Veterinary medicine needs new and innovative green antibiotics

Pierre-Louis Toutain
Ecole Nationale Vétérinaire de Toulouse & INRA,
Toulouse, France
23rd May 2016

Egmond aan Zee, Netherlands
Do we need “new” antibiotics in veterinary medicine?

• From an animal health perspective: No
  – Currently, no major animal health issues

• From a public health perspective: Yes
  – Currently, a major public health issue for vets
  – to manage the link between the human and the veterinary resistome by decreasing our contribution to the overall pool of genes of resistance
One world, one health, one resistome

- Treatment & prophylaxis
- Veterinary medicine
- Animal feed additives
- Agriculture
- Plant protection
- Environment
- Industry
The prudent use of antibiotics

Most recommendations are copy and paste from human medicine. May be counterproductive.
The shortcomings of the paradigm of a prudent use of antibiotics in veterinary medicine

• Selected examples
  – Local route of administration at drying off

A survey of antimicrobial usage on dairy farms and waste milk feeding practices in England and Wales

L. A. Brunton, D. Duncan, N. G. Coldham, L. C. Snow, J. R. Jones

• 93% of respondents used antibiotic intramammary tubes to treat mastitis
• 83% per cent of respondents (413) fed waste milk to calves
WHO and critical AMDs

- It is recommended by the WHO, to reserve to human medicine the most critical antibiotics keeping for veterinary medicine the most outdated substances.
Tetracyclines

• Tetracyclines: half the amount of veterinary antibiotic used worldwide
• Extensively excreted in environment
• Soils may be hot spots for gene transfer,
Q1: But of what resistance are we speaking?
The three types of AMR faced by vet medicine

- **Target pathogen**
  - Efficacy in animal
  - Farm
  - Animal health issue

- **Zoonotic**
  - Efficacy in man
  - Food chain
  - Human health issue

- **Commensal**
  - Global ecological problem
  - Environment
  - Public health issue
Q2: what is the pathways for transmission between animal and human resistomes
“Classical” natural history of bacterial infections (at hospital)

Specific pathogen

Dissemination of pathogens

Dissemination of pathogens

Dissemination of pathogens

Andremont et al, The lancet infection 2011 11 6-8
« New » natural history of bacterial infections (in the community)

Commensal flora of a future patient (1kg)
Colonization/carriage Gene of resistance ESBL, CTX-M…

Disease
Specific pathogen

Dissemination of gene of resistance
Dissemination of genes of resistance

Adapted from Andremont et al, The lancet infection 2011 11 6-8
Most of the prudent use recommendations do not address this question.
Intestinal microbiota is the turnstile between the two medicine

The Human Gut Microbiome as a Transporter of Antibiotic Resistance Genes between Continents

Johan Bengtsson-Palme, Martin Angelin, Mikael Huss, Sanela Kjellqvist, Erik Kristiansson, Helena Palmgren, D. G. Joakim Larsson, Anders Johansson

To have a clean commensal microbiota is public health issue
New Eco-Evo drugs and strategies should be considered when developing new AMD

No impact on gut flora
No release of active substances in the environment
Q3: Where are manufactured genes of resistance having a public health impact
Bacterial load & duration of exposition to antibiotics during treatment

Test tube | Infected Lungs | Digestive tract | Manure Sludge Waste

µg Hours | mg Days | Kg Weeks | Tons Months

Food chain | Environment
Elimination of antibiotics into the environment

• As much as 75% of the antibiotics administered to food producing animals are directly excreted into the environment without any benefit for the animal
Rate of antibiotic degradation in manure, soil, waste...

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>matrix</th>
<th>Dégradation %</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlortétracycline</td>
<td>Cattle manure</td>
<td>24</td>
<td>84</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>Soil+contam manure</td>
<td>0</td>
<td>180</td>
</tr>
<tr>
<td>Beta-lactam</td>
<td>manure</td>
<td>0-50</td>
<td>30</td>
</tr>
<tr>
<td>TMP</td>
<td>Sewage sludge</td>
<td>50</td>
<td>22-41</td>
</tr>
<tr>
<td>Sulfamides</td>
<td>Manure/sludge</td>
<td>0</td>
<td>28</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td>manure</td>
<td>0</td>
<td>30</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>manure</td>
<td>0-30</td>
<td>56-80</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>Sandy loam &amp; manure</td>
<td>77</td>
<td>30</td>
</tr>
<tr>
<td>Tylosine</td>
<td>Pig manure, anaerobic</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Tulathromycin</td>
<td>soil</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>
An ideal AMD in veterinary medicine should not be released in its active form in the environment.
Q5: Why veterinary antibiotic treatments are able to alter the resistome of the animal gut microbiota.
Oral route of administration

