An *in vivo* pharmacokinetic/pharmacodynamic model of bacterial resistance to ciprofloxacin in commensal flora. Application to design a new study of antibiotic resistance

Thu Thuy Nguyen (1,2), Elisabeth Chachaty (3), Jean de Gunzburg (4), Antoine Andremont (1,5,6) and France Mentré (1,2,6)

(1) University Paris Diderot; (2) INSERM, UMR 738, Paris, France; (3) Institut Gustave-Roussy, Paris, France; (4) Da Volterra, Paris, France; (5) EA3964, "Emergence de la résistance bactérienne in vivo", Paris, France; (6) AP-HP, Hospital Bichat, Paris, France

September 20, Rennes

GDR Statistique & Santé 2012

Modelling antibiotic resistance in commensal flora

- Emergence of quinolone resistant bacteria in intestinal flora during treatment: increasing therapeutic problem [1,2]
- Modelling pharmacokinetic/pharmacodynamic (PK/PD) relationship for antibiotics
  - Describing the whole time course of bacteria counts, drug concentrations, and antimicrobial effect
  - Using differential equations
  - Population approach using nonlinear mixed effect models (NLMEM)
    - Analysis of subjects with repeated PK/PD measures, taking into account inter-subject variability
    - Estimation of parameters by likelihood maximisation
      - No analytical expression for the likelihood
      - Approaches without linearisation: SAEM algorithm [3] in MONOLIX [4], Adaptive Gaussian Quadrature in SAS procedure NLMIXED

[1] Lee et al. *J Infect*, 2011.
[2] Nguyen, Chachaty et al. *Antimicrob Agents Chemother*, 2012.
[3] Kuhn and Lavielle. *Comput Stat Data Anal*, 2005.
[4] www.monolix.org.

Design evaluation and optimisation in NLMEM

- Important impact of design (number of subjects and of samples, allocation of times and of doses) on study results (precision of parameter estimates, power of test,...)
- Approach based on population Fisher information matrix (M_F) using first order approximation of the model [1]
  - Implemented in R function PFIM [2,3] and in other software
  - Criterion of design efficacy = determinant$(M_F)^{1/P}$, P = total number of population parameters

[1] Mentré et al. *Biometrika*, 1997.
[2] Bazzoli et al. *Comput Methods Programs Biomed*, 2010.
[3] www.pfim.biostat.fr.

Objectives

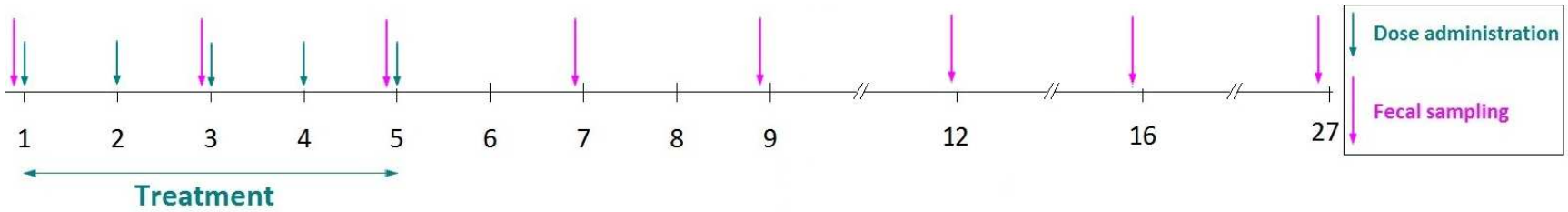
1. To develop a PK/PD model to characterize the link between ciprofloxacin (CIP) concentrations and amounts of CIP resistant EB in feces
2. To design a future study of dynamic of resistance in commensal flora

Study design

- **Prospective study in piglets**: 4 weeks old, not received any antimicrobial since birth
- **Treatment group**: $N = 29$ 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

- **Fecal samplings**



  - **CIP fecal concentrations** determined using microbiological assay (limit of quantification = 0.1 μg/g)
  - **CIP resistant EB** counted on Drigalski agar containing 2 mg/L of ciprofloxacin (limit of quantification = 10^2 CFU/g)

