



Poultry Mycoplasma & Antibiotics 2016: Notes from the Dark Side

Koen De Gussem
Global Technical Director
New Product Development



What am I doing here ?



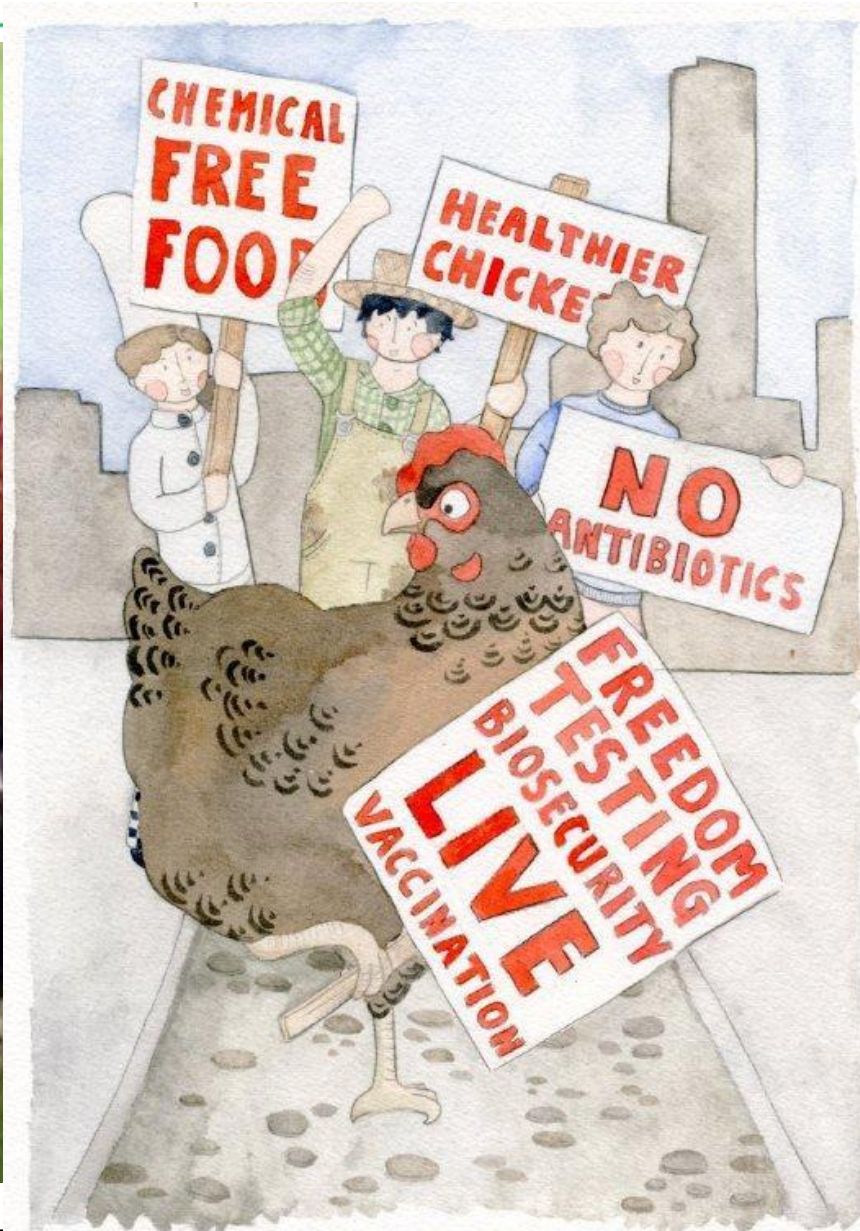
Agenda

- What do 'people' expect from the animal health industry?
 - Consumers, authorities
 - ..and poultry producers
- What are we doing today?
- Where to go from here?

What do they expect ?



How ?



A word cloud of various brand names for food supplements, including:

Lupro-mix, Bio-Add, Euroacid, Broilact, Lisovit, Ovigram, Water Treet, Bioadd,

Crina E, Avigro, Biomus, Biosaf, pre-fec, Salkil, Bolifor, Herban, BMT-mix, Carob,

Termin 8, BiomOS, Salcurb, Greenline, Fastrack, Yucca, Paprika, Uni-sym, Naturex, Selko,

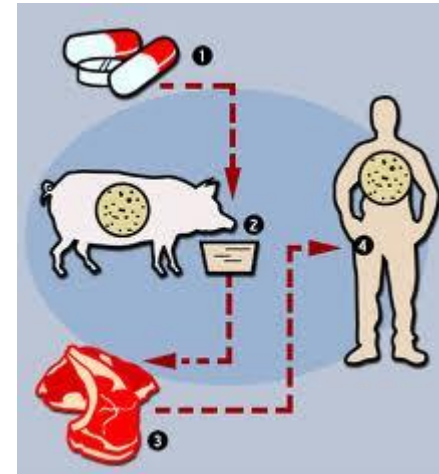
Pre-empt, Sorbic acid, Whey, and Megano.

Courtesy Stephen Lister

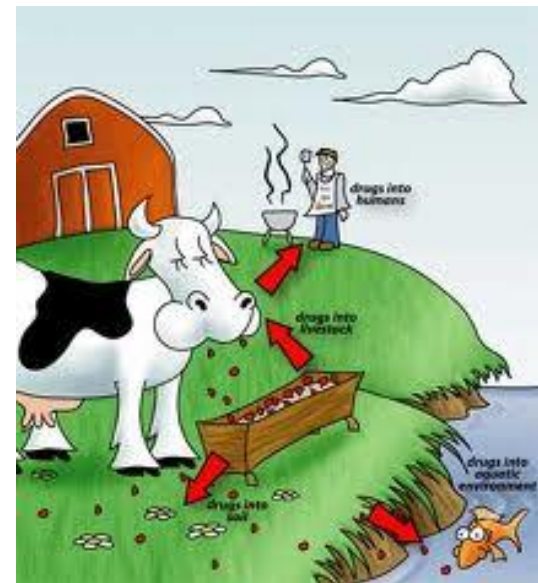
Antibiotics became a threat (if used in animals)



EFSA Website



Superbugs found in chicken causing antibiotic-resistant bladder infections linked to eight million women, researchers say (for USA only). Daily Mail



No 'Global Indications' for antibiotic use

- Obviously this will differ from region to region, country to country, (livestock) company to company and farm to farm
- But maybe 'trends' can be found
- E.g. EU vs. Indian subcontinent
 - 'non specific enteric' usage vs. Specific respiratory (CRD)

EU: ban of AGP's (Antimicrobial Growth Promoters) => huge non specific enteric disorders, combatted with.....(broad spectrum) antibiotics

Indian subcontinent: huge impact of CRD (*Mycoplasma gallisepticum* induced) => combatted with (narrow spectrum) antibiotics

Antibiotic consumption in a Belgian poultry practice 2011

Macrolides, lincosamide, trimethoprim/sulfa and penicillines consumption can +/- completely be attributed to enteric disorders

■ Lincomycine

■ Tiamuline

■ Oxytetracycline

■ Enrofloxacin

■ Tylosine

■ Trimethoprim/sulfa's

■ Doxycycline

■ Flumequine

■ Lincomycine - Spectinomycine

■ Colistine

■ Amoxycilline

■ Fenoxymethylpenicilline kalium

Agenda

- What are we talking about?
- What do 'people' expect from the animal health industry ?
 - Consumers, authorities
 - ...and poultry producers
- What are we doing today?
 - Focus on antibiotics
- Where to go from here?