Gastro-intestinal tract

Proximal

Distal

1-F%

Microbiota
• Zoonotics (salmonella, campylobacter
• Commensal (enterococcus)

F%

Blood

Biophase

Target pathogen

AMD: oral route

Food chain

Environment
Bioavailability of tetracyclins by oral route

• Chlortetracycline:
  – about 20%
• Doxycycline:
  – About 20%
• Oxytetracycline:
  – Pigs: 4.8%
  – Piglets, weaned, 10 weeks of age: by drench: 9%;
  – in medicated feed for 3 days: 3.7%.
• Tetracycline:
  – Pigs fasted: 23%.

• Most of the administered dose is lost for the animal and is only spill in the environment
Non-oral route of administration

Gastro-intestinal tract

Proximal

- Intestinal efflux
- Bile

Distal

- Microbiota
  - Zoonotics (salmonella, campylobacter)
  - Commensal (enterococcus)

Non-oral route

Blood

Biophase

Target pathogen

Food chain

Environment
Genotypic evaluation of ampicillin resistance: copy of $bla_{TEM}$ genes per gram of feces

A significant effect of route of administration on $bla_{TEM}$ fecal elimination ($p<0.001$).
Principles of solution
What could be the ideal pharmacodynamic pharmacokinetic & profile for a veterinary antibiotic to minimize the public health issues
Pharmacodynamics
A major misconception:
To develop in veterinary medicine antibiotics with the highest possible potency
The 3 PD parameters

Emax

Efficacy

ED_{50}

Potency
Potency of Fluoroquinolones
Hydrophobicity vs MIC for *S. aureus*

\[ y = 26.757e^{-2.297x} \]

\[ R^2 = 0.6764 \]

Takenouchi et al AAC 1996

Hydrophobicity (Clog-P)
Potency of fluoroquinolones
Hydrophobicity vs MIC for E coli

\[ y = 1.154e^{-2.003x} \]

\[ R^2 = 0.3719 \]

Takenouchi et al AAC 1996
Fluoroquinolones: XLog-P3 vs. impact on gut flora

Impact gut microbiome

Hydrophobicity (Xlog-P)

y = 0.6708x + 1.9128

R² = 0.4597

Minimal impact

Major impact

Veterinary FQ
Cephalosporins

XLog-P vs. impact on gut flora

Impact gut microbiome

Xlog-P

y = 0.4972x + 2.1543
R² = 0.3397

Veterinary cephalosporins
Wat is the situation of veterinary drugs in terms of lipophilicity
Relationship between lipophilicity and pharmacokinetic parameters for the 10 most used antimicrobial in cattle.

The terminal half-life is positively correlated to the degree of lipophilicity with a coefficient of determination (R²) between lipophilicity and duration of half-life of 0.37.

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Molecular Weight</th>
<th>XLogP3-AA</th>
<th>Vss (L/kg)</th>
<th>Clearance (ml/kg/min)</th>
<th>Half-life (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftiofur</td>
<td>523</td>
<td>0.20</td>
<td>0.30</td>
<td>0.55</td>
<td>7.00</td>
</tr>
<tr>
<td>Danofloxacin</td>
<td>357</td>
<td>-0.30</td>
<td>2.48</td>
<td>8.30</td>
<td>4.01</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>290</td>
<td>0.90</td>
<td>1.50</td>
<td>28.33</td>
<td>1.20</td>
</tr>
<tr>
<td>Tilmicosin</td>
<td>869</td>
<td>3.60</td>
<td>28.20</td>
<td>11.40</td>
<td>28.00</td>
</tr>
<tr>
<td>Tildipirosin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tulathromycin</td>
<td>806</td>
<td>3.80</td>
<td>11.1</td>
<td>3.01</td>
<td>65</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>290</td>
<td>0.90</td>
<td>1.50</td>
<td>28.33</td>
<td>1.20</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>290</td>
<td>0.90</td>
<td>1.50</td>
<td>28.33</td>
<td>1.20</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>290</td>
<td>0.90</td>
<td>1.50</td>
<td>28.33</td>
<td>1.20</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>290</td>
<td>0.90</td>
<td>1.50</td>
<td>28.33</td>
<td>1.20</td>
</tr>
</tbody>
</table>

