FLORVIO™
Pharmacokinetic and pharmacodynamic aspects of soluble florfenicol in swine

David G S Burch

Octagon Services Ltd
www.octagon-services.co.uk
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*
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)

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>No of isolates</th>
<th>MIC 50 (µg/ml)</th>
<th>MIC 90 (µg/ml)</th>
<th>Range (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. pleuropneumoniae</td>
<td>100</td>
<td>0.25</td>
<td>0.5</td>
<td>0.25-1.0</td>
</tr>
<tr>
<td>P. multocida</td>
<td>107</td>
<td>0.5</td>
<td>0.5</td>
<td>0.25-0.5</td>
</tr>
<tr>
<td>S. suis</td>
<td>62</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0-2.0</td>
</tr>
<tr>
<td>S. choleraesuis</td>
<td>36</td>
<td>4.0</td>
<td>4.0</td>
<td>2.0-4.0</td>
</tr>
</tbody>
</table>

Highly susceptible *A. pleuropneumoniae* and *P. multocida*
Less susceptible *S. suis* and *S. choleraesuis*
## Susceptibility of US isolates to florfenicol

(Salmon et al, 2003)

<table>
<thead>
<tr>
<th>Bacteria / antibiotic</th>
<th>No of isolates</th>
<th>MIC 50 (µg/ml)</th>
<th>MIC 90 (µg/ml)</th>
<th>Range (µg/ml)</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. pleuropneumoniae</strong></td>
<td>89</td>
<td>0.25</td>
<td>0.5</td>
<td>&lt;0.06-0.5</td>
<td>0</td>
</tr>
<tr>
<td>Florfenicol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>16</td>
<td>2.0</td>
<td>32</td>
<td>&lt;0.12-64</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.5</td>
<td>32</td>
<td>32</td>
<td>&lt;0.12-64</td>
<td>&gt;10</td>
</tr>
<tr>
<td><strong>P. multocida</strong></td>
<td>186</td>
<td>0.25</td>
<td>0.5</td>
<td>&lt;0.06-4.0</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Florfenicol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>2.0</td>
<td>32</td>
<td>32</td>
<td>0.25-64</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Penicillin</td>
<td>&lt;0.12</td>
<td>&lt;0.12</td>
<td>&lt;0.12</td>
<td>&lt;0.12-64</td>
<td>&lt;10</td>
</tr>
<tr>
<td><strong>S. suis</strong></td>
<td>167</td>
<td>1.0</td>
<td>2.0</td>
<td>0.12-4.0</td>
<td>&lt;10</td>
</tr>
<tr>
<td>Florfenicol</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>0.25-64</td>
<td>&gt;50</td>
</tr>
<tr>
<td>Penicillin</td>
<td>&lt;0.12</td>
<td>0.25</td>
<td>0.25</td>
<td>&lt;0.12-32</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>
### Susceptibility of EU isolates to florfenicol
(VetPath III, 2013)

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

<table>
<thead>
<tr>
<th>Species / ref.</th>
<th>No of isolates</th>
<th>MIC 50 (µg/ml)</th>
<th>MIC 90 (µg/ml)</th>
<th>Range (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Haemophilus parasuis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VetPath III, 2013 (EU)</td>
<td>61</td>
<td>≤0.12</td>
<td>0.5</td>
<td>≤0.12-0.5</td>
</tr>
<tr>
<td><strong>Actinobacillus suis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jackson et al, 2000</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Bordetella bronchiseptica</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VetPath III, 2013 (EU)</td>
<td>126</td>
<td>2.0</td>
<td>4.0</td>
<td>1.0-32</td>
</tr>
<tr>
<td><strong>Mycoplasma hyopneumoniae</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maes et al, 2007</td>
<td>21</td>
<td>0.25</td>
<td>0.5</td>
<td>≤0.12-1.0</td>
</tr>
<tr>
<td><strong>Mycoplasma hyorhinis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Florfenicol spectrum of activity – enteric

<table>
<thead>
<tr>
<th>Species / ref.</th>
<th>No of isolates</th>
<th>MIC 50 (µg/ml)</th>
<th>MIC 90 (µg/ml)</th>
<th>Range (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella Typhimurium</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DANMAP 2006, 2007</td>
<td>509</td>
<td>4.0</td>
<td>8.0</td>
<td>2.0-128</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DANMAP 2006, 2007</td>
<td>148</td>
<td>8.0</td>
<td>8.0</td>
<td>2.0-128</td>
</tr>
<tr>
<td><strong>Lawsonia intracellularis (I/C MIC - Enterisol)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grosse Liesner &amp; Keller, 2014</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
</tbody>
</table>
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** (jejenum, 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)

Graph showing:
- Cmax (Injection, bolus)
- Area under the curve (AUC)
- Steady state (feed, water)
- MIC for bacterium
- Time > MIC

Y-axis: Antimicrobial conc (µg/ml)
X-axis: Hours

Lines:
- Injection: Blue
- Water/feed: Pink
- MIC: Green dotted
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 $28.31\mu g/ml$; $Css = 1.18\mu g/ml$. 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)

Css of florfenicol 1.18µg/ml

[Bar chart showing the number of isolates (MICs) for different species: A. pleuropneumoniae, P. multocida, S. suis]
Susceptibility patterns of EU isolates
(VetPath III, 2013; Maes et al, 2007)

A. pleuropneumoniae (n=160)
P. multocida (n=152)
S. suis (n=156)
H. parasuis (n=61)
M. hyopneumoniae (n=21)

Florfenicol 1.18µg/ml 1 day med
Florfenicol 2.0µg/ml 5 day med
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)

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Depressed pigs (%)</th>
<th>Dyspneic pigs (%)</th>
<th>Coughing pigs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated controls</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>82</td>
<td>66</td>
<td>16</td>
</tr>
<tr>
<td>Day 5</td>
<td>62</td>
<td>61</td>
<td>25</td>
</tr>
<tr>
<td>Day 7</td>
<td>58</td>
<td>56</td>
<td>20</td>
</tr>
<tr>
<td>Florfenicol treated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>79</td>
<td>67</td>
<td>13</td>
</tr>
<tr>
<td>Day 5</td>
<td>25*</td>
<td>24*</td>
<td>9*</td>
</tr>
<tr>
<td>Day 7</td>
<td>21*</td>
<td>19*</td>
<td>6*</td>
</tr>
</tbody>
</table>

* 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)

- Depressed (%)
  - Controls: Day 0 (82), Day 5 (62), Day 7 (58)
  - Florfenicol: Day 0 (79), Day 5 (25), Day 7 (21)

- Dyspneic (%)
  - Controls: Day 0 (66), Day 5 (61), Day 7 (56)
  - Florfenicol: Day 0 (67), Day 5 (24), Day 7 (19)

Percentage pigs affected.
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)

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

*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 45 days (15 kg) used in trial
  – Treatments (via drinking water)
    • Untreated control
    • Doxycycline 10 mg/kg bwt
    • Florfenicol 10 mg/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)

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

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)

- **Mortality (%)**
  - Negative Control: 75%
  - Doxycycline 10mg/kg bwt: 18.75%
  - Florfenicol 10mg/kg bwt: 12.5%

- **Lung injury/pleurisy (%)**
  - Negative Control: 81.3%
  - Doxycycline 10mg/kg bwt: 43.8%
  - Florfenicol 10mg/kg bwt: 12.5%
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.