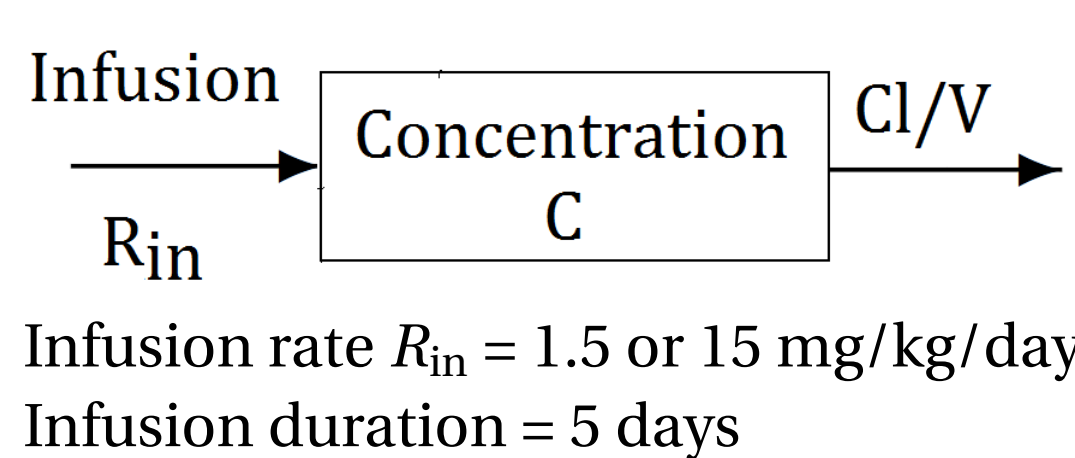


• Microbiological analysis

- **CIP fecal concentrations**
 - * Microbiological assay using antibiotic medium 5 at pH 8.0 and Escherichia coli ATCC 25922 as indicator strain
 - * Fecal samples ten-fold diluted in HCl 0.1N, then diluted in phosphate buffer pH 8.0
 - * Limit of quantification = 0.1 µg/g of feces
- **CIP resistant EB counts**
 - * Fecal samples serially diluted in saline, then plated on Drigalski agar, supplemented or not with 2 mg/l of ciprofloxacin
 - * Susceptibility to quinolones of enterobacteria assayed by agar disk diffusion assay
 - * Limit of quantification = 10² CFU/g

• PK/PD modelling

- **PK model of fecal concentrations**
 - * Assimilated to a one compartment model with intravenous infusion and first order elimination, parameterized by volume of distribution (V) and clearance (Cl)

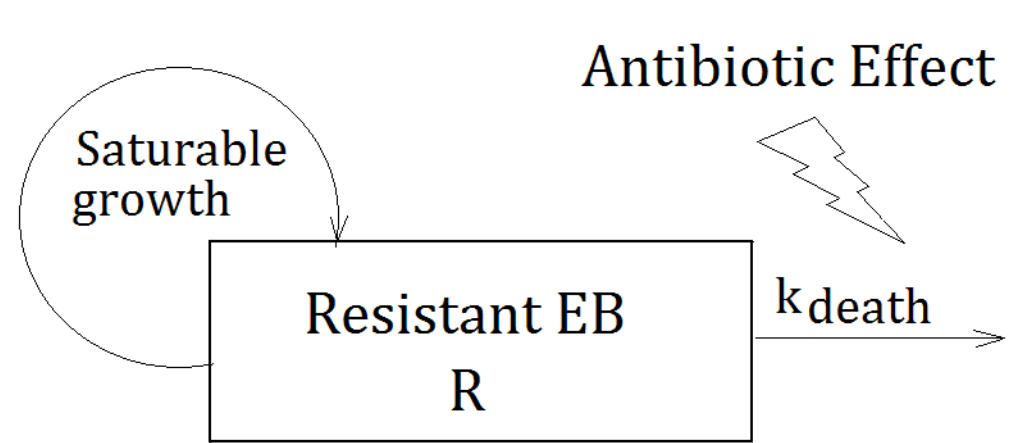


Model equation

$$\begin{aligned} \text{During treatment } \frac{dC}{dt} &= \frac{R_{in}}{V} - \frac{Cl}{V} C \\ \text{After treatment } \frac{dC}{dt} &= -\frac{Cl}{V} C \\ \text{Initial condition } C_0 &= 0 \end{aligned}$$