Strategies for Mycoplasma control

- 1 perfect strategy
- A few other ones...

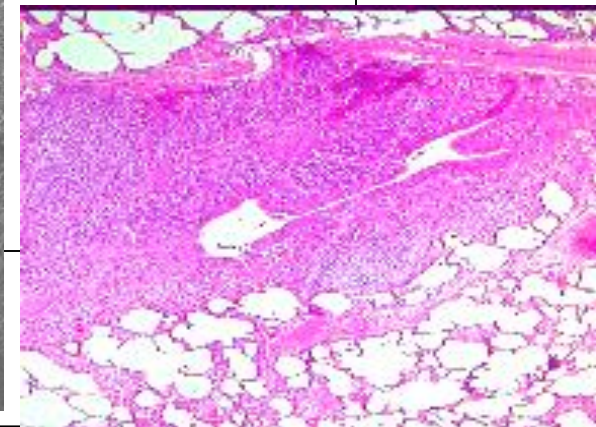
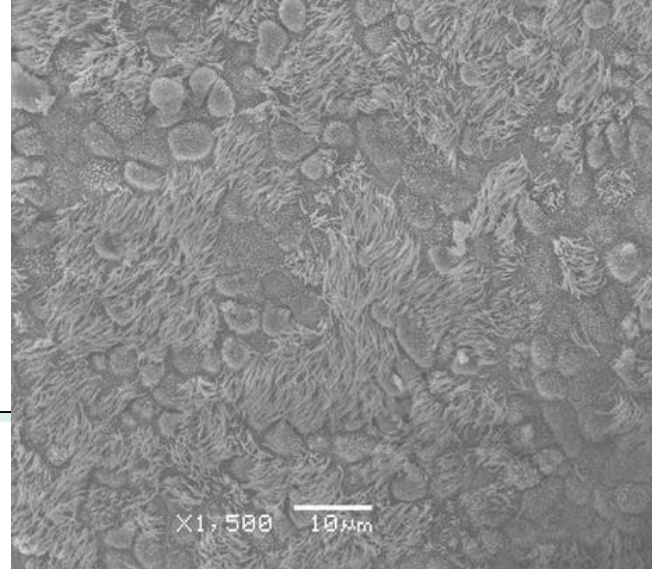
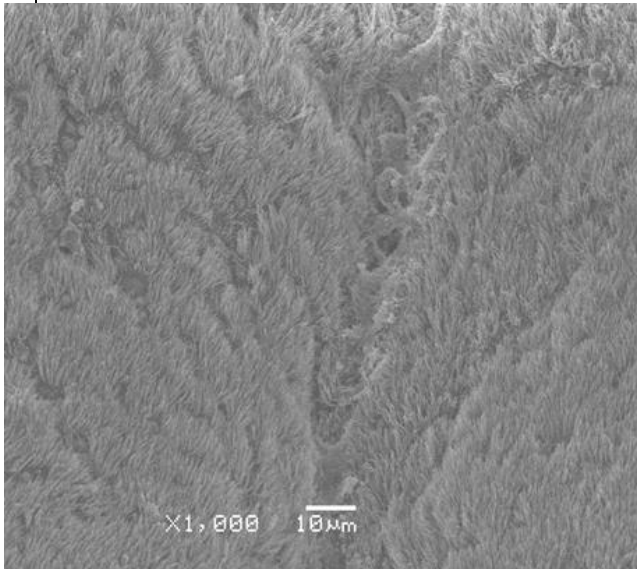
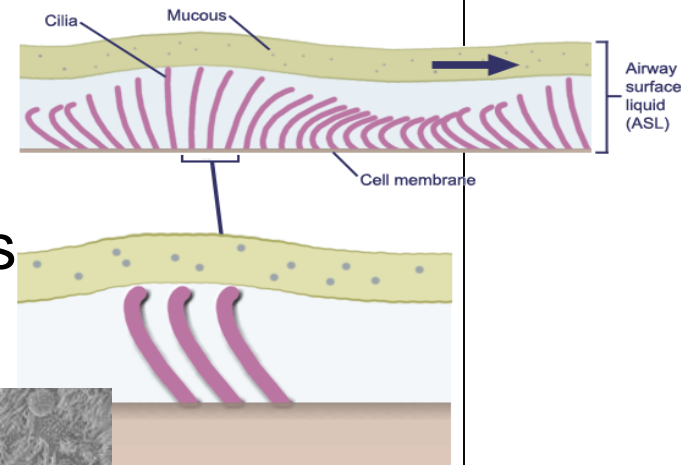
FREEDOM ! No Mycoplasma