Data

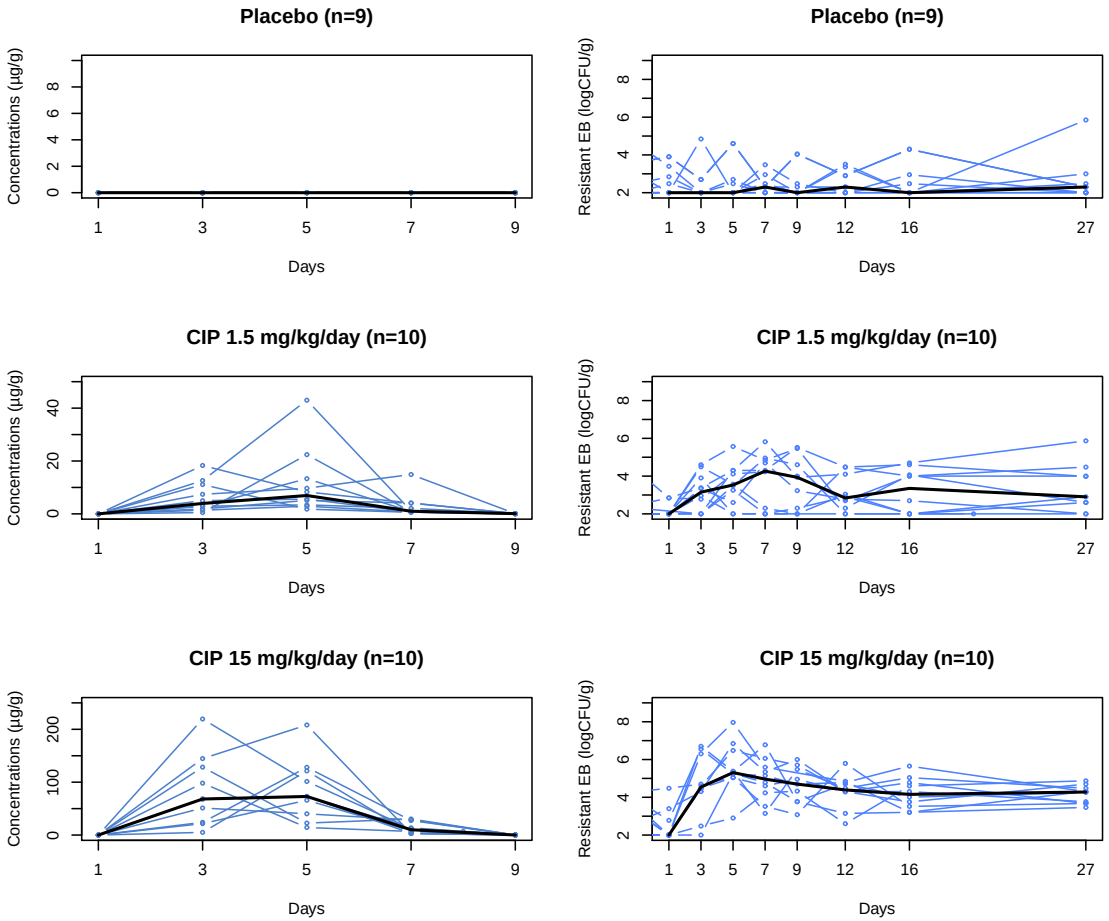
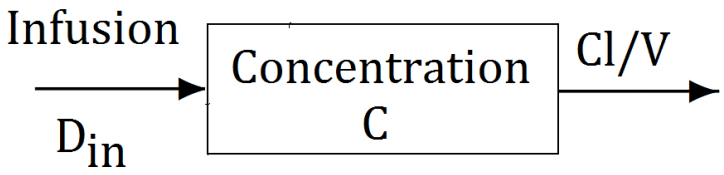


Fig: Experimental data from individual animals (blue) and medians (black)

PK/PD model

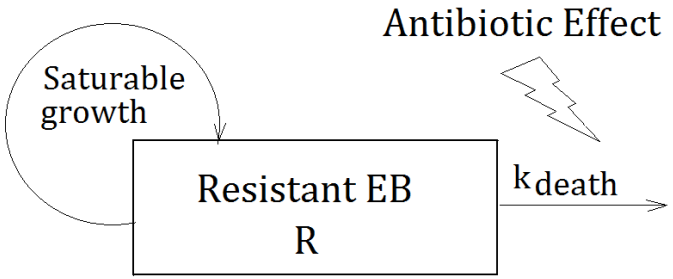
- **PK model of fecal concentrations (C)**
  - Assimilated to a one compartment model with intravenous infusion (rate D_{in} = 1.5 or 15 mg/kg/day, duration = 5 days)