- **PD model of resistant EB counts**

- * CIP effect supposed to inhibit the natural elimination rate (k_{death}) of resistant bacteria counts (R)



Model equation

$$\frac{dR}{dt} = \frac{V_{max}}{R_{50} + R} R - k_{death} \left(1 - \frac{C}{C_{50} + C}\right) R$$

Initial condition $R_0 = V_{max}/k_{death} - R_{50}$

⇒ Models written with MLXTRAN in MONOLIX 4.1.1 [9]

• Population analysis

- Joint modelling of data of 20 piglets receiving CIP by nonlinear mixed effects model
 - * Exponential model for the random effects
 - * Diagonal variance-covariance matrix of diagonal terms ω^2 (no variability on V_{max} and R_{50})
 - * Combined error model for fecal CIP concentrations (parameterized in σ_{add}^{CIP} and σ_{prop}^{CIP}) and constant error model for log₁₀ of resistant EB counts (parameterized in σ_{add}^{EB})
- Estimation of population parameters & their variability by SAEM algorithm [10] in MONOLIX 4.1.1
- Data below limit of quantification (BLQ) taken into account in SAEM [11]
- Model evaluation via goodness-of-fit plots

RESULTS

• Model fitting and goodness-of-fit evaluation

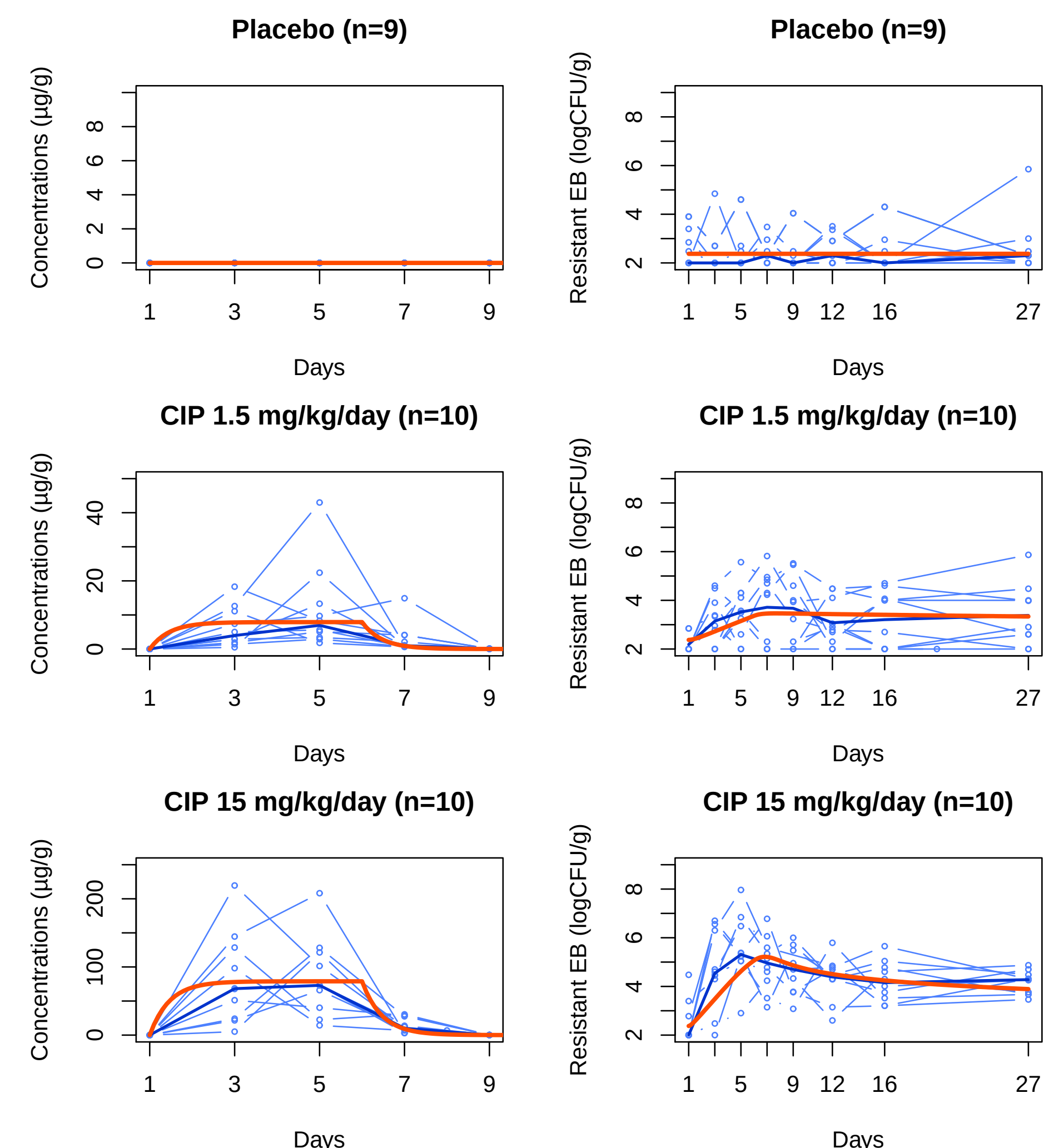


Fig 1: Experimental data from individual animals (light blue) and medians (deep blue) versus model predicted curves (orange)