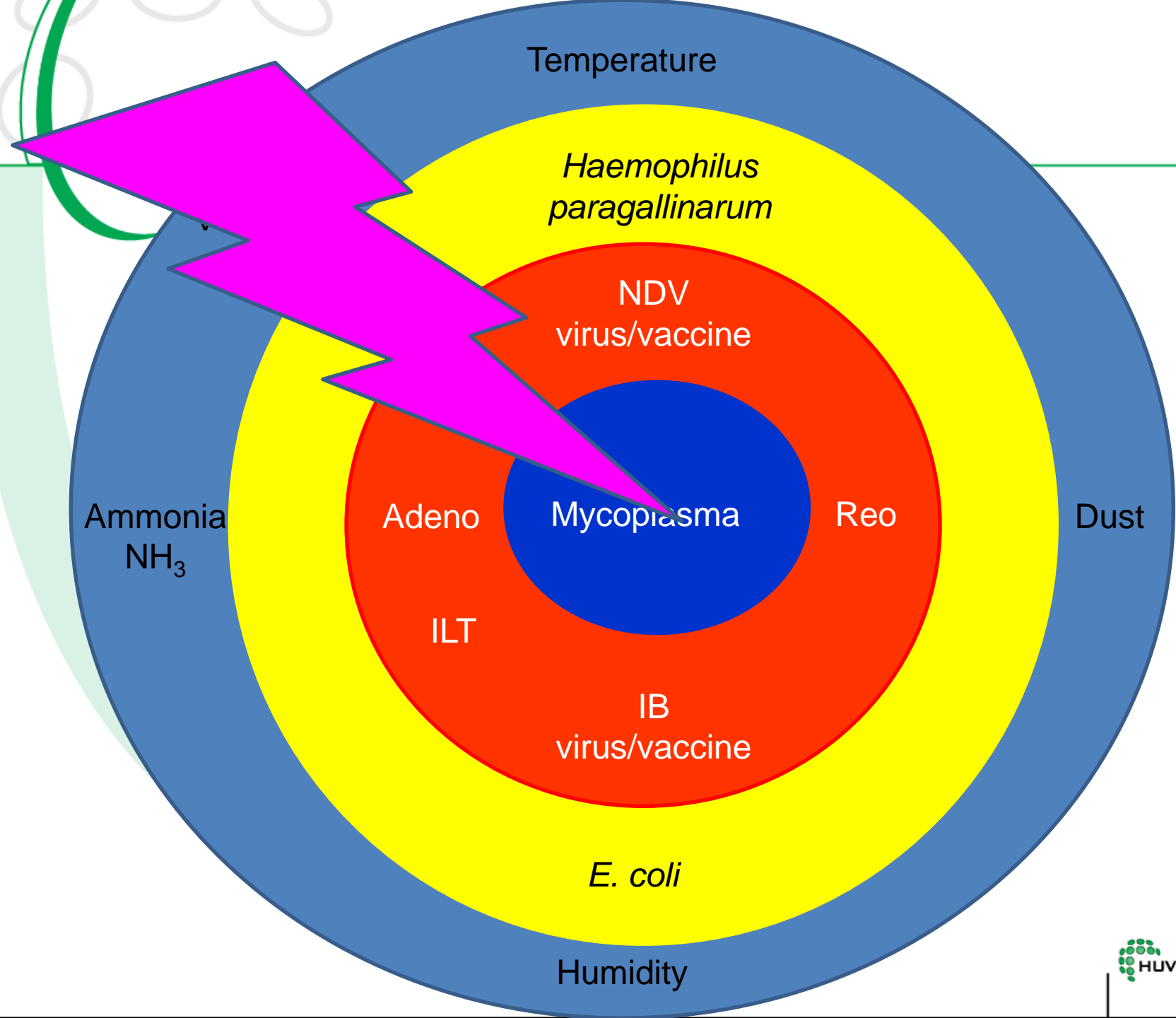
DAMAGE CONTROL

- Antibiotics
- Vaccines
- ...

Interactions with other (secondary) pathogens !!

- Pathogenesis of *M. gallisepticum*
 - Adhesion on ciliated epithelia
 - Ciliostasis – Loss of cilia
 - Accumulation of inflammatory cells
 - Alteration of immune response





Factors determining the efficacy of an antibiotic treatment

The role of antibiotics is to eradicate the causative organisms from the site of infection

Factors determining the efficacy of an antibiotic treatment

- **Pharmacokinetics** : the behaviour of the antibiotic in the body (absorption, distribution, elimination)
- **Pharmacodynamics** : the behaviour of the antibiotic against the bacterium

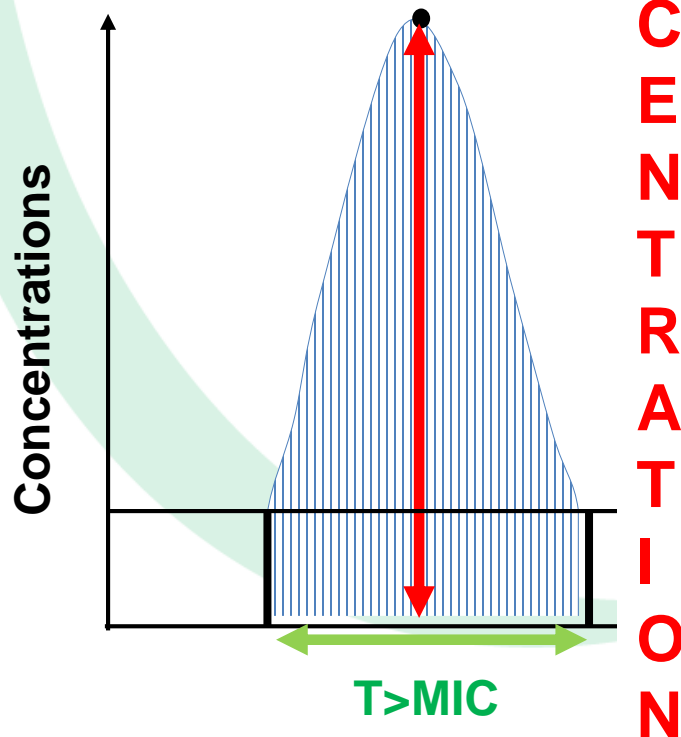
Pharmacokinetics

- Bio-availability
 - Poor resorption after oral application ?
(e.g. aminoglycosides (apramycin))
 - Good resorption after oral application ?
(e.g. macrolides (tylosin))
- Distribution to the target tissue
- Speed of elimination (biotransformation or excretion)

PK/PD predictors of efficacy

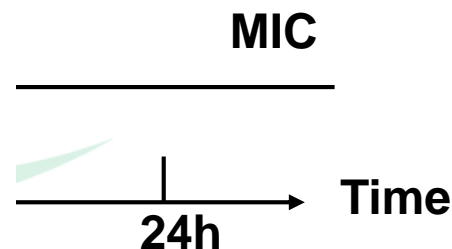
- **C_{max}/MIC** : aminoglycosides

- **AUC/MIC** : quinolones, tetracyclines, azithromycins,
- **T>MIC** : penicillins, cephalosporins, macrolides,
pleuromutilines



