FLORVIO ™
Pharmacokinetic and pharmacodynamic aspects of soluble florfenicol in swine

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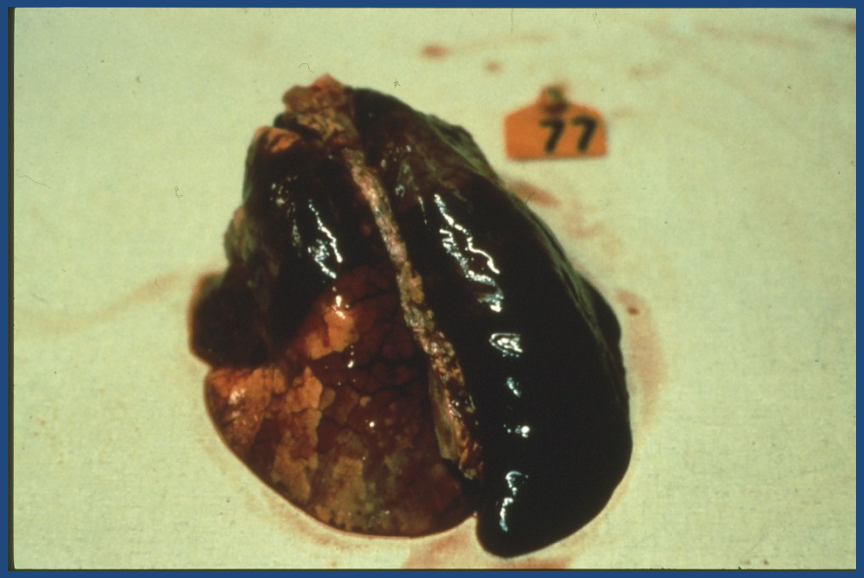
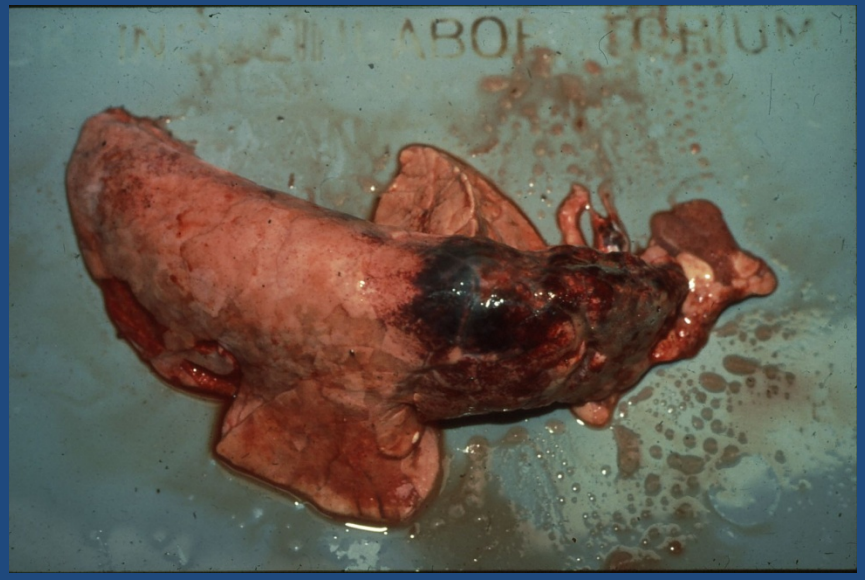
Indications in the US (generic)

- *FLORVIO* (Florfenicol – An antimicrobial 2.3% concentrate solution) (23mg/ml)
- For oral use in swine drinking water only
- For use by or on the order of a licensed veterinarian
- Indicated for the treatment of swine respiratory diseases associated with:
  - *Actinobacillus pleuropneumoniae*
  - *Pasteurella multocida*
  - *Streptococcus suis*
  - *Salmonella choleraesuis*