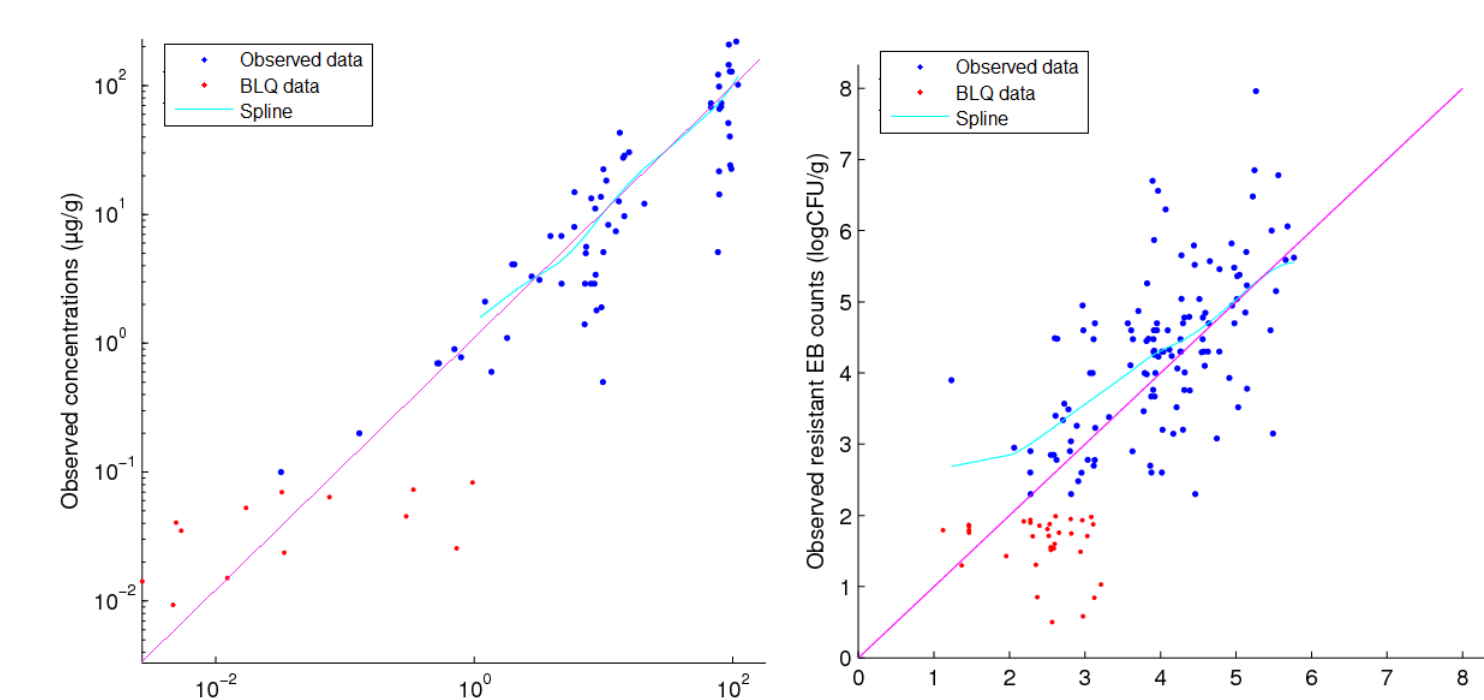


Fig 2: Observations versus predictions

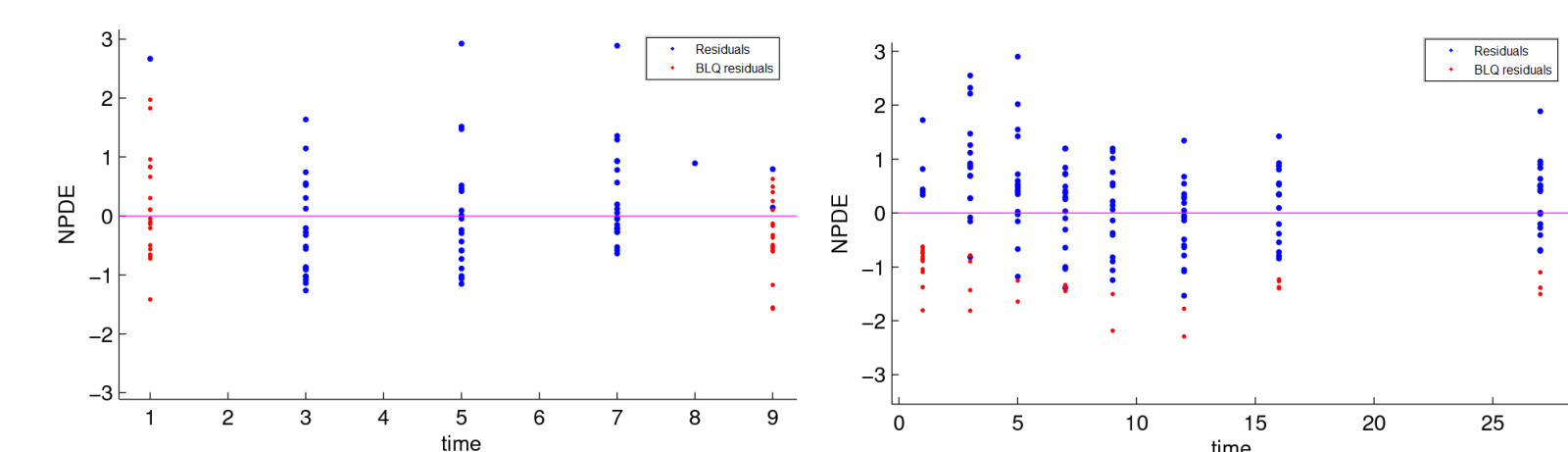


Fig 3: Normalized prediction distribution errors for PK (left) & PD (right) responses versus time [12]

- The chosen PK/PD model adequately describes CIP fecal concentrations and resistant EB counts

• Parameter estimation and relative standard errors (RSE%)

Fixed effects	Estimates (RSE%)	Variabilities	Estimates (RSE%)
V (L kg ⁻¹)	0.08 (10)	ω_V	0.29 (34)
Cl (L kg ⁻¹ day ⁻¹)	0.16 (8)	ω_{Cl}	0.26 (26)
V_{max} (CFU g ⁻¹ day ⁻¹)	1.95 10 ⁵ (0.38)	$\omega_{C_{50}}$	1.80 (19)
$\log_{10} R_{50}$ (logCFU g ⁻¹)	5.40 (0.005)	σ_{add}^{CIP} (µg g ⁻¹)	0.03 (49)
k_{death} (day ⁻¹)	0.77 (0.05)	σ_{prop}^{CIP}	0.76 (12)
C_{50} (µg g ⁻¹)	20.2 (47)	σ_{add}^{EB} (logCFU g ⁻¹)	1.06 (6)