$$\frac{dC}{dt} = \frac{D_{in}}{V} - \frac{Cl}{V} C$$

Initial condition $C_0 = 0$

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



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

Initial condition

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

Population analysis

- Joint modelling of data of **20** piglets receiving CIP by nonlinear mixed effects model
  - Exponential model for the random effects, diagonal covariance matrix of diagonal terms ω^2
  - Combined error model for fecal CIP concentrations ($\sigma^{CIP}_{\text{add}}$ and $\sigma^{CIP}_{\text{prop}}$)
  - Constant error model for $\log_{10}$ of resistant EB counts (σ^{EB}_{add})
- Estimation of population parameters & their variability by SAEM algorithm in MONOLIX 4.1.1 [1], taking into account data below limit of quantification (BLQ) as left-censored data [2]
- Model evaluation via goodness-of-fit plots

[1] www.monolix.org.
[2] Samson, Lavielle and Mentré. *Comput Stat Data Anal*, 2006.

Model fitting and goodness-of-fit evaluation

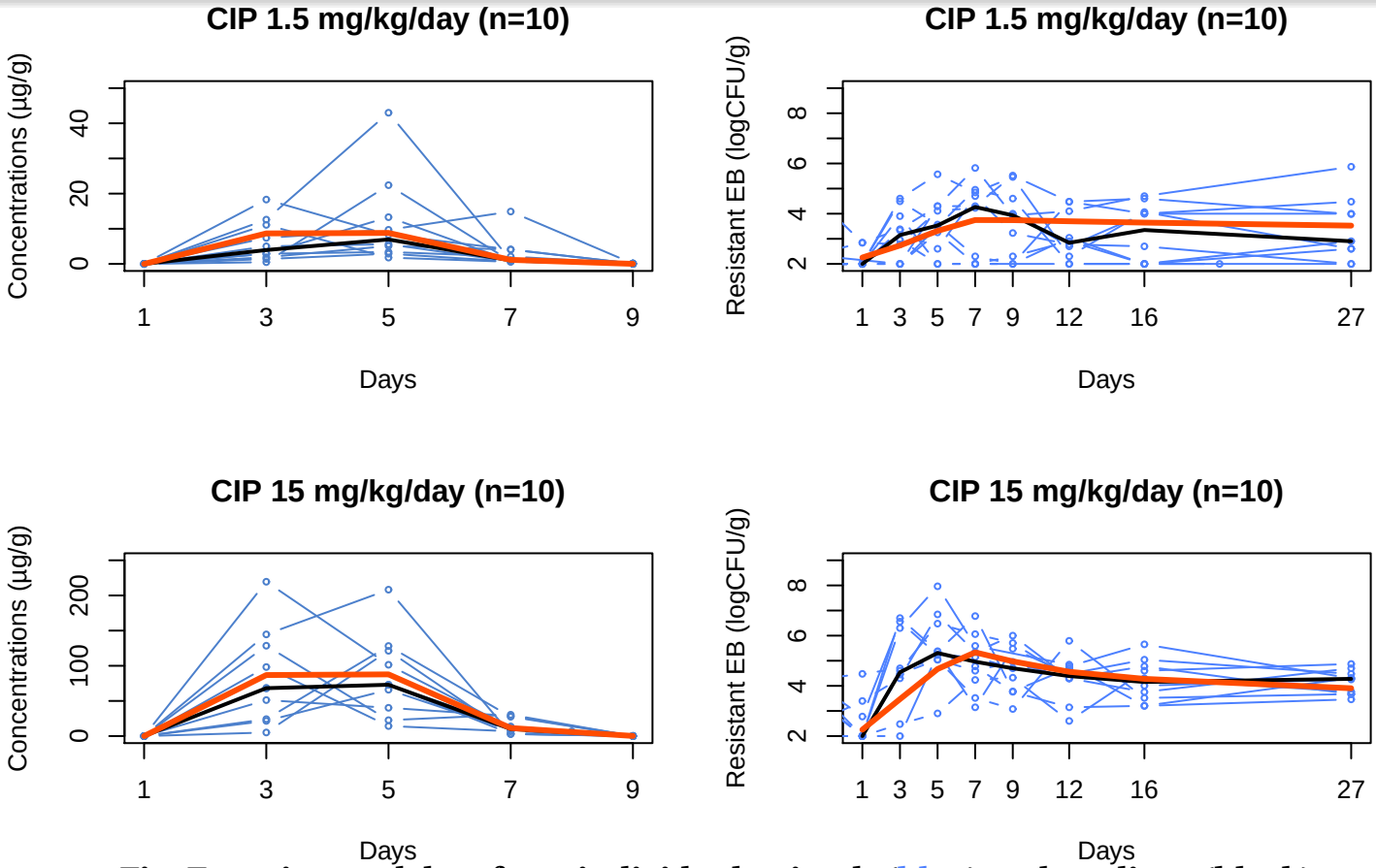


Fig: Experimental data from individual animals (blue) and medians (black) *versus* model median prediction curves (red)

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

Parameter estimation and relative standard errors

Fixed effects	Estimates (RSE%)
V ($\mathrm{L\,kg^{-1}}$)	0.08 (10)
Cl ($\mathrm{L\,kg^{-1}\,day^{-1}}$)	0.16 (8)
$V_{\max}$ ($\mathrm{CFU\,g^{-1}\,day^{-1}}$)	$1.95\ 10^5$ (0.38)
$\log_{10} R_{50}$ ($\log \mathrm{CFU\,g^{-1}}$)	5.40 (0.005)
k_{death} ($\mathrm{day^{-1}}$)	0.77 (0.05)
C_{50} ($\mu \mathrm{g\,g^{-1}}$)	20.2 (47)

Variabilities	Estimates (RSE%)