A. pleuropneumoniae

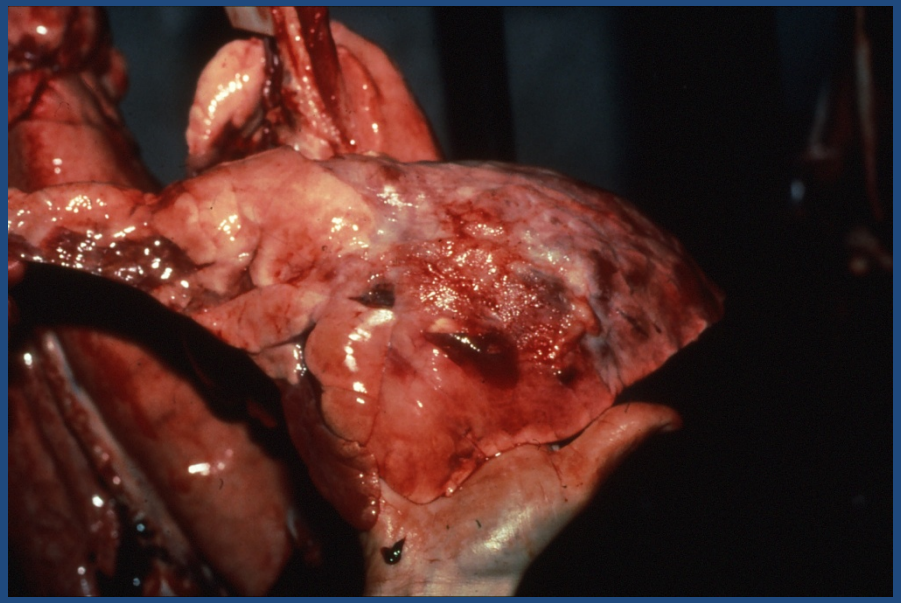


Per-acute infection - death

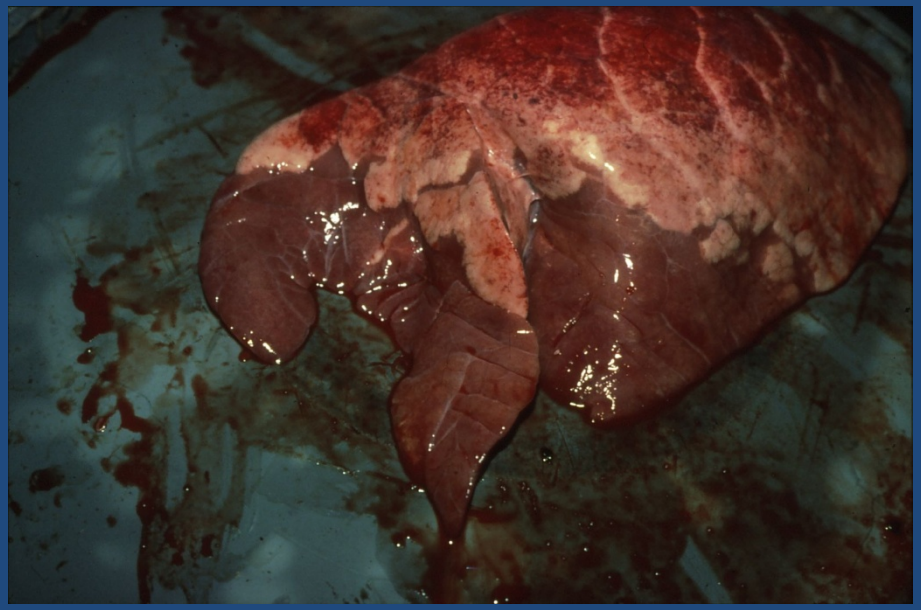


Chronic infection lesions + pleurisy

Pleurisy and enzootic pneumonia



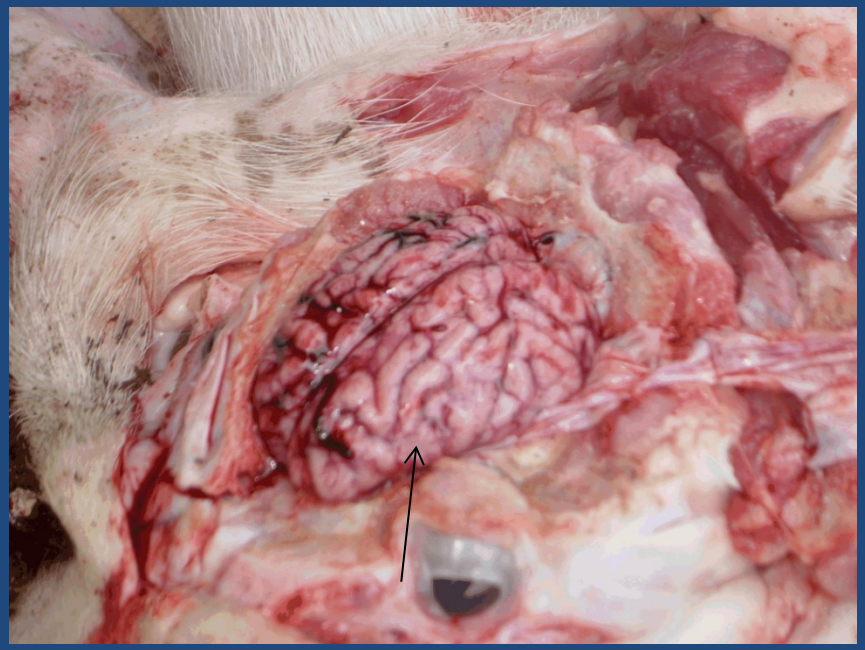
Extensive APP pleurisy



EP lesions –
Mycoplasma and *P. multocida*

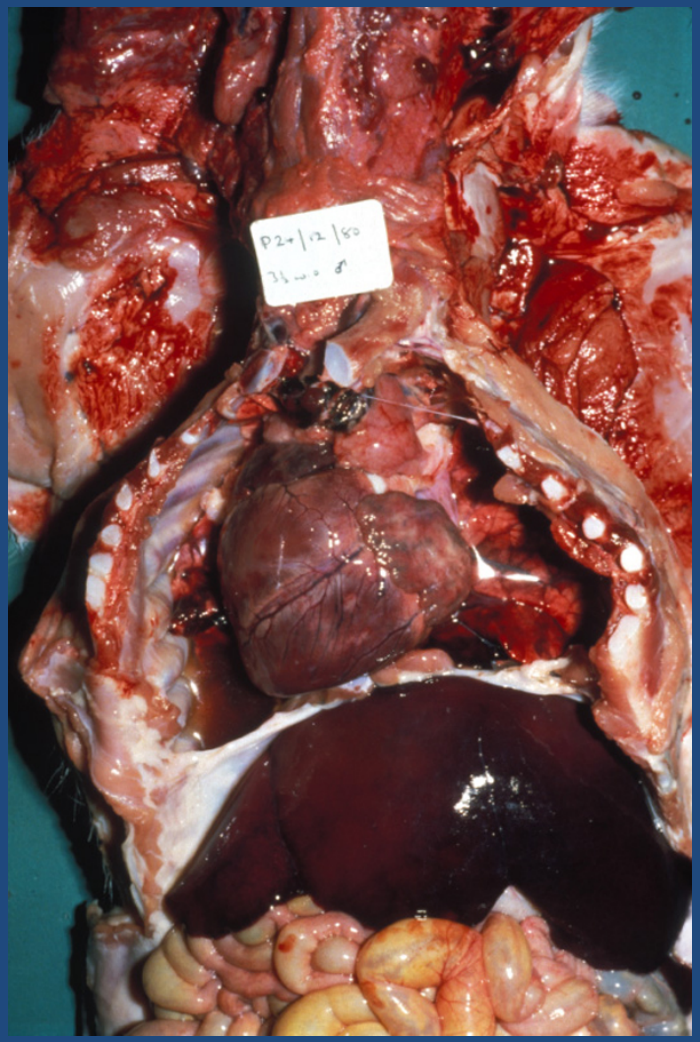
S. suis - meningitis

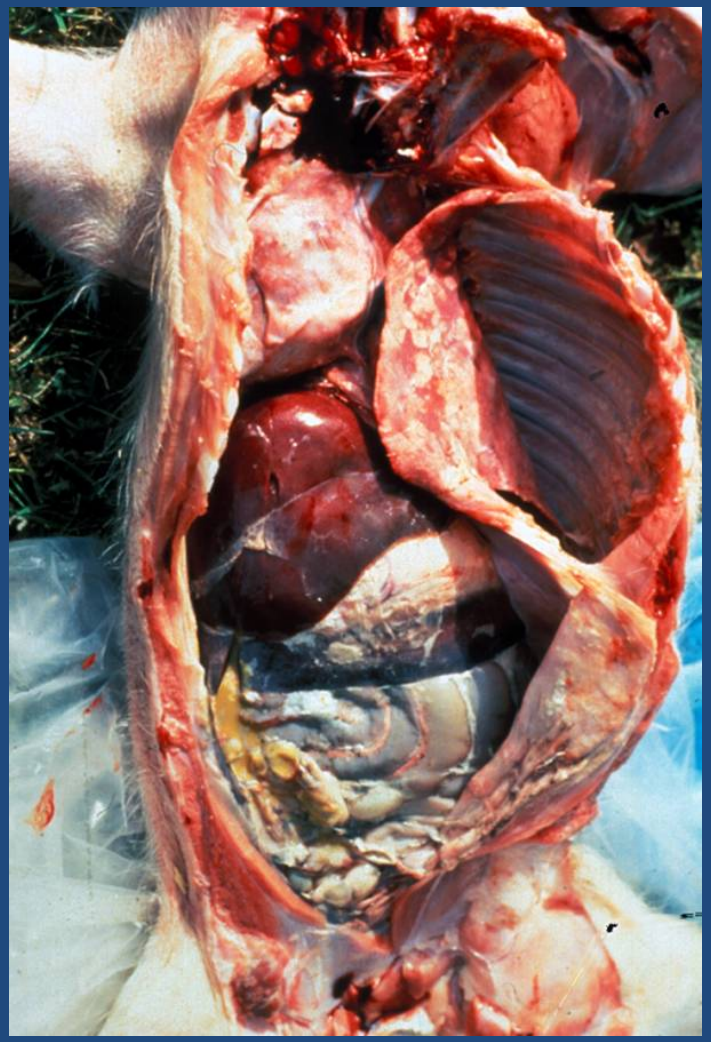
Dead or paddling pigs commonly found



Encephalitis / meningitis

Pneumonia and polyserositis “suiscides”





S. choleraesuis – pigs and lungs

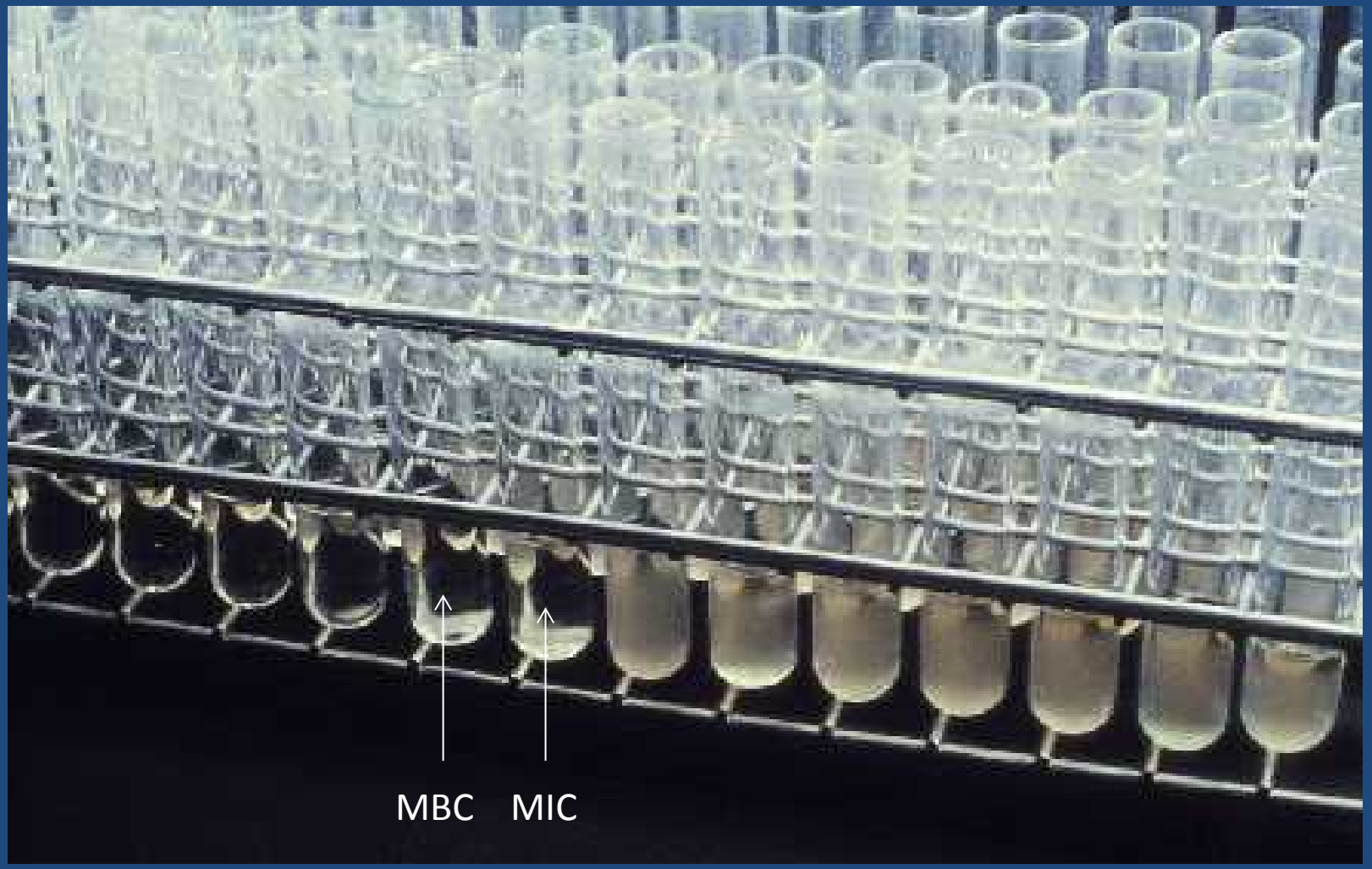
Septicaemic pigs

Haemorrhages in lung, liver, spleen

Pharmacodynamics

- Susceptibility testing:
  - MIC (minimum inhibitory concentration) the lowest concentration which will inhibit the growth of a bacterium. Florfenicol is classed as a bacteriostatic drug
  - MBC (minimum bactericidal concentration) the lowest concentration which will kill the bacterium. For florfenicol the MBC is approximately 2 times the MIC – for *A. pleuropneumoniae* and *P. multocida* (Etore et al, 2004)
  - Killing curves – shows how the drug kills the bug. Is it time dependent, concentration dependent or both?– Florfenicol activity is dependent upon time above the MIC (Etore et al, 2004) - these results are consistent with product effectiveness.