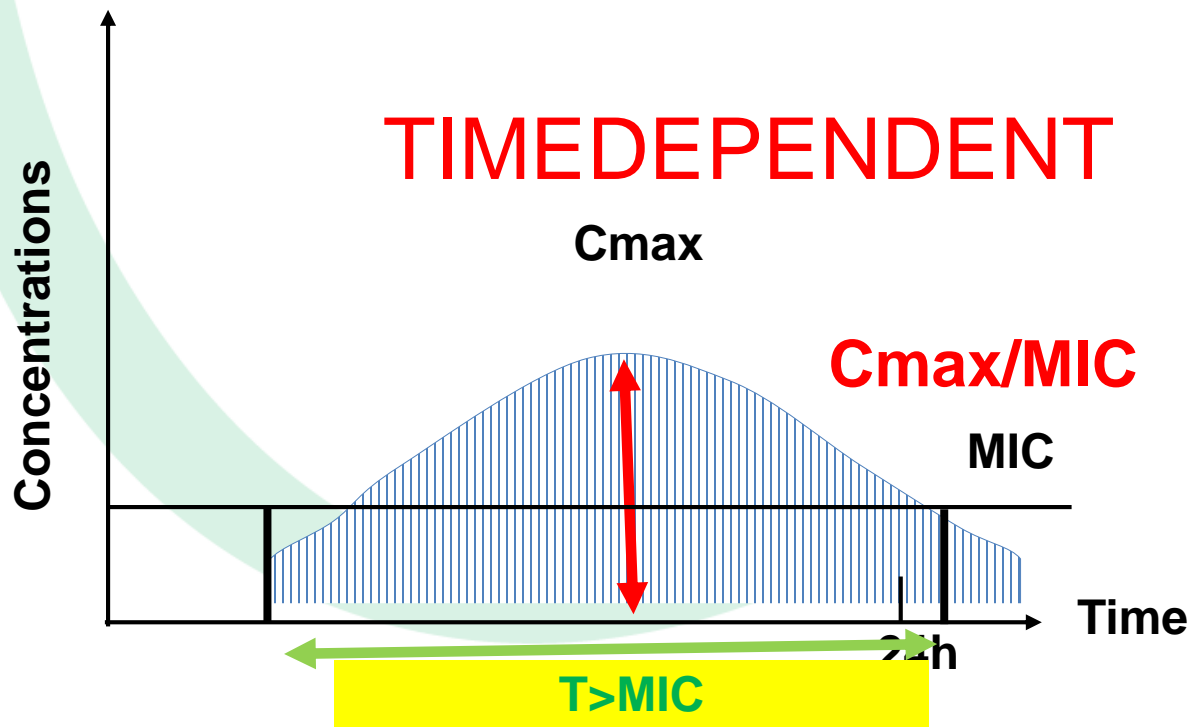
C_{max}/MIC

$$AUIC = \frac{AUC}{MIC}$$



PK/PD predictors of efficacy

- **C_{max}/MIC** : aminoglycosides
- **AUC/MIC** : quinolones, tetracyclines, azithromycins,
- **T>MIC** : penicillins, cephalosporins, macrolides, pleuromutilines



$$AUIC = \frac{AUC}{MIC}$$

Factors determining the efficacy of an antibiotic treatment

Pharmacodynamics:

- What is the intrinsic activity ?
 - Gr-,
 - Gr+,
 - (an)aerobic
 - Mycoplasma
- What is the actual activity ? Is there acquired resistance ?

1. Cell wall inhibitors

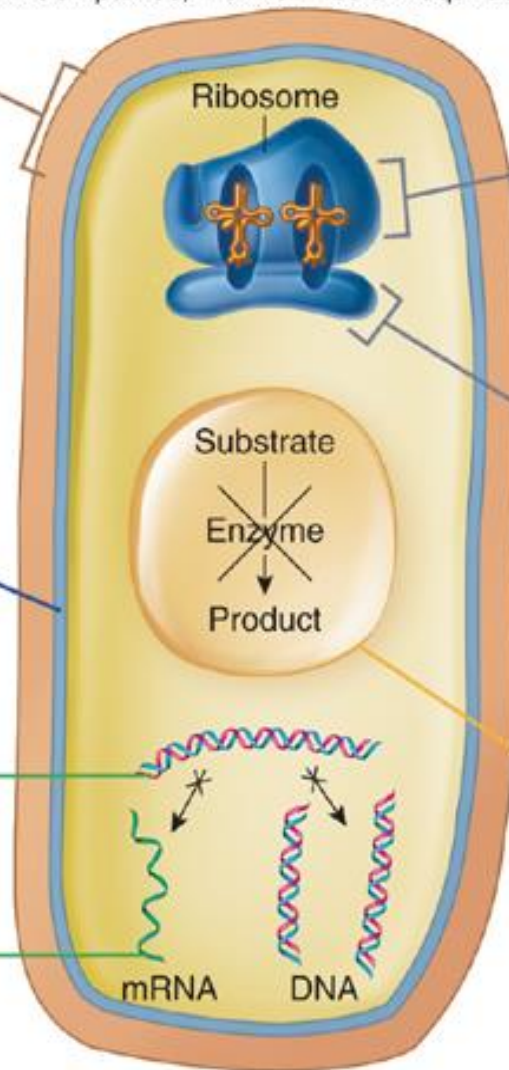
Block synthesis and repair
Penicillins
Cephalosporins
Vancomycin
Bacitracin
Monobactams/carbapenems
Fosfomycin
Cycloserine
Isoniazid

2. Cell membrane

Cause loss of selective permeability
Polymyxins

3. DNA/RNA

Inhibit replication and transcription
Inhibit gyrase (unwinding enzyme)
Quinolones (ciprofloxacin)
Inhibit RNA polymerase
Rifampin