- **MW**=371.6
- **ClogP** = -0.18.
Selectivity of antimicrobial drugs

Selectivity

PD

Large vs Narrow spectrum

PK

Selective distribution of the AB to its biophase
Pharmacodynamic selectivity

• Narrow spectrum
  – Gram positive vs. Gram negative
  – Advantages
    • Limit the risk of AMR including on the gut flora
  – Limits
    • Segmentation of the market
    • Require an accurate diagnostic
Selectivity of antimicrobial drugs in veterinary medicine

Selectivity

PD
- Rather Low potency
- Narrow spectrum

PK
- Selective distribution of the AB to its biophase
PK selectivity: oral route

Proximal

1 - F = 0%

F = 100% = lower dose

Biophase

Target pathogen

Blood

Distal

Trapping, inactivation (betalactamase)

100%

Renal elimination

Microbiome
- Zoonotics
- Commensal

Food chain

Environment

AB: oral route

Trapping, inactivation (betalactamase)
Objective 1:
Improve the oral bioavailability for oral antibiotics
PK Variability

Doxycycline
F≈20%

n = 215
Ideal AMD: oral route

• A high oral bioavailability is required:
  – To minimize or suppress a uselessly impact of the gut microbiota
  – To decrease the dose
  – To decrease the intersubject variability
How to get a high Bioavailability

- Appropriate logP: Lipinsky rule
- Optimization of formulation (including in water as micellar formulations)
- No interference with food (binding to cellulosis)
- No taste (no influence on feeding behavior)
- No active transport (as for peptides like AMDs)
- No PGP... substrate
- No first-pass effect (but prodrugs)
Intestinal vs Bacterial membrane

Intestine

Gram-positive

Murein/peptidoglycan

Betalactams: Site of action

Cell Wall

Cytoplasmic membrane

Intracellular site of action

Gram-negative

Lipinski rule
LogP=2

Aquaporin

Outer membrane

MDR system
Efflux pump

Cytoplasmic membrane

Intracellular site of action
Why hydrophilic

• To escape MDR pumps
  – hydrophobic cations are the preferred substrates and highly hydrophobic compounds in general are also well recognized, whereas anions are not good substrates.
• To favor penetration
  – relatively hydrophilic compounds with a mass <600 Da are also favoured for penetration, probably owing to their ability to pass through porins of the outer membrane.
  – Furthermore, it seems that the inclusion of atoms that are not frequently found in natural compounds (such as fluorine and boron) is good for penetration, possibly as MDR pumps have not been exposed to them.
  – Another consideration regards targets: the broad-spectrum β-lactams are fairly hydrophilic enter through porins and do not need to be amphipathic, as their target is in the periplasm
The produg approach

Intestine
- Lipophilic prodrug

Liver

First pass effect

Hydrophilic drug

Gram-negative
- Aquaporin
- MDR system
- Efflux pump

Cell Wall

Outer membrane

Cytoplasmic membrane

Intracellular site of action
The produg approach

• Podrugs as pivampicillin, bacampicillin, ceforuxime axetil has been shown to be favorable from an ecological point of view
How to manage the non-absorbed fraction

- Trapping: activated charcoal, smectite...
- Inactivation
  - Destruction: betalactamases
  - Decrease potency with pH sensitive AMD
    - A relatively low pH is generally found in the caecum (6.0-6.4) and colon (6.1-6.6)
    - Several classes of AMD are pH-sensitive
Desirable pharmacokinetic properties for antibiotic administered by the non-oral route in food producing animals
PK selectivity: systemic route

Trapping, inactivation

Proximal

Biliary & intestinal clearance = 0

Distal

microbiome
• Zoonotics
• commensal

Food chain

Environment

Renal elimination = 100%

Stop

Administration

Target pathogen

Blood
The % of urinary excretion decreased or fecal excretion increased for the drugs with C log P>0

Figure 1. Dependence of urinary excretion of drug-related material following intravenous administration on C log P.