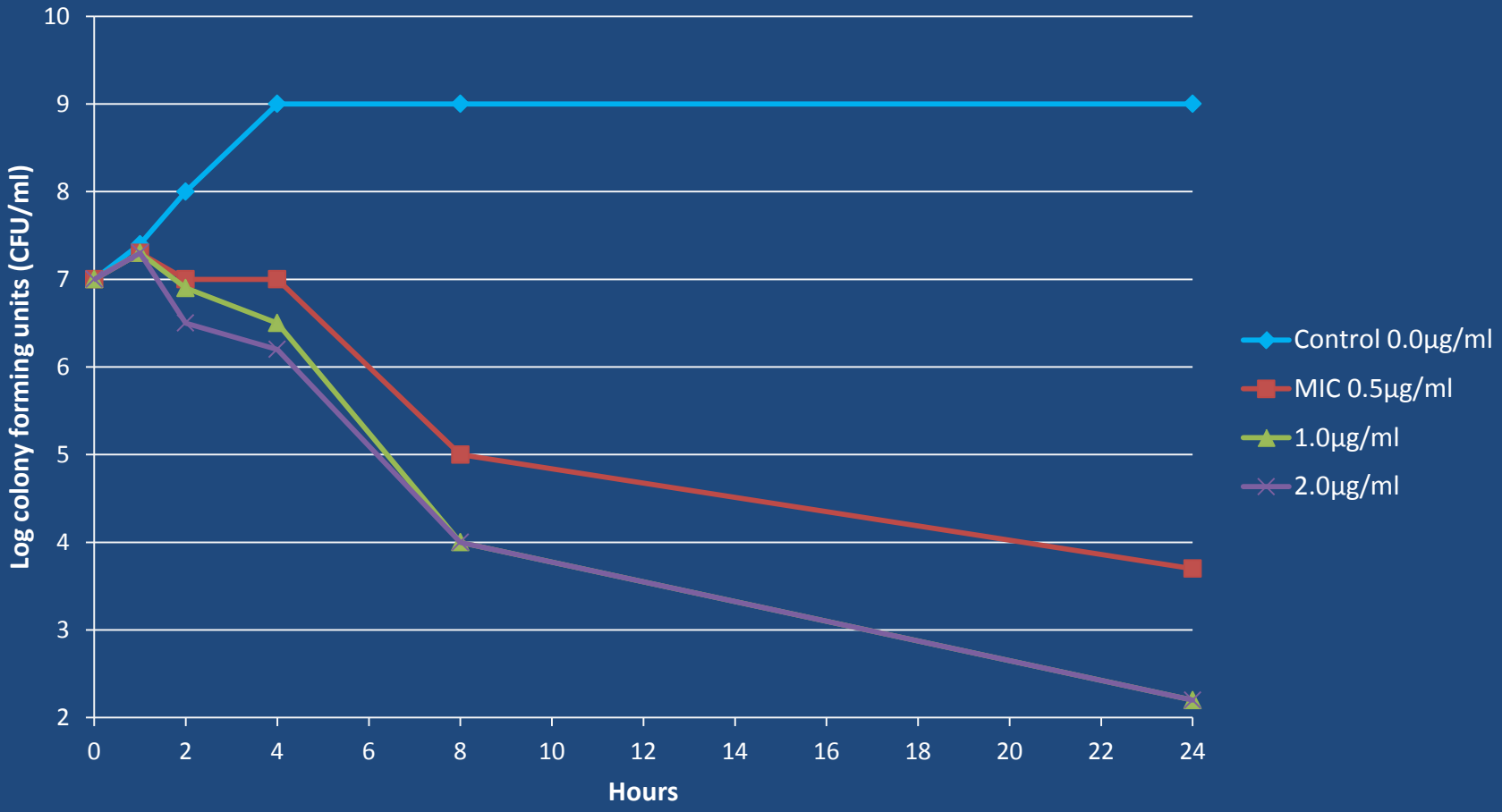
MIC determination – broth dilution



Minimum inhibitory concentrations (MIC) - where the bug stops growing in the drug
Minimum bactericidal concentration (MBC) – where bug is killed

Killing curves for *P. multocida* & florfenicol

(Etore et al, 2004)



Bactericidal effect occurs at 2 times MIC but does not increase from there

Time dependent killing curve; similar for *A. pleuropneumoniae*

Susceptibility of US isolates to florfenicol

(Zolynas et al, 2003)

Bacteria	No of isolates	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
A. pleuropneumoniae	100	0.25	**0.5**	0.25-1.0
P. multocida	107	0.5	**0.5**	0.25-0.5
S. suis	62	2.0	**2.0**	1.0-2.0
S. choleraesuis	36	4.0	**4.0**	2.0-4.0

Highly susceptible *A. pleuropneumoniae* and *P. multocida*

Less susceptible *S. suis* and *S. choleraesuis*

Susceptibility of US isolates to florfenicol

(Salmon et al, 2003)

Bacteria / antibiotic	No of isolates	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)	Resistance (%)
A. pleuropneumoniae	89				
Florfenicol		0.25	**0.5**	<0.06-0.5	0
Tetracycline		16	32	<0.12-64	**>50**
Penicillin		0.5	32	<0.12-64	>10
P. multocida	186				
Florfenicol		0.25	**0.5**	<0.06-4.0	<10
Tetracycline		2.0	32	0.25-64	>10
Penicillin		<0.12	<0.12	<0.12-64	<10
S. suis	167				
Florfenicol		1.0	**2.0**	0.12-4.0	<10
Tetracycline		64	64	0.25->64	**>50**
Penicillin		<0.12	0.25	<0.12-32	<10

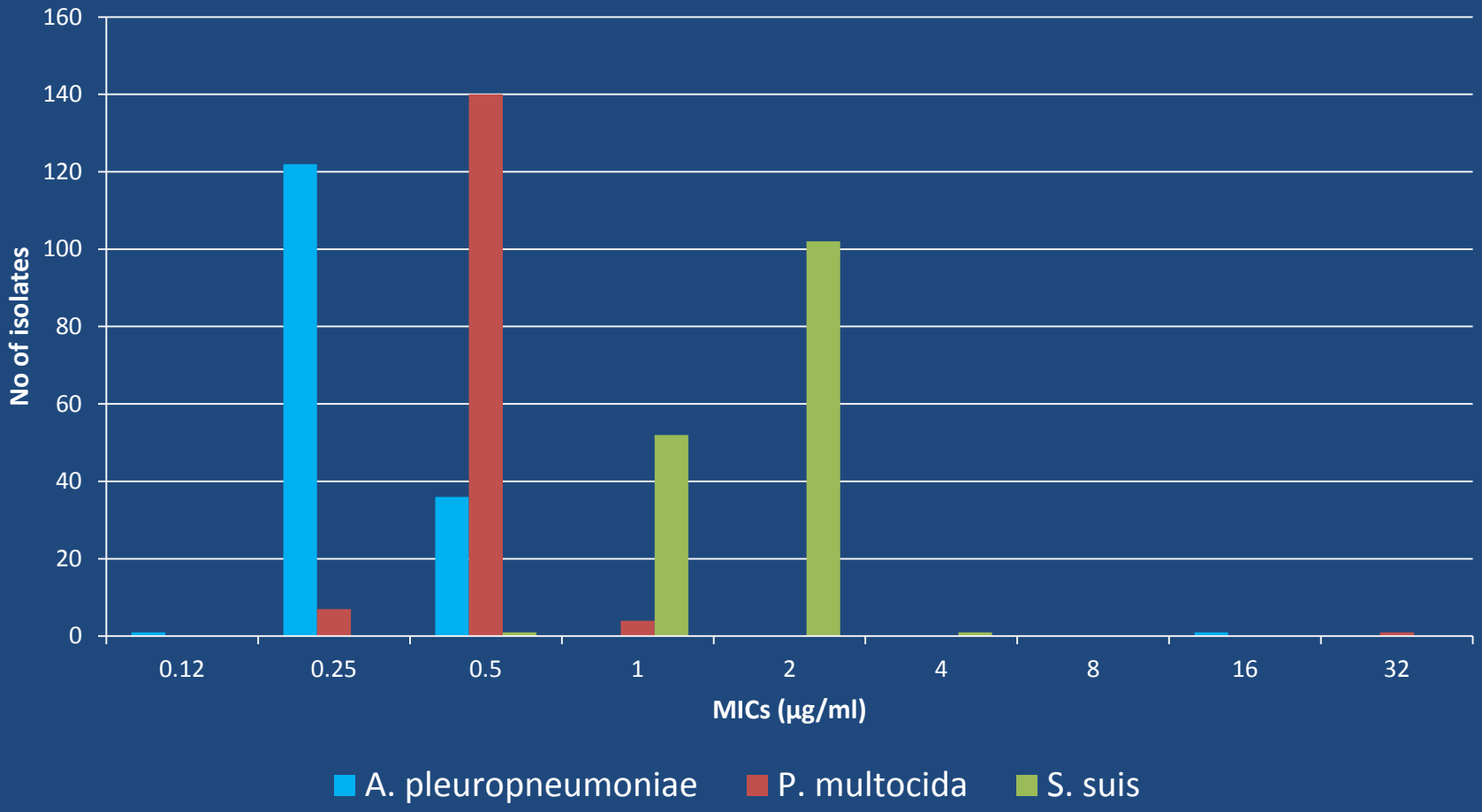
Susceptibility of EU isolates to florfenicol

(VetPath III, 2013)

Bacteria / antibiotic	No of isolates	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)	Resistance (%)
A. pleuropneumoniae	160				
Florfenicol		0.25	**0.5**	0.12-16	0.6
Tetracycline		0.5	16	0.25-128	**23.1**
Tilmicosin		16	16	2.0-32	0.6
P. multocida	152				
Florfenicol		0.5	**0.5**	0.25-32	0.7
Tetracycline		0.5	2.0	0.12-128	**20.4**
Tilmicosin		8.0	16	2.0-32	3.0
S. suis	156				
Florfenicol		2.0	**2.0**	0.5-4.0	0
Tetracycline		32	64	0.25-128	**87.8**
Tilmicosin		>64	>64	0.5-128	**66.7**