4. Protein synthesis inhibitors

Site of action
50S subunit

Chloramphenicol
Erythromycin
Clindamycin
Oxazolidinones

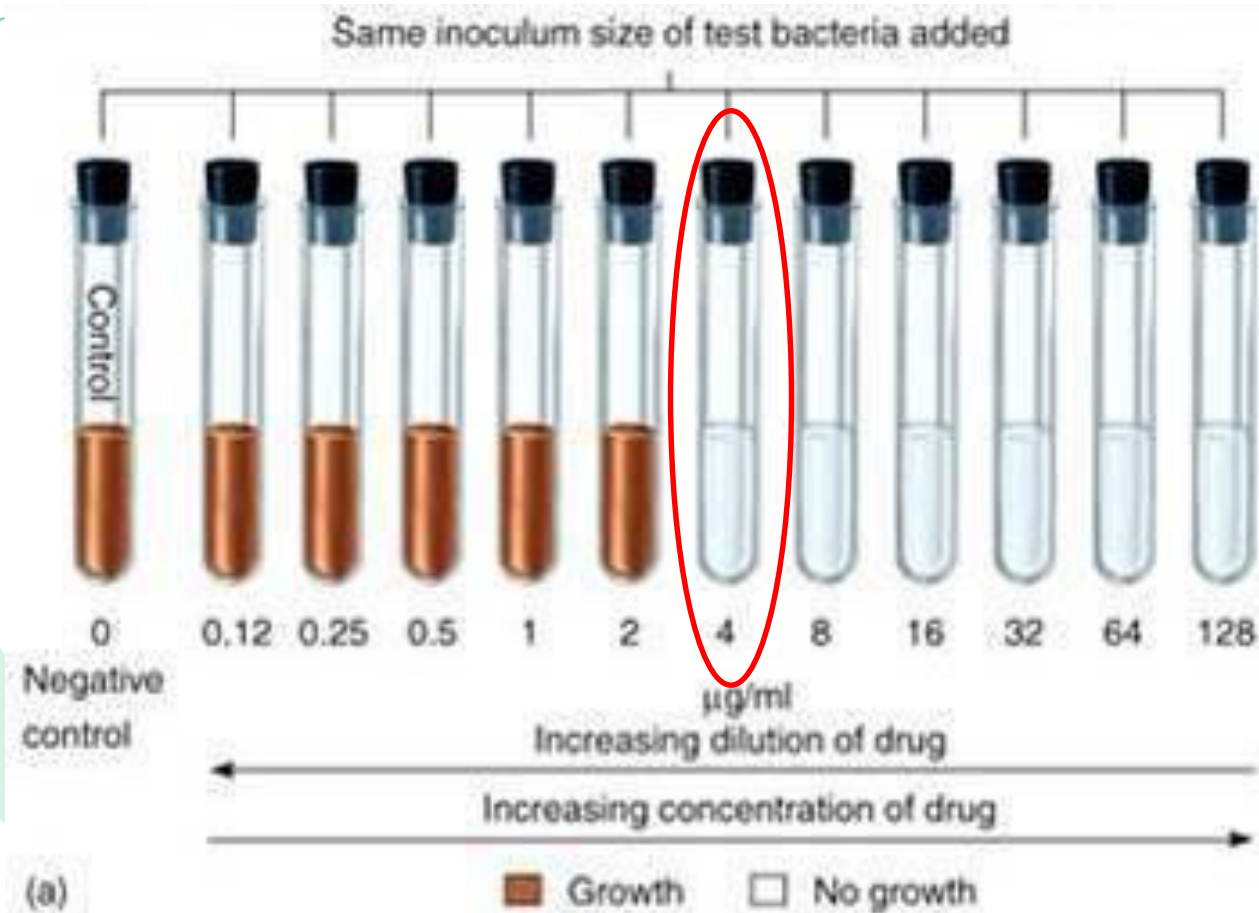
Site of action
30S subunit

Aminoglycosides
Tetracyclines
Streptomycin
Amikacin

5. Metabolic products

Block pathways and inhibit metabolism
Sulfonamides (sulfa drugs)
Trimethoprim

Mycoplasma: MIC



MIC: lowest concentration producing no visible growth

Number of publications on MIC values of *Mycoplasma* are scarce

Avian Pathology (August 2008) 37(4), 415–420



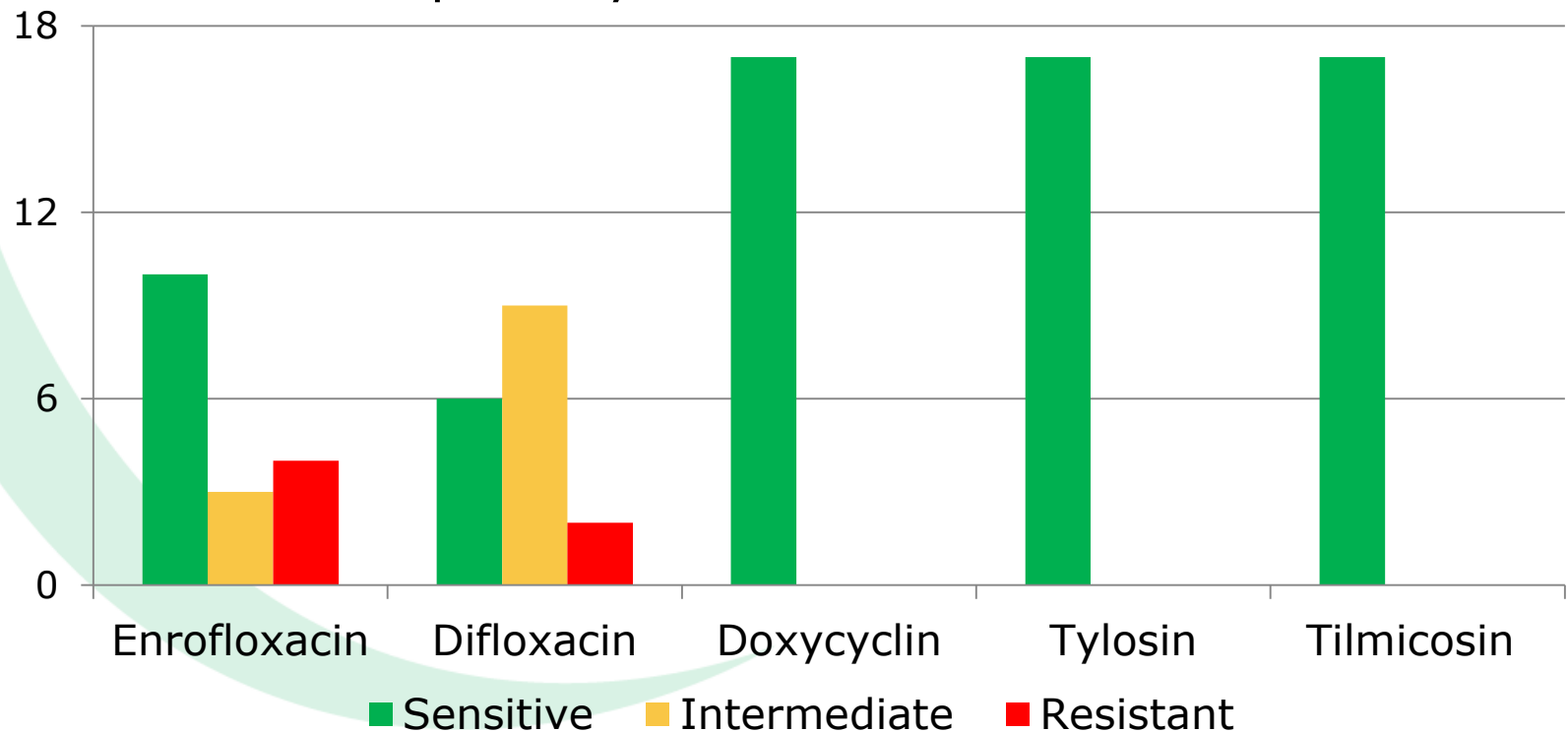
In vitro antibiotic susceptibility of Dutch *Mycoplasma synoviae* field isolates originating from joint lesions and the respiratory tract of commercial poultry

W. J. M. Landman^{1*}, D. J. Mevius², K. T. Veldman² and A. Feberwee¹

¹Animal Health Service (GD), P.O. Box 9, 7400 AA Deventer, the Netherlands, and ²Central Institute for Animal Disease Control (CDIC-Lelystad), Lelystad, the Netherlands