ω_V	0.29 (34)
ω_{Cl}	0.26 (26)
$\omega_{C_{50}}$	1.80 (19)
$\sigma^{CIP}_{\mathrm{add}}$ ($\mu \mathrm{g\,g^{-1}}$)	0.03 (49)
$\sigma^{CIP}_{\mathrm{prop}}$	0.76 (12)
$\sigma^{EB}_{\mathrm{add}}$ ($\log \mathrm{CFU\,g^{-1}}$)	1.06 (6)

Parameter estimation and relative standard errors

Fixed effects	Estimates (RSE%)
V ($\mathrm{L\,kg^{-1}}$)	0.08 (10)
Cl ($\mathrm{L\,kg^{-1}\,day^{-1}}$)	0.16 (8)
$V_{\max}$ ($\mathrm{CFU\,g^{-1}\,day^{-1}}$)	$1.95\ 10^5$ (0.38)
$\log_{10} R_{50}$ ($\log \mathrm{CFU\,g^{-1}}$)	5.40 (0.005)
k_{death} ($\mathrm{day^{-1}}$)	0.77 (0.05)
C_{50} ($\mu \mathrm{g\,g^{-1}}$)	20.2 (47)

Variabilities	Estimates (RSE%)
ω_V	0.29 (34)
ω_{Cl}	0.26 (26)
$\omega_{C_{50}}$	1.80 (19)
$\sigma^{CIP}_{\mathrm{add}}$ ($\mu \mathrm{g\,g^{-1}}$)	0.03 (49)
$\sigma^{CIP}_{\mathrm{prop}}$	0.76 (12)
$\sigma^{EB}_{\mathrm{add}}$ ($\log \mathrm{CFU\,g^{-1}}$)	1.06 (6)

Design of a new study

Using PFIM 3.2 [1] based on model and estimated parameters to study the influence of different design variables

- Comparison of predicted SE (using M_F) and criteria between different designs
  - Two dose groups vs. one dose group
  - Original rich design vs. optimal sparse design (same design in all subjects)
- Optimisation of design with 2 doses using Fedorov-Wynn algorithm

Dose allocation	$N = 30$, $n_{PK} = 5$, $n_{PD} = 8$	$N = 50$, $n_{PK} = 3$, $n_{PD} = 5$
	$n_{tot} = 390$	$n_{tot} = 400$
1 dose group: 15mg	PK (1, 3, 5, 7, 9)	samples
2 dose groups: 1/2 1.5mg, 1/2 15mg	PD (1, 3, 5, 7, 9, 12, 16, 27)	to be defined
2 dose groups: optimised		samples & structure to be defined

Table: Different studied designs

[1] www.pfim.biostat.fr.