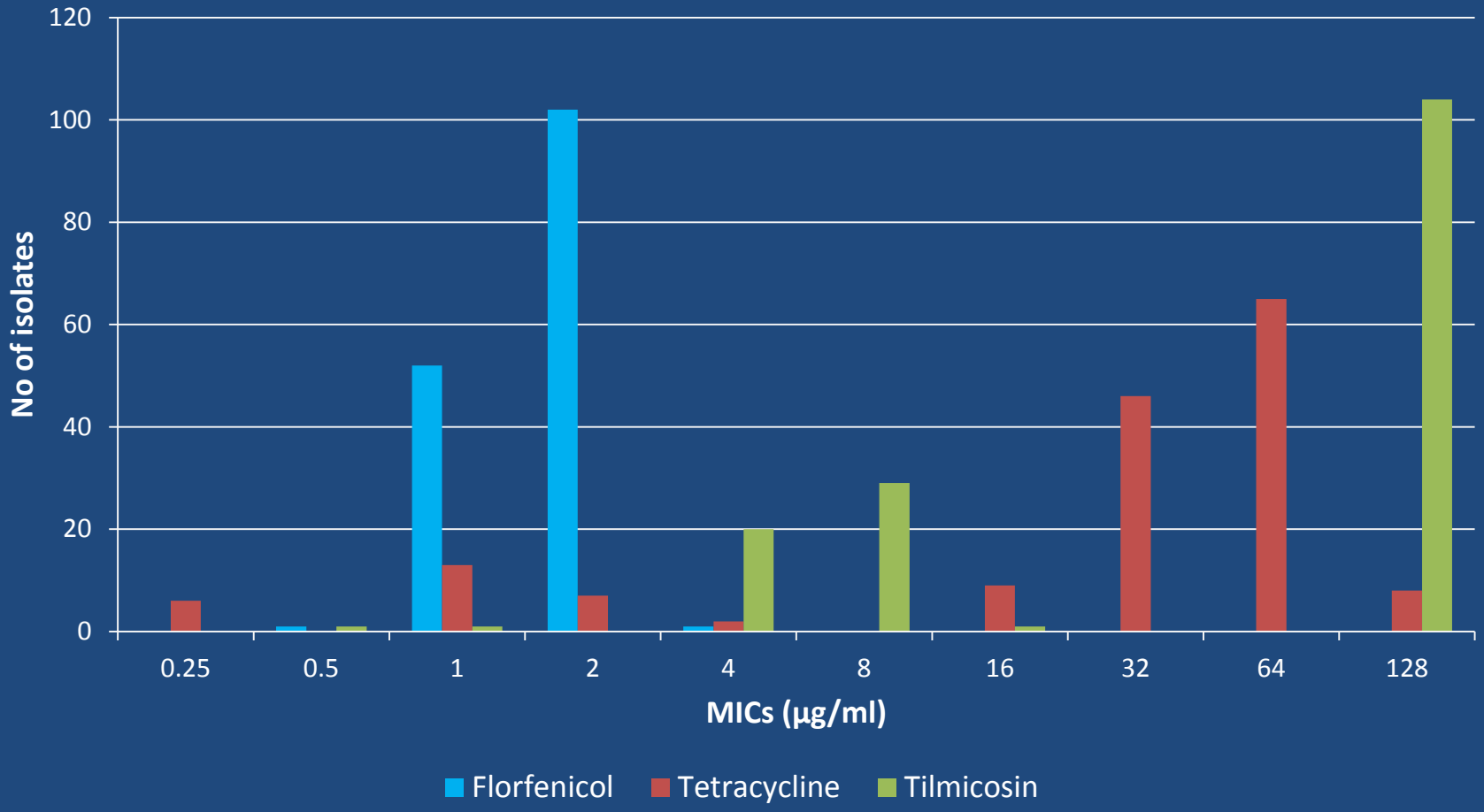
Susceptibility patterns of EU isolates

(VetPath III, 2013)



Susceptibility patterns of 156 EU *S. suis*

(VetPath III)



Susceptible pattern of florfenicol; resistance patterns of tetracyclines and tilmicosin

Florfenicol spectrum of activity – respiratory / systemic

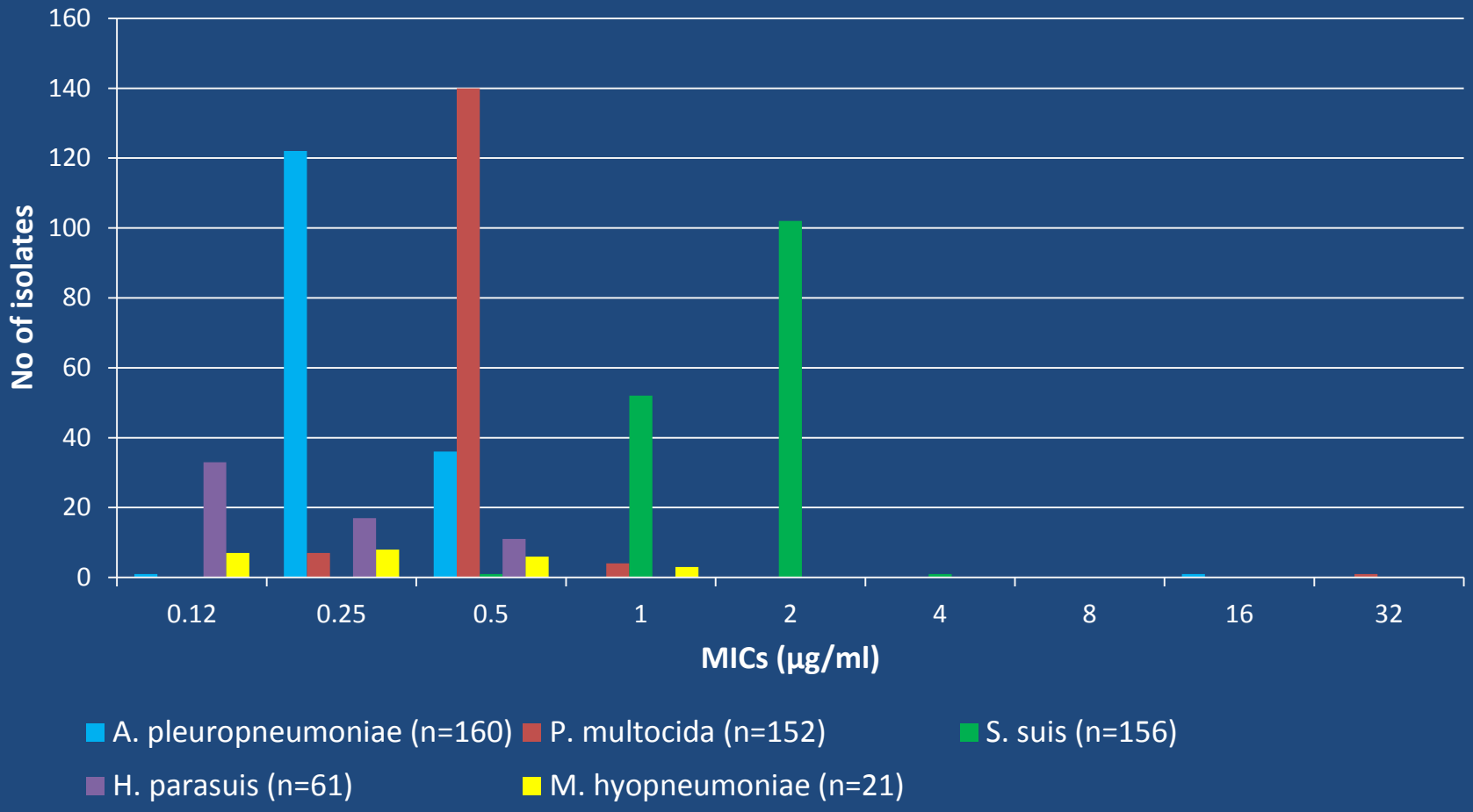
Species / ref.	No of isolates	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Haemophilus parasuis				
VetPath III, 2013 (EU)	61	≤0.12	**0.5**	≤0.12-0.5
Actinobacillus suis				
Jackson et al, 2000	2	-	-	**0.5**
Bordetella bronchiseptica				
VetPath III, 2013 (EU)	126	2.0	4.0	1.0-32
Mycoplasma hyopneumoniae				
Maes et al, 2007	21	0.25	**0.5**	≤0.12-1.0
Mycoplasma hyorhinis				
Thongkamkoon et al, 2010	20	3.12	3.12	1.56-3.12

Florfenicol spectrum of activity – enteric

Species / ref.	No of isolates	MIC 50 (µg/ml)	MIC 90 (µg/ml)	Range (µg/ml)
Salmonella Typhimurium				
DANMAP 2006, 2007	509	4.0	8.0	2.0-128
Escherichia coli				
DANMAP 2006, 2007	148	8.0	8.0	2.0-128
***Lawsonia intracellularis* (I/C MIC - Enterisol)**				
Grosse Liesner & Keller, 2014	1	-	-	0.5

Susceptibility patterns of EU isolates

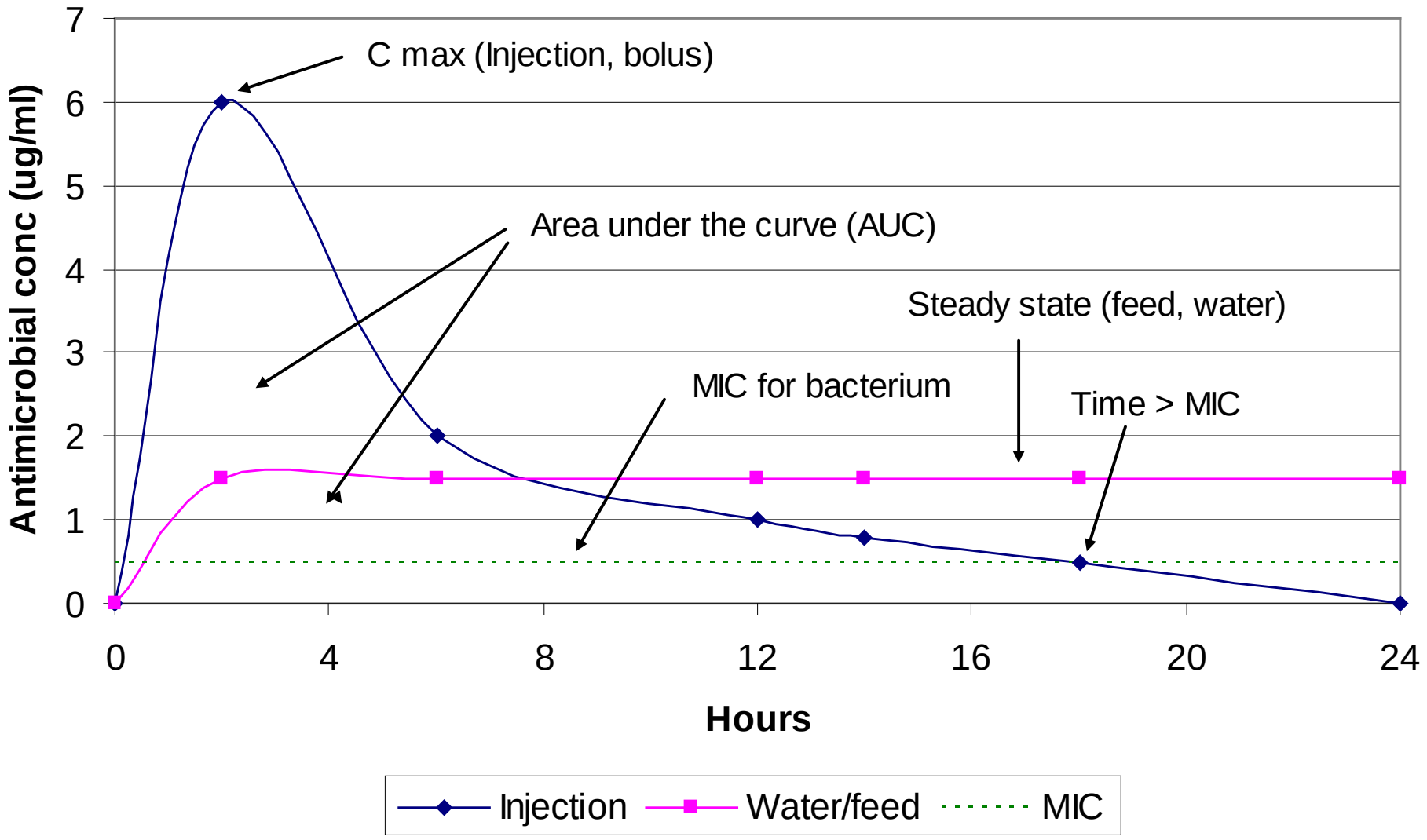
(VetPath III, 2013; Maes et al, 2007)