Pharmasin®— Clinical applications in poultry

- Determination of MIC values of 17 strains of *Mycoplasma synoviae*
 - 3 joint lesions
 - 14 respiratory lesions



Results for tylosin (Huvepharma Research 2013)

Bacterial species	Number of strains with tylosin MIC values (µg/ml) of												
	≤0.0078	≤0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8	16>32
<i>Mycoplasma synoviae</i> (n=17)		7	5	3	1		1						

No acquired resistance
Very low MIC's (chickens and turkeys)

¹Huvepharma, Uitbreidingstraat 80, 2600 Antwerp, Belgium

²Istituto Zooprofilattico Sperimentale delle Venezie, Viale dell'Università 10, 35020 Legnaro (PD), Italy

Results for tylosin chickens

(Huvepharma Research 2013)

Bacterial species

Number of strains with tylosin MIC values ($\mu\text{g/ml}$) of

≤ 0.0078 ≤ 0.015 0.03 0.06 0.12 0.25 0.5 1 2 4 8 >8 16 >32

*Mycoplasma
gallisepticum* (n=7)

1

6

Mg strains isolated from chickens have low MIC values

Results for Enrofloxacin

Bacterial species	Number of strains with enrofloxacin MIC values (µg/ml) of									
	< 0.125	0.25	0.5	1	2	4	8	16	> 16	
<i>Mycoplasma gallisepticum</i> (n=7)				1				1	5	

Except for one strain (from turkeys, isolated in 2013), all tested strains resistant

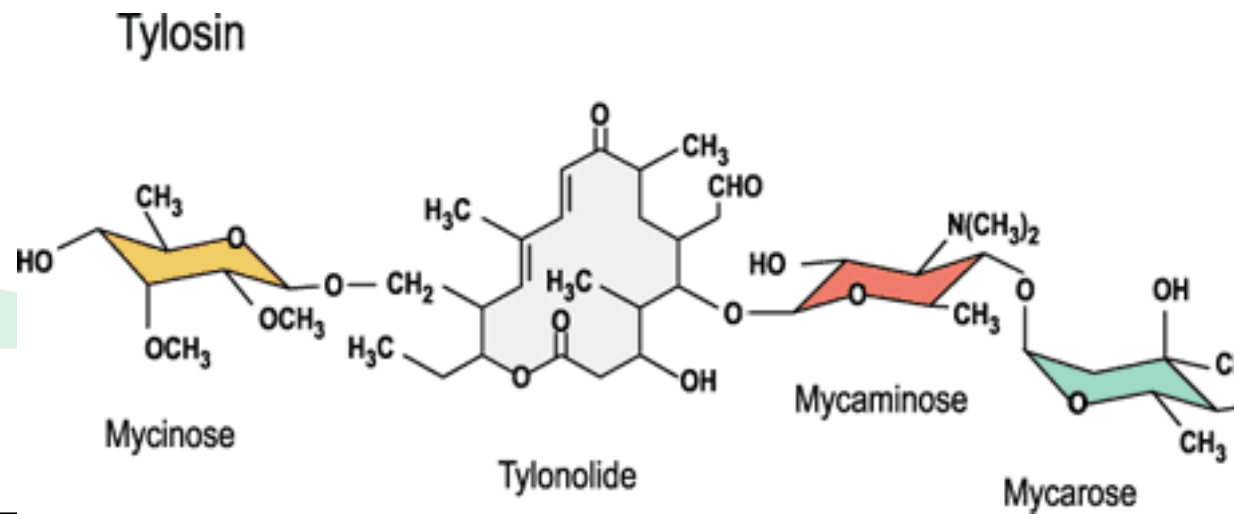
Results for Enrofloxacin

Bacterial species	Number of strains with enrofloxacin MIC values ($\mu\text{g/ml}$) of									
	< 0.125	0.25	0.5	1	2	4	8	16	> 16	
<i>Mycoplasma synoviae</i> (n=7)										7

All tested strains resistant

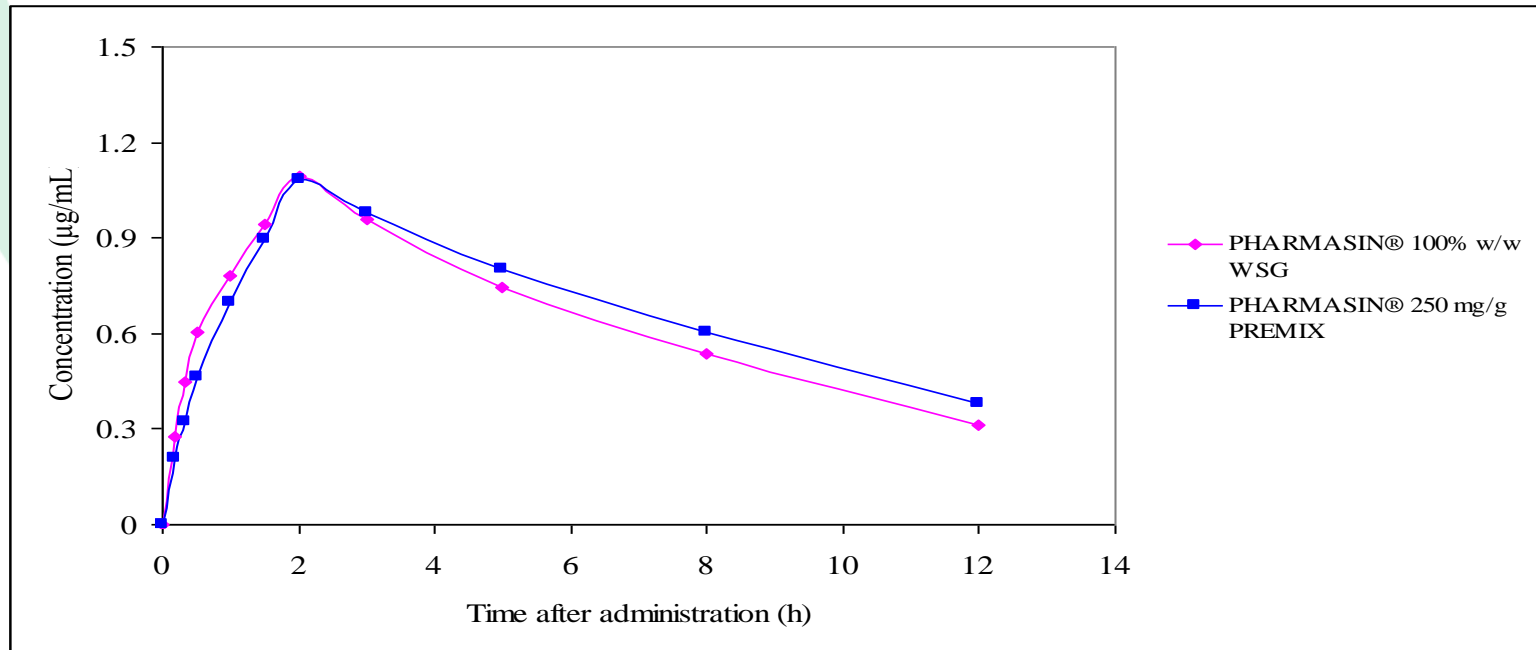
Pharmasin® – Key points

- Tylosin : macrolide produced by *Streptomyces fradiae*
- Inhibits protein synthesis of susceptible bacteria
 - *Mycoplasma spp.*
 - Gram-positive bacteria (including *Clostridium perfringens*)