Design of a new study

Using PFIM 3.2 [1] based on model and estimated parameters to study the influence of different design variables

- Comparison of predicted SE (using M_F) and criteria between different designs
  - Two dose groups vs. one dose group
  - Original rich design vs. optimal sparse design (same design in all subjects)
- Optimisation of design with 2 doses using Fedorov-Wynn algorithm

Dose allocation	$N = 30$, $n_{PK} = 5$, $n_{PD} = 8$	$N = 50$, $n_{PK} = 3$, $n_{PD} = 5$
	$n_{tot} = 390$	$n_{tot} = 400$
1 dose group: 15mg	PK (1, 3, 5, 7, 9)	samples
2 dose groups: 1/2 1.5mg, 1/2 15mg	PD (1, 3, 5, 7, 9, 12, 16, 27)	to be defined
2 dose groups: optimised		samples & structure to be defined

Table: Different studied designs

[1] www.pfim.biostat.fr.

Design of a new study

Using PFIM 3.2 [1] based on model and estimated parameters to study the influence of different design variables

- Comparison of predicted SE (using M_F) and criteria between different designs
  - Two dose groups vs. one dose group
  - Original rich design vs. optimal sparse design (same design in all subjects)
- Optimisation of design with 2 doses using Fedorov-Wynn algorithm

Dose allocation	$N = 30$, $n_{PK} = 5$, $n_{PD} = 8$	$N = 50$, $n_{PK} = 3$, $n_{PD} = 5$
	$n_{tot} = 390$	$n_{tot} = 400$
1 dose group: 15mg	PK (1, 3, 5, 7, 9)	samples
2 dose groups: 1/2 1.5mg, 1/2 15mg	PD (1, 3, 5, 7, 9, 12, 16, 27)	to be defined
2 dose groups: optimised		samples & structure to be defined

Table: Different studied designs

[1] www.pfim.biostat.fr.

Design of a new study

Using PFIM 3.2 [1] based on model and estimated parameters to study the influence of different design variables

- Comparison of predicted SE (using M_F) and criteria between different designs
  - Two dose groups vs. one dose group
  - Original rich design vs. optimal sparse design (same design in all subjects)
- Optimisation of design with 2 doses using Fedorov-Wynn algorithm

Dose allocation	$N = 30$, $n_{PK} = 5$, $n_{PD} = 8$	$N = 50$, $n_{PK} = 3$, $n_{PD} = 5$
	$n_{tot} = 390$	$n_{tot} = 400$
1 dose group: 15mg	PK (1, 3, 5, 7, 9)	samples
2 dose groups: 1/2 1.5mg, 1/2 15mg	PD (1, 3, 5, 7, 9, 12, 16, 27)	to be defined
2 dose groups: optimised		samples & structure to be defined

Table: Different studied designs

[1] www.pfim.biostat.fr.

Design of a new study

Dose allocation	$N = 30$, $n_{PK} = 5$, $n_{PD} = 8$			$N = 50$, $n_{PK} = 3$, $n_{PD} = 5$		
	RSE(C_{50})	RSE(k_{death})	Criterion	RSE(C_{50})	RSE(k_{death})	Criterion
1 group: 15mg *	179.3%	47.1%	40.7	*NA*	*NA*	41.2
2 groups: 1/2 1.5mg, 1/2 15mg **	76.8%	30.6%	42.9	66.6%	25.0%	44.1
2 groups: optimised ***				74.6%	24.7%	54.3

Table: Predicted RSE(%) and criteria by PFIM with different designs
NA: prediction not available for sparse design with only one dose

- Optimised design *: {PK (1, 3, 9), PD (1, 5, 7, 9, 27)}
- Optimised design **: {PK (3, 5, 9), PD (1, 5, 9, 16, 27)}
- Optimised design ***:
  34 piglets with 15 mg
  24/34 {PK (3, 5, 9), PD (1, 5, 7, 16, 27)}; 10/34 {PK (1, 3, 9), PD (1, 5, 9, 16, 27)}
  16 piglets with 1.5 mg
  8/16 {PK (1, 3, 7), PD (1, 3, 9, 12, 27)}; 8/16 {PK (1, 3, 9), PD (1, 3, 9, 12, 27)}

Design of a new study

Dose allocation	$N = 30$, $n_{PK} = 5$, $n_{PD} = 8$			$N = 50$, $n_{PK} = 3$, $n_{PD} = 5$		
	RSE(C_{50})	RSE(k_{death})	Criterion	RSE(C_{50})	RSE(k_{death})	Criterion
1 group: 15mg *	179.3%	47.1%	40.7	*NA*	*NA*	41.2
2 groups: 1/2 1.5mg, 1/2 15mg **	76.8%	30.6%	42.9	66.6%	25.0%	44.1
2 groups: optimised ***				74.6%	24.7%	54.3