- The more hydrophobic is a drug, the more likely it is to be excreted in the feces.
## Renal clearance of different quinolones

<table>
<thead>
<tr>
<th>Drugs</th>
<th>% of total clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ofloxacin</td>
<td>80-95%</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>65</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>50</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>13</td>
</tr>
<tr>
<td>Grepafloxacin</td>
<td>10</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>5-10</td>
</tr>
</tbody>
</table>

Adapted from Hooper DC, CID 2000;30:243-254
Ideal AMD for a non-oral route of administration

- non-oral route of administration raised the specific question of the **duration of activity** of the antibiotic;
- indeed if the antibiotic can be easily administered on a daily basis, this is not the case of the non-oral route and here veterinary drug companies are prompted to select **long-acting products** in order to obtain a cure with a single dose administration; typically, for a bronchopneumonia, an at least 5-days duration of action is desirable;
A long half-life (HL) is desirable for convenience in vet medicine: two possible options

- A substance property
- A formulation property
How to get a long Half-life: formulation

Formulation (e.g. old AMD)

High clearance
  Slow absorption

Local tolerance; residues;

Betalactams/sulfamides
How to get a long Half-life: substance

Substance (new AMD)

- Low clearance
  - Renal
  - Inactive

- Large volume of distribution
  - STOP
  - Macrolides/FQ

- Metabolic
  - STOP

- Intestinal, Bile
  - STOP
  - active
  - inactive
How to get a long Half-life

- Formulation (e.g. old AMD)
  - High clearance
  - Slow absorption
  - Local tolerance; residues;

- Substance (new AMD)
  - Low clearance
  - Large volume of distribution
  - Intestinal, Bile
  - Metabolic
  - Renal
  - Active
  - Inactive

- Macrolides/FQ
- Betalactams/sulfamides
Pharmacokinetic parameters (Size & route of administration)

- Too large
- Not low
- Small
- Low
- Long Half-life
- Slow release formulation
- Only Oral

Animal (clearance mechanisms)

- Intestinal
- Hepatic (metabolism)
- Biliary
- Renal
- Metabolite Active
- Metabolite Inactive

Environment (fate)

- Degradation (hydrolyse...)
- Sorption
- STOP

Degradation (hydrolyse...)

Non-oral
Degradation or inactivation of AB and gene of resistance in the environment
The ideal antibiotics: PK

1. Oral: High oral bioavailability
   - *no first pass effect but prodrugs; no affinity for efflux pumps, no interference with diet; No influence on feeding behavior*
2. Non oral: slow absorption
   - *LA formulations > LA substances*
3. Small volume of distribution
4. Slow metabolic clearance
   - *giving hydrophilic inactive metabolites*
5. Renal clearance (substance & inactive metabolites)
6. No bile and/or intestinal clearance
7. Rapid degradation in the environment
The ideal antibiotics: PD properties

1. Full efficacy
   • *including against persisters, biofilms*..
2. Rather low potency
   • *especially in acidic condition (no activity in gut)*
3. Microbiological selectivity: rather narrow spectrum
4. No effect on eucaryote cells
   • *safety issue; e.g. action on bacterial wall rather intracellular proteins*
5. Prodrugs converted inside the pathogen by specific bacterial enzymes
6. Non specific intracellular mechanism of action or dual mechanism of action (e.g. FQ)
7. Others properties:
   • *immunostimulation, anti-inflammatory, quorum sensing*
Is there a successful antibiotic development complying with Eco-Evo concept i.e. green antibiotics?
Ecological impact of some new AMD

Ceftobiprole; Ceftaroline Telavancin; Dalbavancine

Anaerobe 18 (2012) 249–253

Clinical microbiology

Effect of new antimicrobial agents on the ecological balance of human microflora

Mamun-Ur Rashid, Andrej Weintraub, Carl Erik Nord*

Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska University Hospital, Karolinska Institutet, 141 86 Stockholm, Sweden
And now a last question

What is the best AMD in terms of public health

1. An old broad spectrum tetracycline, with a 10% bioavailability, impacting the gut flora, with a long half-life in the environment and able to coselect for resistance to multiple late-generation human therapeutic antibiotics encoded on tetracycline resistance plasmids captured from uncultivated stream and soil bacteria.

2. A third generation hydrophilic cephalosporin with an 80% oral bioavailability, of a rather low potency, with no impact on gut flora and rapidly degraded in the environment?
Veterinary medicine needs green antibiotics

Veterinary medicine needs new and innovative green antimicrobial drugs