Pharmacokinetics

- PK of drug - concentrations in plasma/serum main parameter that is used
  - But ...what if the bug is not in the blood/plasma – e.g. extracellular fluids, bronchial fluids, lung, joint fluids – all plasma linked though
  - Plasma protein binding – high binding reduces effect – florfenicol is considered a low binder (USP, 2003)
  - Intracellular penetration – epithelial cells , leucocytes (Actinobacillus?) – lipid solubility important
  - Intestinal contents concentration (jejunum, ileum, colon) - where is the bug? – effect of faecal binding?
  - Influence of absorption, feed interference
  - Excretion of microbiologically active metabolites or parent compound – via urine or out via the bile. Florfenicol excreted 63% urine and the rest via the liver (Nuflor Technical Monograph)

Basic pharmacokinetics in plasma (Burch, 2013)

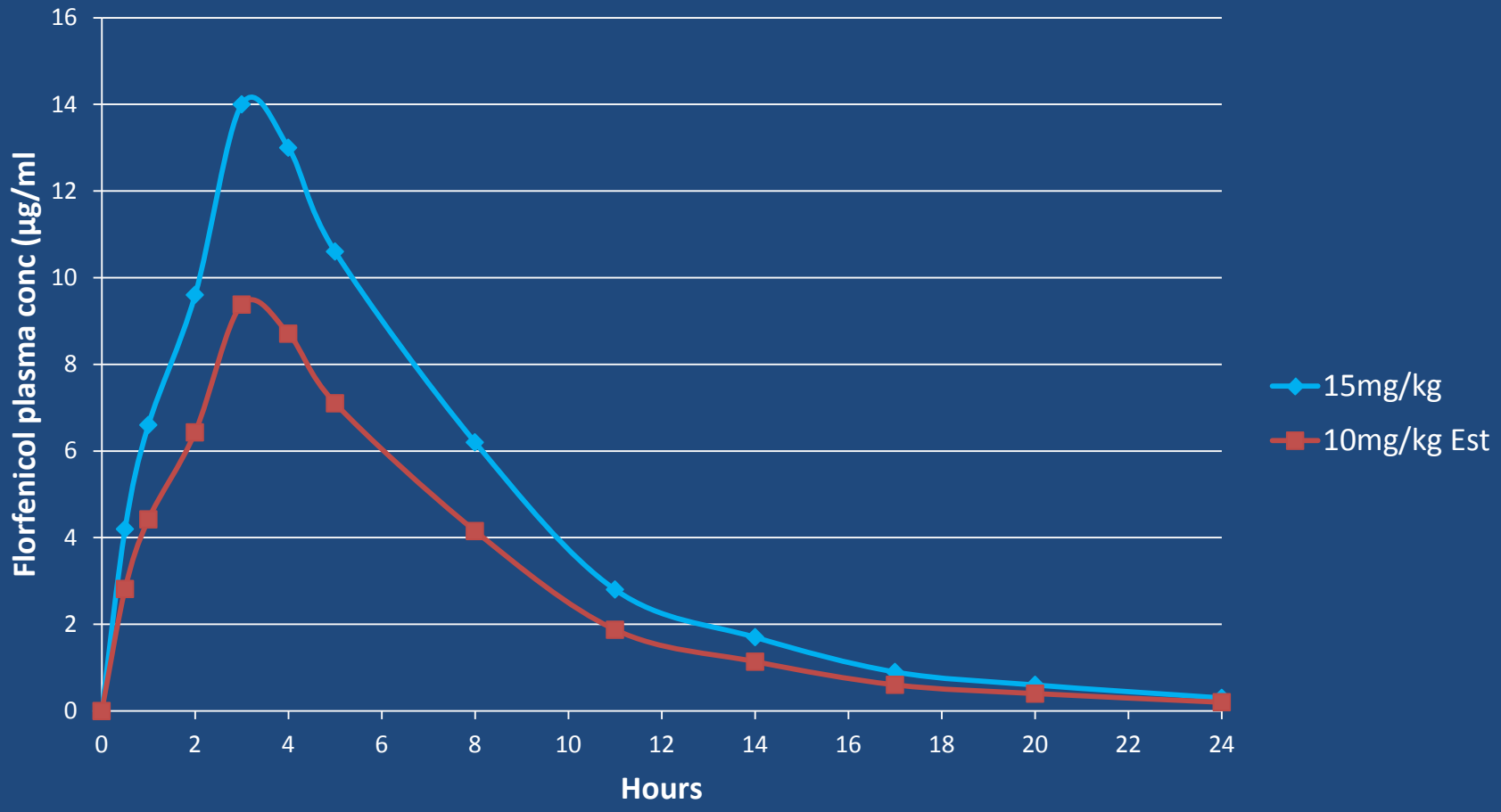


Basic pharmacokinetics

- **Cmax** – maximum concentration – mainly after injection
  - For bactericidal antimicrobials (aminoglycosides, fluoroquinolones)
  - Look for Cmax/MIC ratio of 10-12: 1 for clinical kill of bacterium
  - MIC & MBC similar
- **AUC $_{24h}$** – area under the curve (time & concentration factors)
  - For bactericidal antimicrobials (penicillins, trimethoprim/sulphas)
  - AUC/MIC ratio of 100-120 over 24 hours for clinical kill of bacterium
  - Equivalent to 4-5 times MIC (MIC & MBC similar)
  - Inhibitory effect above MIC (AUC/MIC ≥24h is inhibitory)
- **Css –** concentration steady state – usually AUC_{24h}/24h (following feed and water medication), useful approximation can relate to MICs
- **Time >MIC** – mainly for penicillins, cephalosporins and florfenicol
  - Useful for bacteriostatic antibiotics (tiamulin, tetracyclines, macrolides) aim for >18h + post antibiotic effect (**PAE**) 6h = 24h
  - Florfenicol' s **PAE** approx 3-7h at 10 x MIC; post antibiotic sub-MIC effect (**PASME**) 12->24h at 0.6 x MIC (Wilhelm & Thomas, 2012)

Pharmacokinetics florfenicol – oral gavage

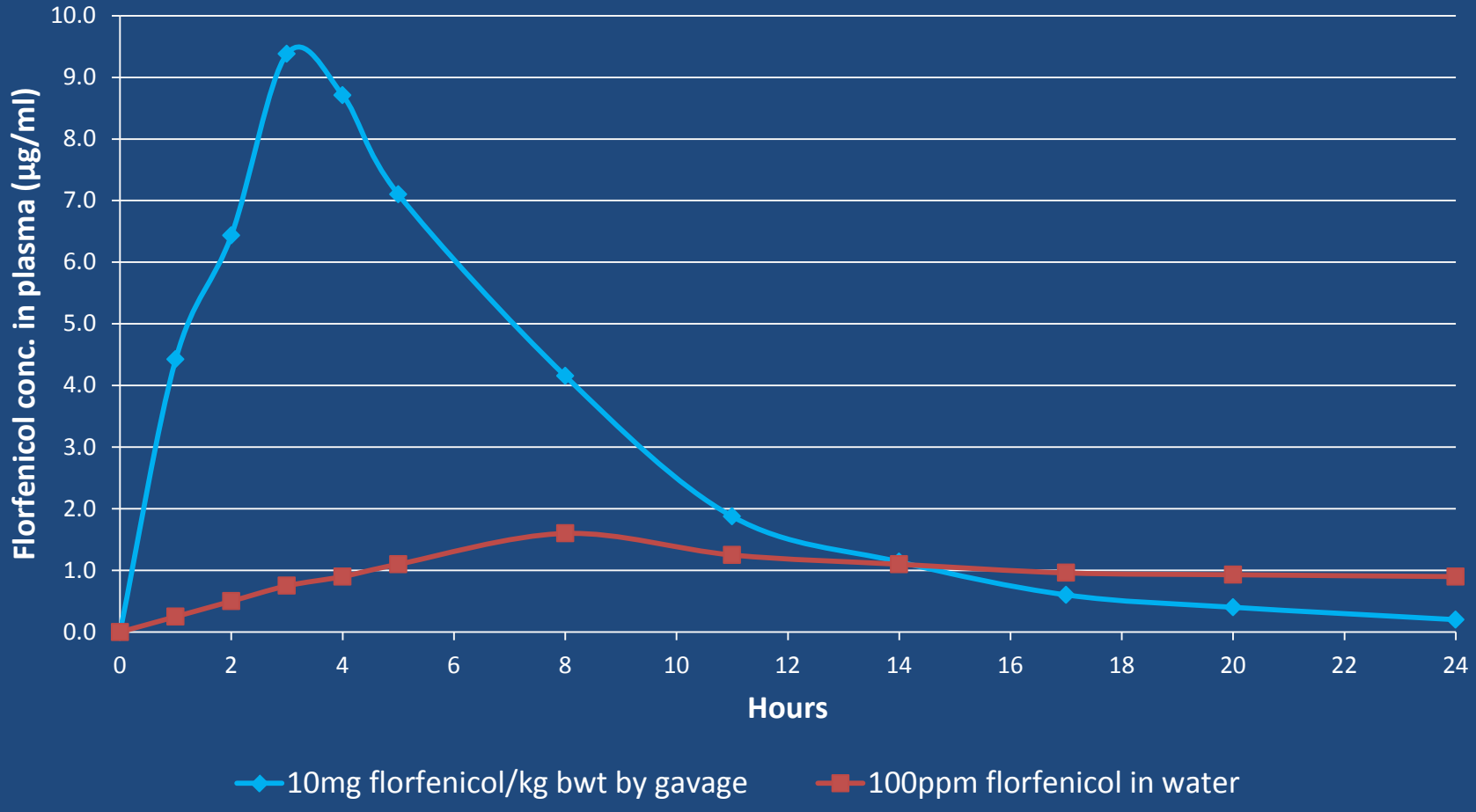
(Voorspoels et al, 1999)