Pharmacokinetics

- **T max: 2 hours**
 - Fast uptake
- **T1/2 orally: 4-6 hours**
 - Withdrawal time: **short** 0 days eggs and meat (country specific)
 - Excretion mainly intestine
- **Bioavailability: +/-70%**

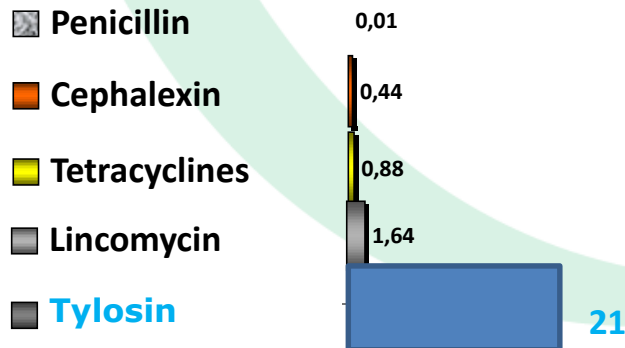


Pharmacokinetics

- Tylosin is highly lipid soluble, resulting in:
 - *High tissue distribution Vd (3)*
 - *High intracellular concentrations (Mycoplasma!)*

$$V_D = \frac{\text{total amount of drug in the body}}{\text{drug blood plasma concentration}}$$

Ratio intracellular – extracellular concentration



Pharmasin® – Clinical applications in poultry

- Respiratory

- Control of *M. synoviae* in chickens.
- Control of *M. gallisepticum* in chickens / turkeys.

- Enteric

- Control of gut health
- As an aid in the control of Necrotic Enteritis (clinical and subclinical)
- Dysbacteriosis

Amount(s) to be administered and administration route

Turkeys:

- For the treatment of **respiratory disease:**
 - 75 – 100 mg tylosin per day (corresponding to 82.5 – 110 mg of the veterinary medicinal product per kg BW) for 3 – 5 days

Chickens:

- For the treatment of **respiratory disease:**
 - 75 – 100 mg tylosin per day (corresponding to 82.5 – 110 mg of the veterinary medicinal product per kg BW) for 3 – 5 days
- For the treatment of **necrotic enteritis:**
 - 20 mg tylosin per kg BW per day (corresponding to 22 mg of the veterinary medicinal product) for 3 days

WRONG

Dose determination (example)

- General equation:
- $\text{Dose} = \text{Cl} \times \text{Target Concentration} / F$
- Cl over 24 h is 37 L/kg ($0.025 \times 60 \times 24$),
Target Concentration depends on germ susceptibility (MIC90), e.g. 0.5 mcg/ml, $F = 0.7$ (70%):
- $\text{Dose} = 37 \times 0.5 / 0.7 = 25 \text{ mg/kg/day}$

The answer: dose titration study

- First challenge study in a long time
- Using chickens in isolators
- Using Mg strain recently isolated in Italy with 'normal' MIC's

WVPAC2013 - 19-23 august 2013 - Nantes FRANCE

SEP09

Efficacy of tylosin tartrate at different dose levels in controlling *Mycoplasma gallisepticum* in a challenge model in chickens