Table: Predicted RSE(%) and criteria by PFIM with different designs
NA: prediction not available for sparse design with only one dose

- Optimised design *: {PK (1, 3, 9), PD (1, 5, 7, 9, 27)}
- Optimised design **: {PK (3, 5, 9), PD (1, 5, 9, 16, 27)}
- Optimised design ***:
  34 piglets with 15 mg
  24/34 {PK (3, 5, 9), PD (1, 5, 7, 16, 27)}; 10/34 {PK (1, 3, 9), PD (1, 5, 9, 16, 27)}
  16 piglets with 1.5 mg
  8/16 {PK (1, 3, 7), PD (1, 3, 9, 12, 27)}; 8/16 {PK (1, 3, 9), PD (1, 3, 9, 12, 27)}

Design of a new study

Dose allocation	$N = 30$, $n_{PK} = 5$, $n_{PD} = 8$			$N = 50$, $n_{PK} = 3$, $n_{PD} = 5$		
	RSE(C_{50})	RSE(k_{death})	Criterion	RSE(C_{50})	RSE(k_{death})	Criterion
1 group: 15mg *	179.3%	47.1%	40.7	*NA*	*NA*	41.2
2 groups: 1/2 1.5mg, 1/2 15mg **	76.8%	30.6%	42.9	66.6%	25.0%	44.1
2 groups: optimised ***				74.6%	24.7%	54.3

Table: Predicted RSE(%) and criteria by PFIM with different designs
NA: prediction not available for sparse design with only one dose

- Optimised design *: {PK (1, 3, 9), PD (1, 5, 7, 9, 27)}
- Optimised design **: {PK (3, 5, 9), PD (1, 5, 9, 16, 27)}
- Optimised design ***:
  34 piglets with 15 mg
  24/34 {PK (3, 5, 9), PD (1, 5, 7, 16, 27)}; 10/34 {PK (1, 3, 9), PD (1, 5, 9, 16, 27)}
  16 piglets with 1.5 mg
  8/16 {PK (1, 3, 7), PD (1, 3, 9, 12, 27)}; 8/16 {PK (1, 3, 9), PD (1, 3, 9, 12, 27)}