Florfenicol is well absorbed, good bioavailability (usually 97%) better than by injection

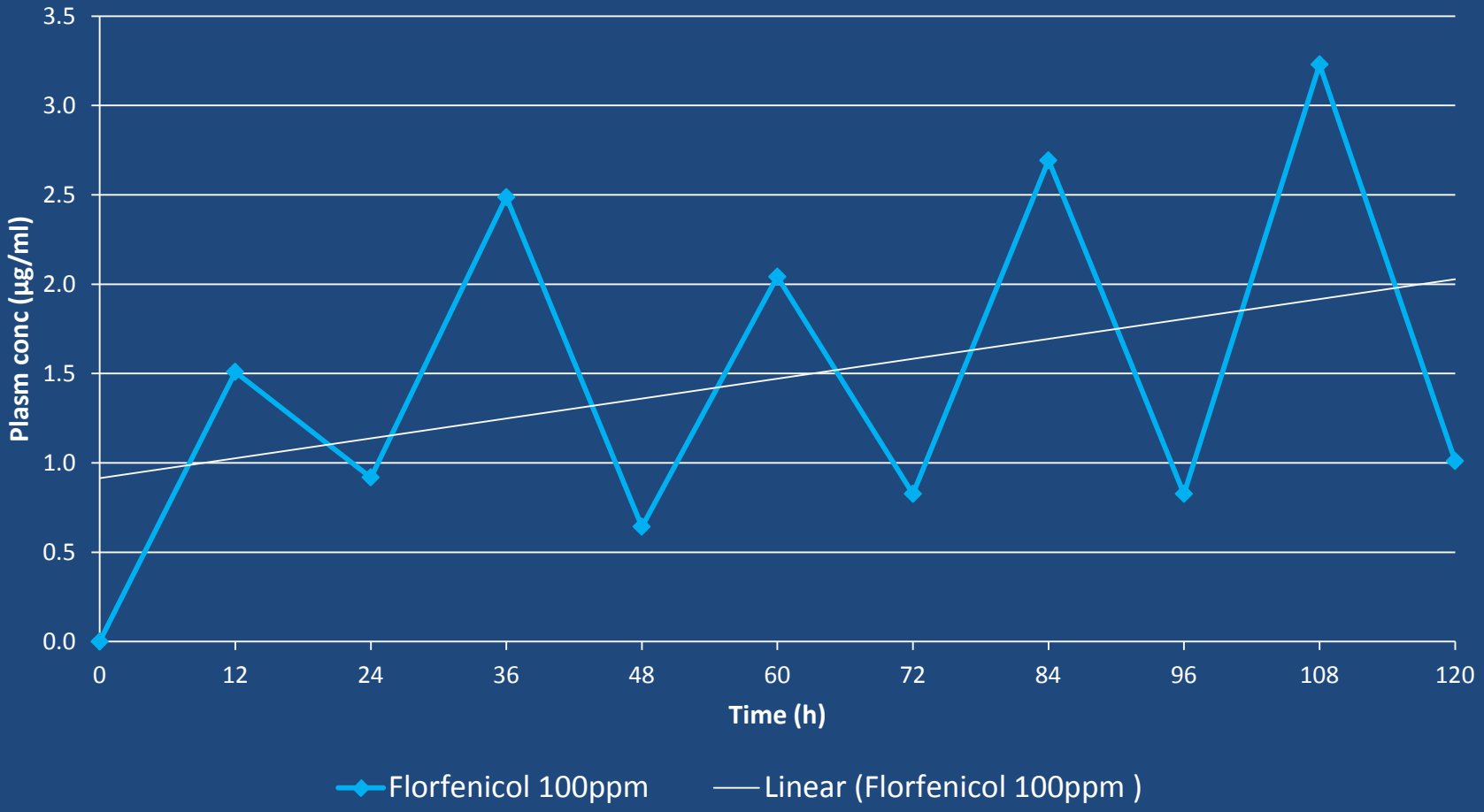
At 10mg florfenicol/kg bwt (estimate) - AUC 24h = 67.3µg.h/ml; Css 2.81µg/ml

Comparison florfenicol by gavage and in drinking water (Gutierrez et al, 2011)



AUC in water is 28.31µg/ml; Css = 1.18µg/ml. There was a marked drop in water intake reported but water only given for 15 h/day in study.

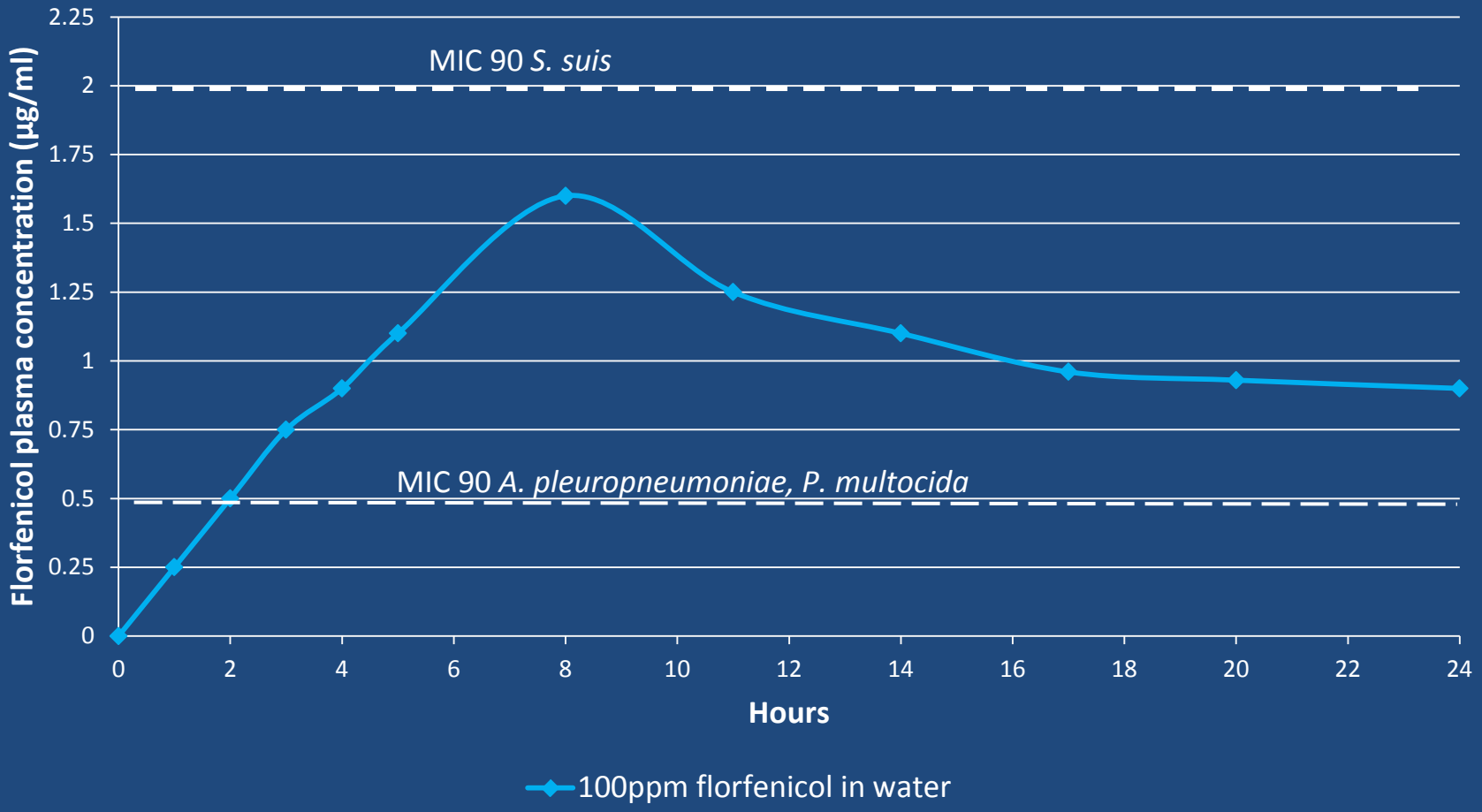
Florfenicol PK in drinking water at 100ppm for 5 days (Perozo et al, 2014)