- Parameters are well estimated with good precision

• Prediction for various ciprofloxacin doses

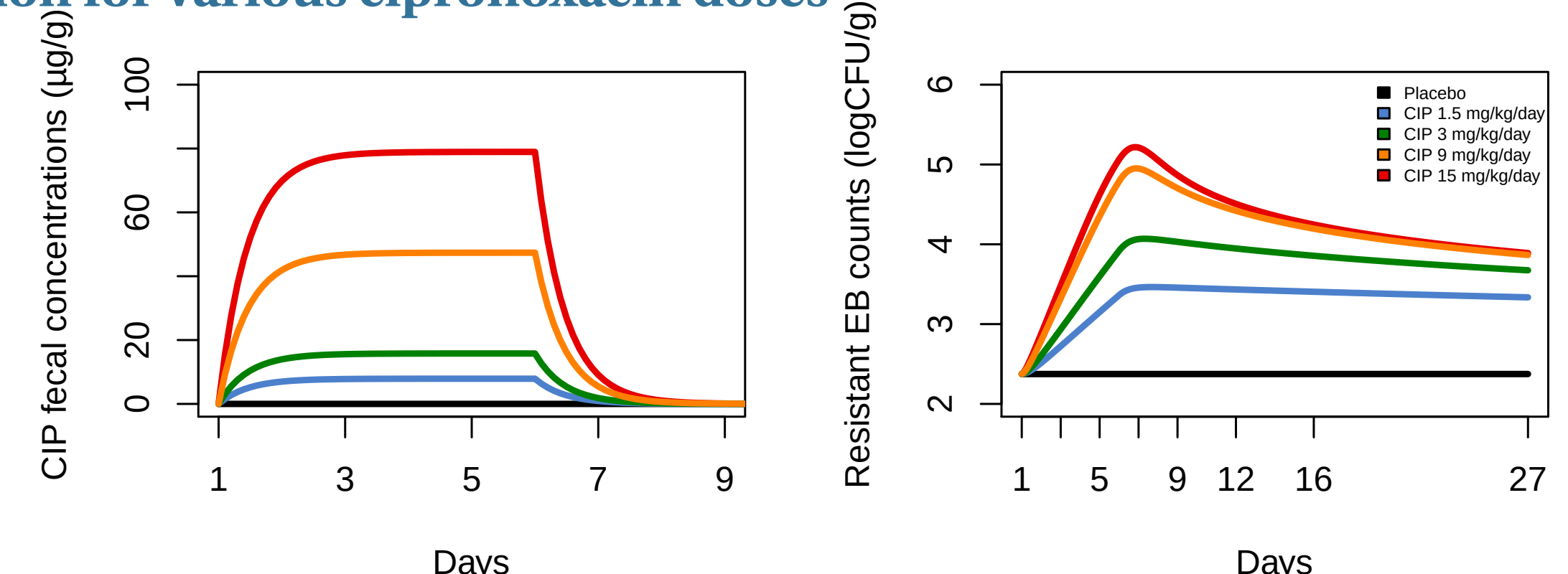


Fig 4: CIP concentrations and resistant EB counts predicted by the chosen model for various doses

- CIP fecal concentrations increase sharply with dose
- Resistant EB amounts increase nonlinearly with concentrations (as found by standard analysis of areas under the curve [4]) and after treatment return to a value higher than baseline

CONCLUSION

- The proposed PK/PD model adequately describes jointly CIP fecal concentrations and resistant EB counts for different CIP doses
- To our knowledge, this is the first model developed on *in vivo* data to study the dynamic of resistance in colonic commensal flora
- Next step: to model these data jointly with the susceptible EB counts
- This modelled relationship suggests that removing residual colonic quinolone can help control the emergence and dissemination of quinolone resistance [13]

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