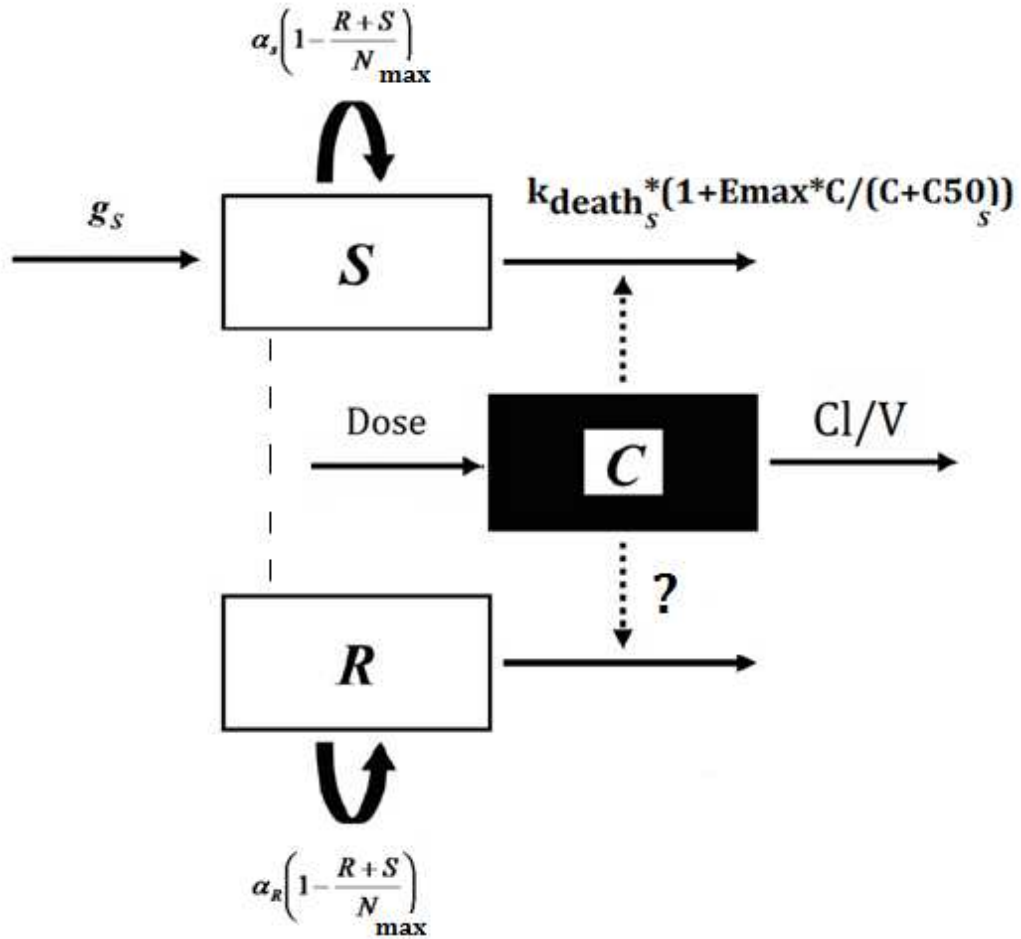
Discussion

Summary

- This empirical PK/PD model adequately describes jointly CIP fecal concentrations and resistant bacteria counts for different CIP doses
- To our knowledge, this is the first modelling on *in vivo* data to study the dynamic of resistance in colonic commensal flora
- The use of NLMEM and of the obtained results with PFIM allows to efficiently design future studies
  - Good balance between number of subjects and of samples per subject as well as appropriate choice of dose and time allocation allow sparse design and improve the estimation precision of parameters
- Next step: to model these data jointly with the total bacteria counts (= susceptible S + resistant R counts)

Discussion

On-going work: Modelling of both total and resistant bacteria counts with a more physiological model *(collaboration with Jérémie Guedj, INSERM UMR 738)*



THANK YOU FOR YOUR ATTENTION !

Back-up

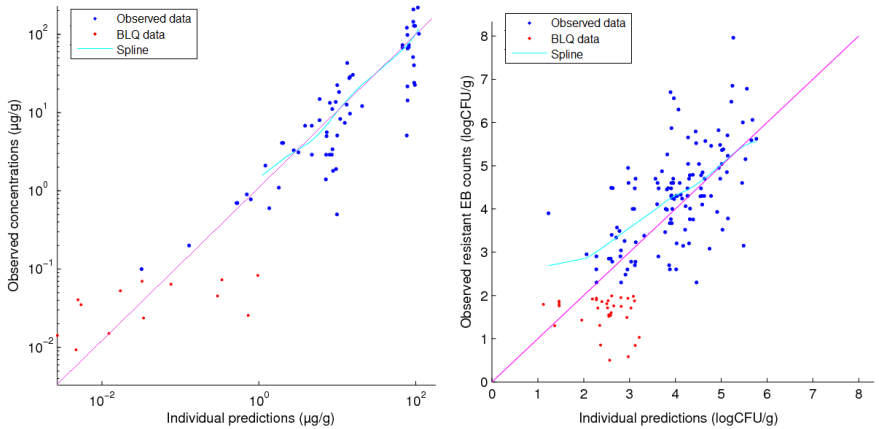


Fig: Observations *versus* predictions

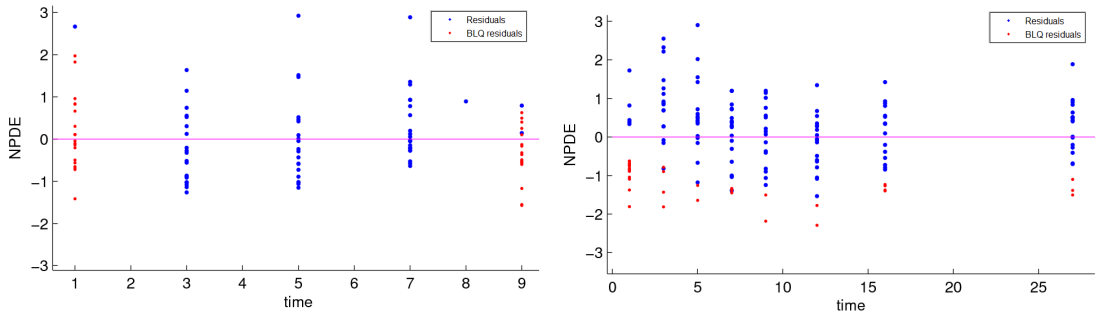


Fig: Normalized prediction distribution errors for PK (left) & PD (right) responses *versus* time [1]

[1] Brendel et al. *Pharm Res*, 2006.