Mean dose 9.18mg/kg bodyweight – accumulation effect in plasma

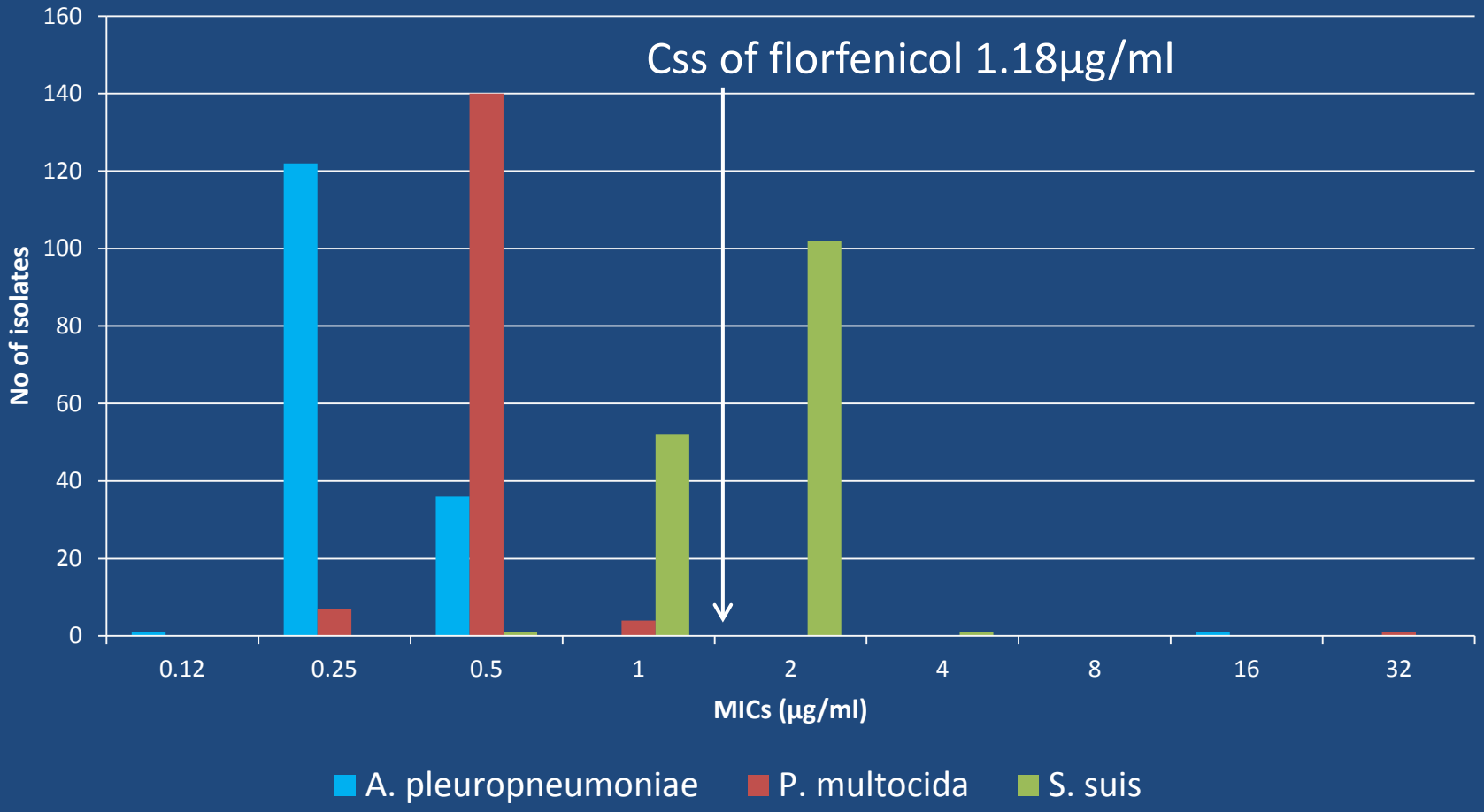
AUC mean 5 days = 36.73µg.h/ml; Rolling mean = 1.53µg/ml

Comparison florfenicol plasma concentration and MIC 90's for target bacteria (Gutierrez et al, 2011)



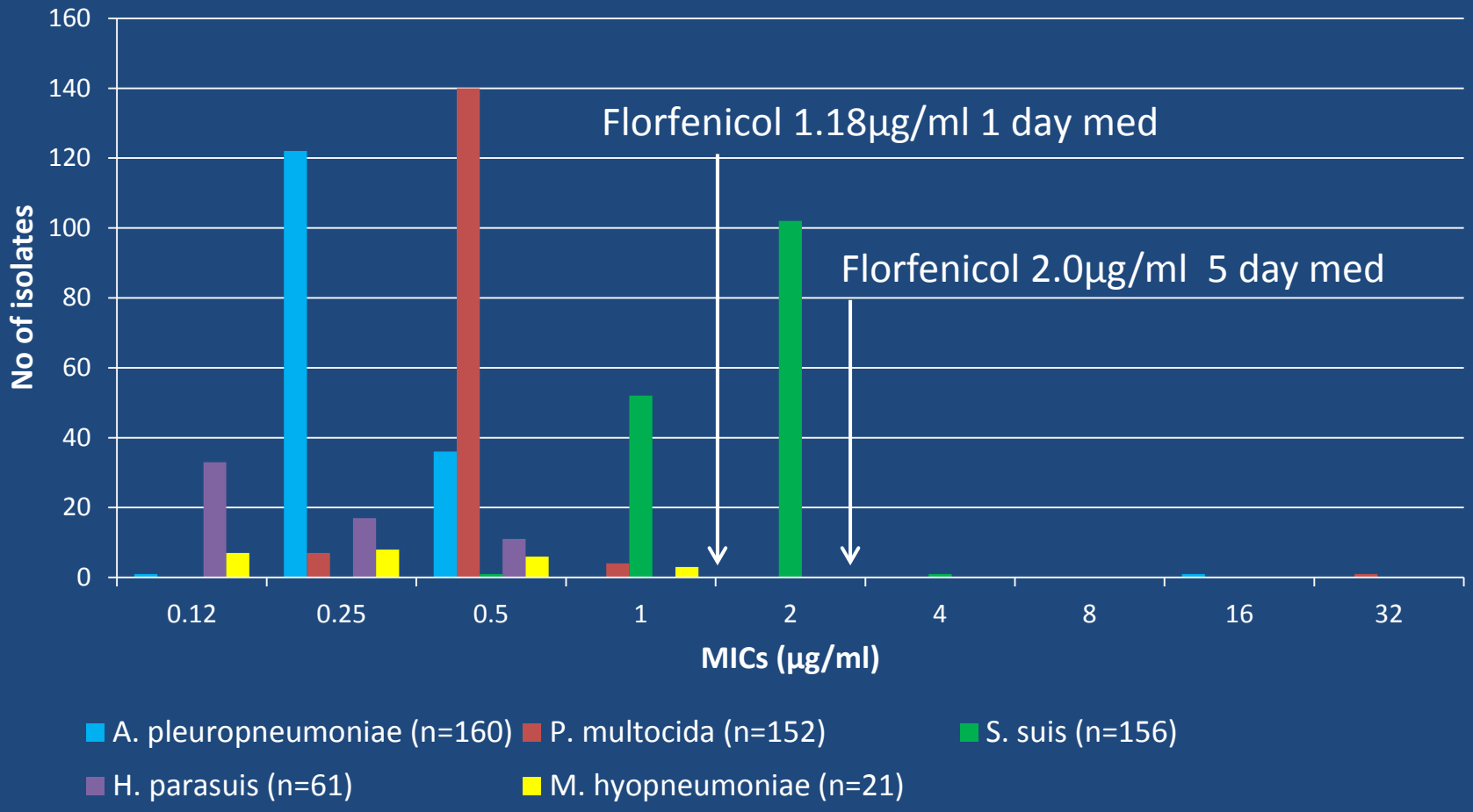
Looks good for *A. pleuropneumoniae* and *P. multocida* at 0.5µg/ml; Perozo et al (2014) demonstrated an accumulation effect over 5 days up to 2.0µg/ml for *S. suis*

PK/PD comparison of florfenicol with EU isolates (VetPath, 2013; Gutierrez et al, 2011)



Susceptibility patterns of EU isolates

(VetPath III, 2013; Maes et al, 2007)



Other PK aspects (Nuflor Technical Monograph)

- Possibly, higher florfenicol concentrations are achieved in the gut contents, which would be sufficient to inhibit *S. choleraesuis* with an MIC 90 of 4.0µg/ml as >35% excreted via the bile. No data given.
- Concentrations in lung are similar/slightly higher (112%) to plasma
- Concentrations in joints tend to be lower than plasma – approximately 88% and brain 32% - blood brain barrier.
- Florfenicol is primarily excreted in the urine (63%) and is also metabolised in the liver and excreted via the bile into the gut mainly as florfenicol and florfenicol amine (inactive metabolite) (24%).
- Liu et al, 2002 showed numerical increase in AUC (approx 10%) in APP infected pigs – metabolic effect?

Adverse effects

- Reduced water intake? Perozo et al, 2014 – approx 10%
- Do not use in swine intended for breeding. The effects of florfenicol on swine reproductive performance, pregnancy and lactation have not been determined.
- Perianal inflammation may occur transiently following treatment with florfenicol given orally. Glattleider et al (2000) described as many as 30% of pigs were affected following injection. Rectal eversion in 46% and 1% reversible rectal prolapse was also reported. A lower incidence was reported with oxytetracycline injection, 3%, 10% and 2%, respectively. The precise cause is uncertain but it has been described for a number of antibiotics including tylosin, tiamulin, valnemulin and carbadox. It is thought it may be associated with endotoxin release when the antimicrobial drug kills the bacteria, or product irritation?

Field Trial Results (Jackson et al, 2000)

- Multi-site trial – Minnesota, Nebraska, Ohio, S. Dakota
- Florfenicol administered in the drinking water at 10mg/kg bodyweight for 5 days
- Pigs enrolled when Temperature 40.3°C
- 223 pigs treated florfenicol; 226 pigs remained untreated
- Monitored daily days 0-7
  - Temperature
  - Depression (score 0-normal to 3-severe)
  - Dyspnea (score 0-normal to 3-severe)
  - Coughing (score 0-absent to 3-severe)
  - Mortality
  - Other observations
- Nasal swabs taken day 0 from 33% of pigs
- Lungs samples tested from dead pigs