Back-up

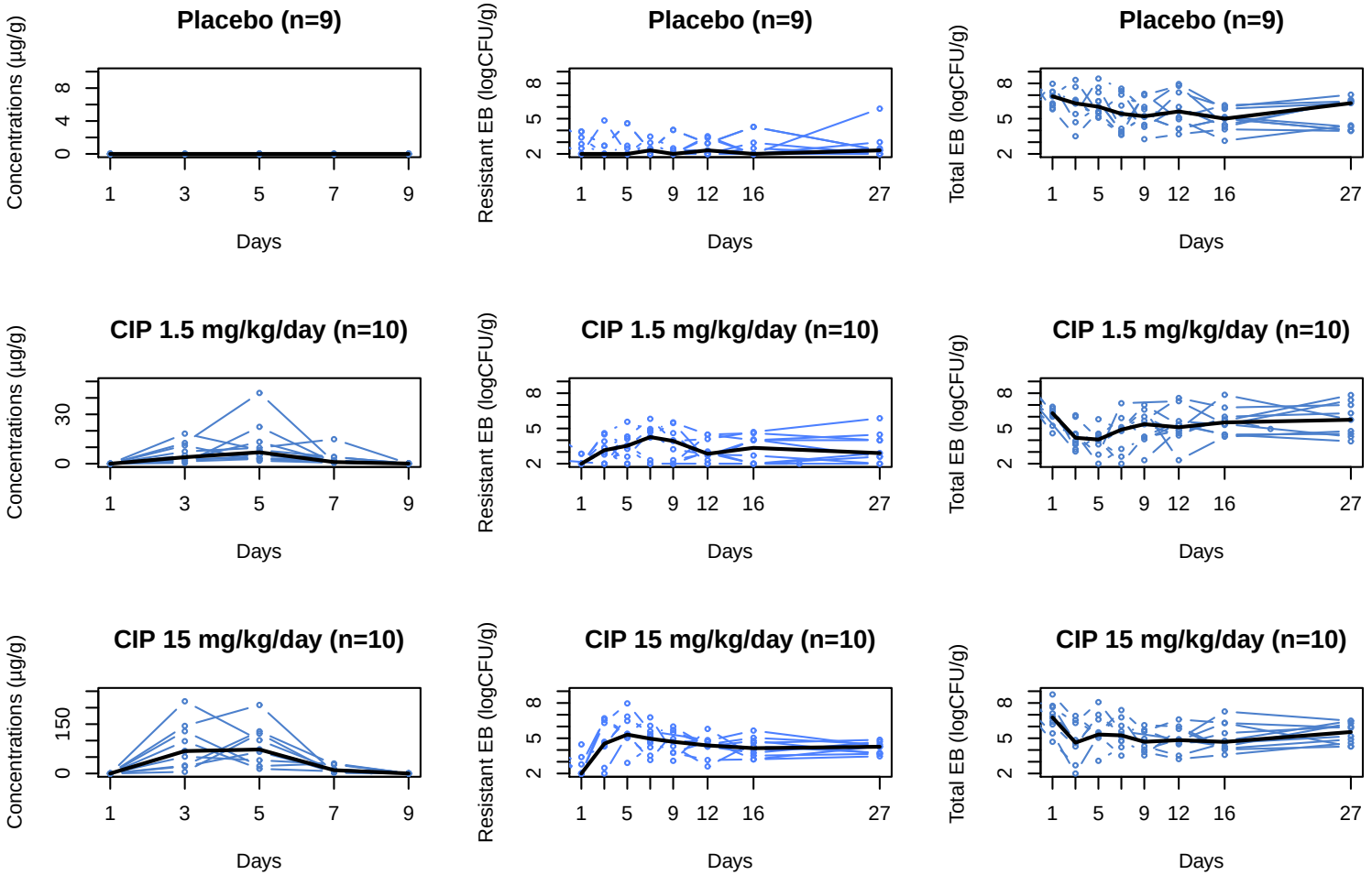


Fig: Experimental data from individual animals (light blue) and medians (deep blue)