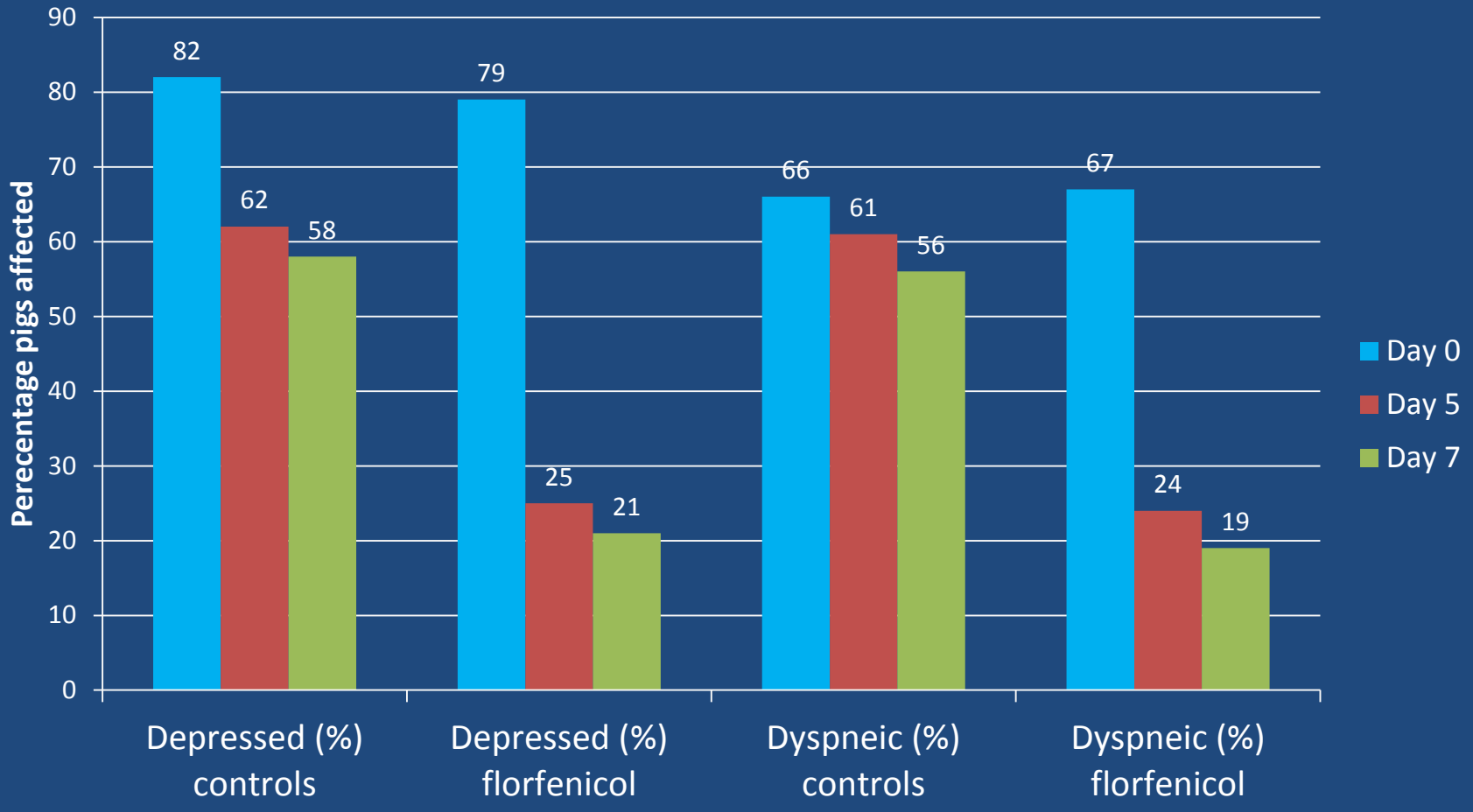
Field Trial Results (Jackson et al, 2000)

Treatment group	Depressed pigs (%)	Dyspneic pigs (%)	Coughing pigs (%)
Untreated controls			
Day 0	82	66	16
Day 5	62	61	25
Day 7	58	56	20
Florfenicol treated			
Day 0	79	67	13
Day 5	25*	24*	9*
Day 7	21*	19*	6*

* Significantly different

Mortality 9.7% for untreated control, 7.4% for the florfenicol treated pigs
Perianal inflammation 3.1% for untreated control; 36.4% for the florfenicol treated pigs
Swab isolates – *P. multocida* (39), *S. suis* (82 – 1, 2, 5), *A. pleuropneumoniae* (53), *A. suis* (2)

Field Trial Results (Jackson et al, 2000)



Artificial infection study – *H. parasuis*
(Iglesias et al, 2002)

- Artificial challenge study: -
  - Piglets were weaned and acclimatised
  - Challenge Day 0, 7 pigs/treatment group – untreated infected controls, florfenicol treated drinking water 100ppm for 5 days
  - Piglets anaesthetised and *H. parasuis* given endotracheally
  - Treatment started when 2 piglets showed clinical signs of infection and treated for 5 days
  - Necropsy Day 12-14 post infection
  - Monitored during the trial for clinical signs related to infection

Artificial infection study – *H. parasuis*
(Iglesias et al, 2002)

Parameter	Untreated control	Florfenicol 100ppm	Difference (%)
Mortality (%)	28.5 (2/7 pigs)	0*	100
Meningitis index	1.57	0.28*	82
Polyserositis index	1.57	0.57*	64
Pericarditis index	2.85	1.28*	56
Polyarthritis index	1.57	0.85*	46
Lung lesion (%)	7.57	5.00	34
Clinical index	7.29	6.46	11

*Significant difference

Major reduction in mortality (day 4 & 5)
Significant reduction in meningitis, polyserositis, pericarditis and polyarthritis

Artificial infection study – *A. pleuropnemoniae* ST1 (Dolso et al, 2014)

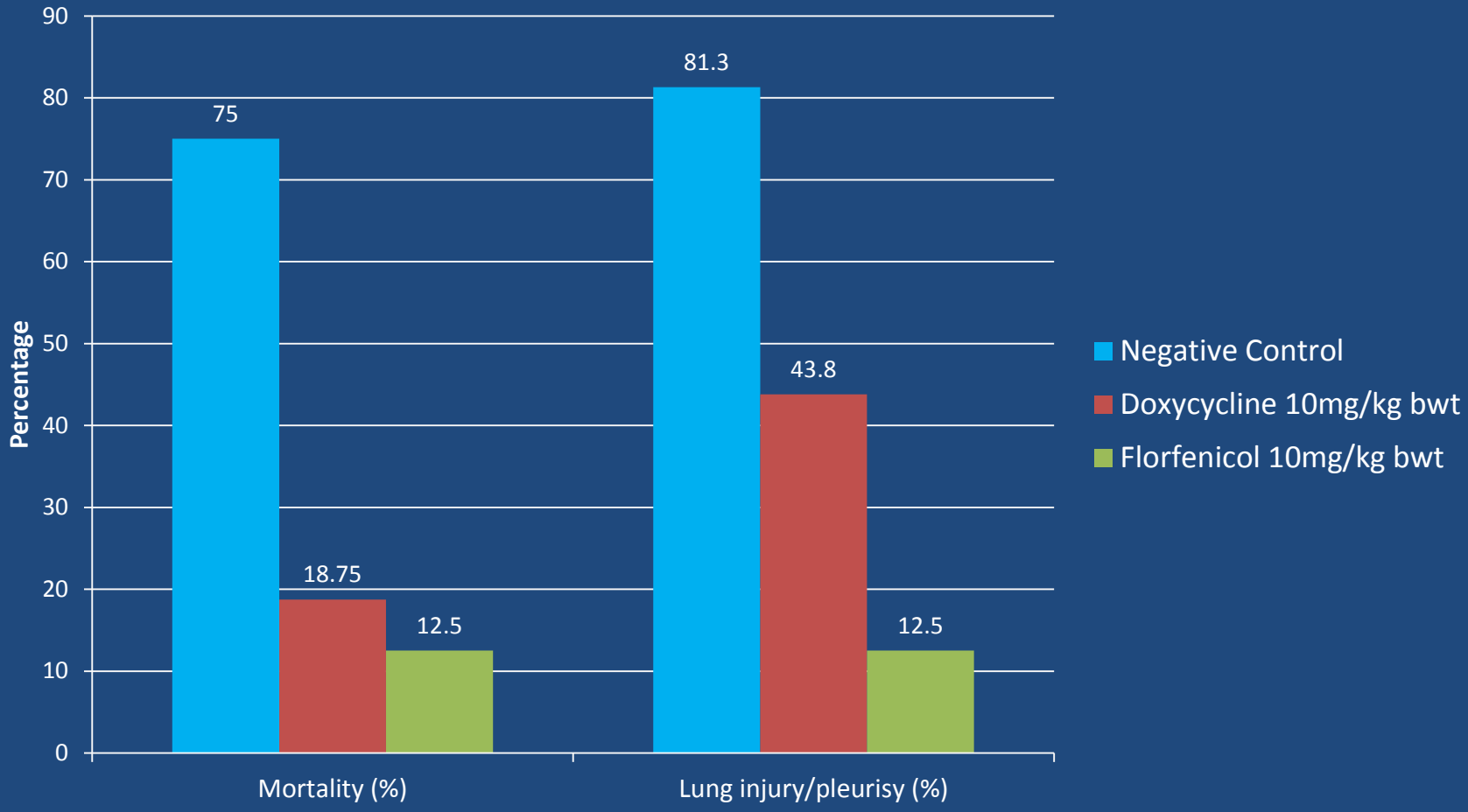
- Artificial challenge study (prevention App)
  - 48 pigs aged 45days (15kg) used in trial
  - Treatments (via drinking water)
    - Untreated control
    - Doxycycline 10mg/kg bwt
    - Florfenicol 10mg/kg bwt
    - Given 1 day before infection for 5 days.
  - Observed for 6 days post infection – then necropsied

Artificial infection study – *A. pleuropnemoniae* ST1 (Dolso et al, 2014)

	Untreated controls	Doxycycline 10mg/kg bwt 5 days	Florfenicol 10mg/kg bwt 5 days
Mortality (%)	75	18.75*	12.5*
Anorexia(%)	36.6	4.0*	0.0*
Cough (%)	19.5	5.0*	1.0*
Lung injury/pleurisy (%)	81.3	43.8*	12.5*
Temperature 24h PI (°C)	40.2	39.5*	39.3*

Both treated groups significantly improved survival and reduced disease effects of App challenge
This was a severe challenge model with 75% mortality
Florfenicol showed improved responses over Doxycycline
Prevention study but severe challenge

Artificial infection study – *A. pleuropneumoniae* ST1 (Dolso et al, 2014)



Conclusions

- Florfenicol is well absorbed from the gut when given orally and achieves significant plasma concentrations and has low plasma-protein binding.
- It is very active against *A. pleuropneumoniae* and *P. multocida* but less active against *S. suis* and *S. choleraesuis*.
- It is primarily classed as a bacteriostatic drug but can be bactericidal at twice the MIC. (Etore et al, 2004)
- There appears to be very little resistance development (Vetpath III, 2013).
- Florfenicol kills in a time-dependent way
- Sufficient plasma concentrations are achieved when given in drinking water and florfenicol is highly effective for the treatment of *A. pleuropneumoniae* and *P. multocida* and against *S. suis*, in field reports, in spite of higher MICs.