DE GUSSEM Koen¹, DEPONDT Wouter¹, VERECKEN Monita¹

¹Huvepharma, Uitbreidingstraat 80, 2600 Antwerp, Belgium

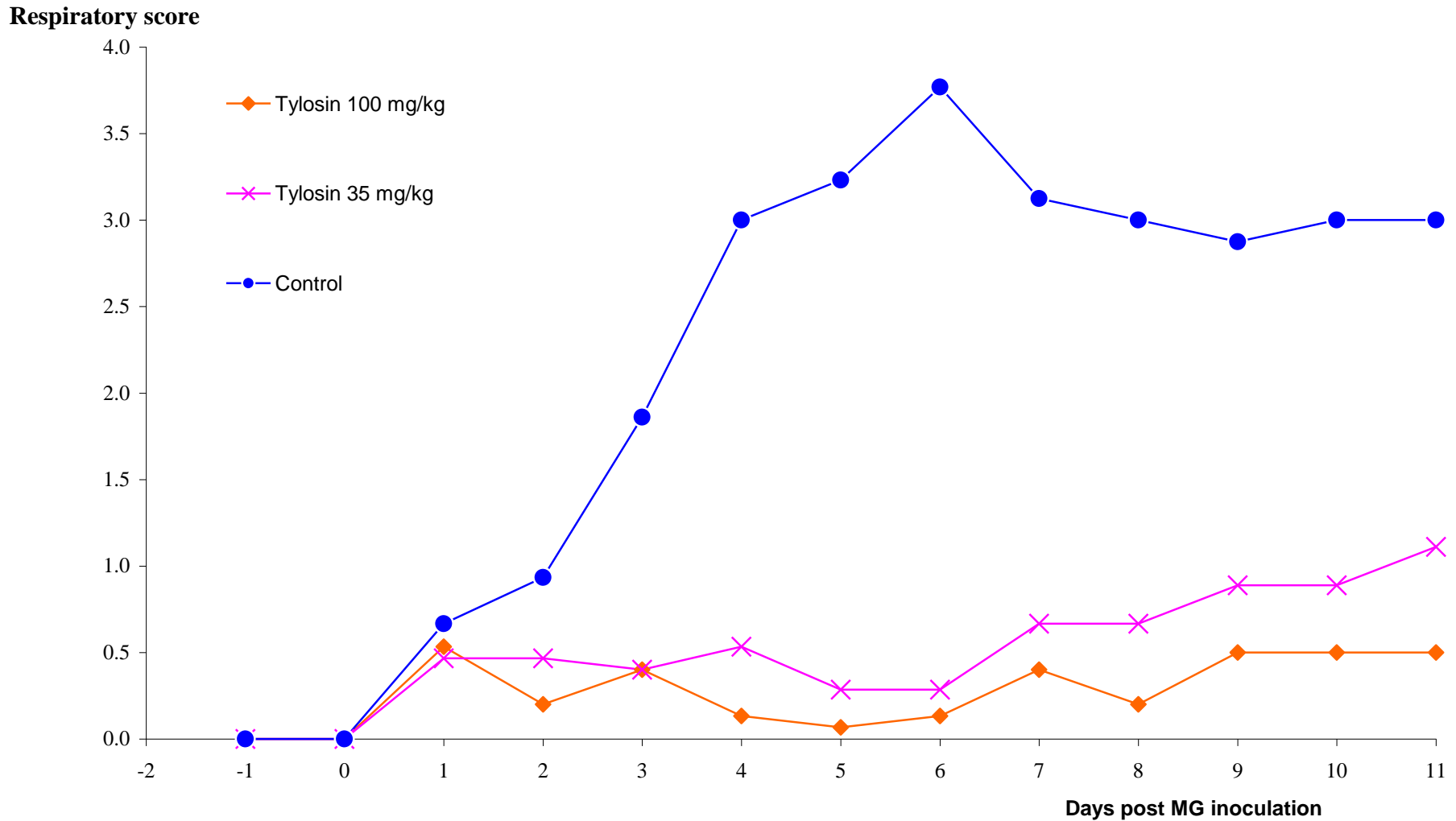
Mycoplasma gallisepticum

- Strain was isolated from Layers
- Parameters:
 - Clinical signs
 - Body weight

Score	Clinical sign
0	No respiratory signs
+ 1	Tracheal reflex
+ 1	Breathing noise
+ 1	Dyspnea only after manipulation
or	or
+2	Obvious signs of dyspnea

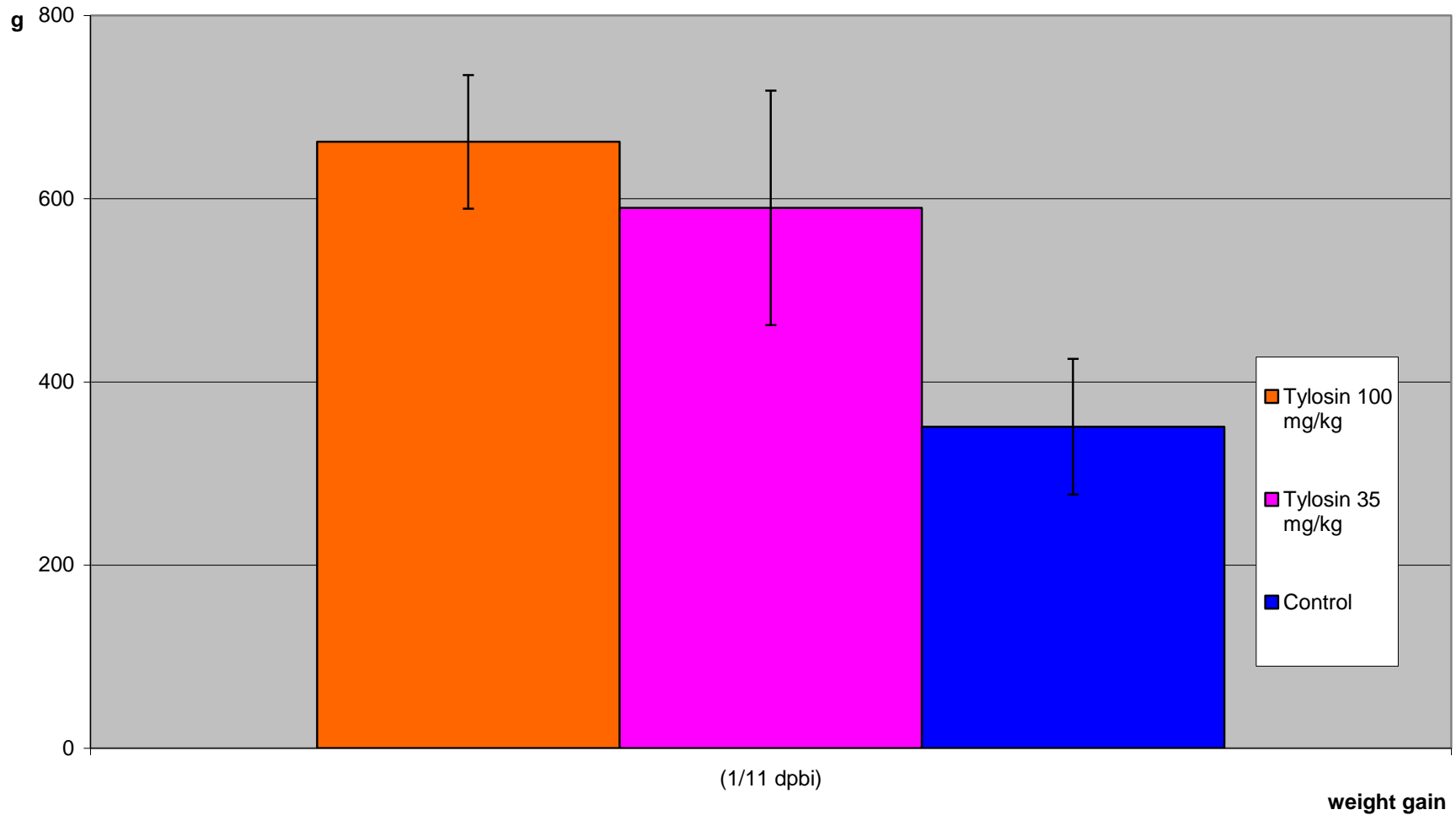
Dose titration study

Figure 1. Mean respiratory scores



Dose titration study

Figure 4. Mean weight gains



Conclusions on in vivo study

- Based on calculations with PK/PD parameters- tylosine tartrate at 35mg/kg BW should be as good as 100 mg/kg BW.
- Confirmed by in vivo study
 - Clinical signs and mortality completely controlled with Pharmasin[®]
 - Small differences in performance and bacterial load however could be seen (in this study)
- But in many SPC's worldwide: 100 mg/kg BW
- Very often: treatment duration too short
- In many countries...underdosed

Mycoplasma synoviae: Laying birds Egg Apex Abnormalities

AVIAN DISEASES 54:961–964, 2010

|Case Report—

Treatment of Eggshell Abnormalities and Reduced Egg Production Caused by *Mycoplasma synoviae* Infection

Salvatore Catania,^{AC} Dania Bilato,^A Federica Gobbo,^A Anna Granato,^A Calogero Terregino,^A Luciano Iob,^A and Robin A. J. Nicholas^B

^AIstituto Zooprofilattico Sperimentale delle Venezie, viale dell'Università, 10-35020 Legnaro (Padova), Italy

^BVeterinary Laboratory Agency (Weybridge), New Haw, Addlestone, Surrey KT15 3NB, United Kingdom

Summary:

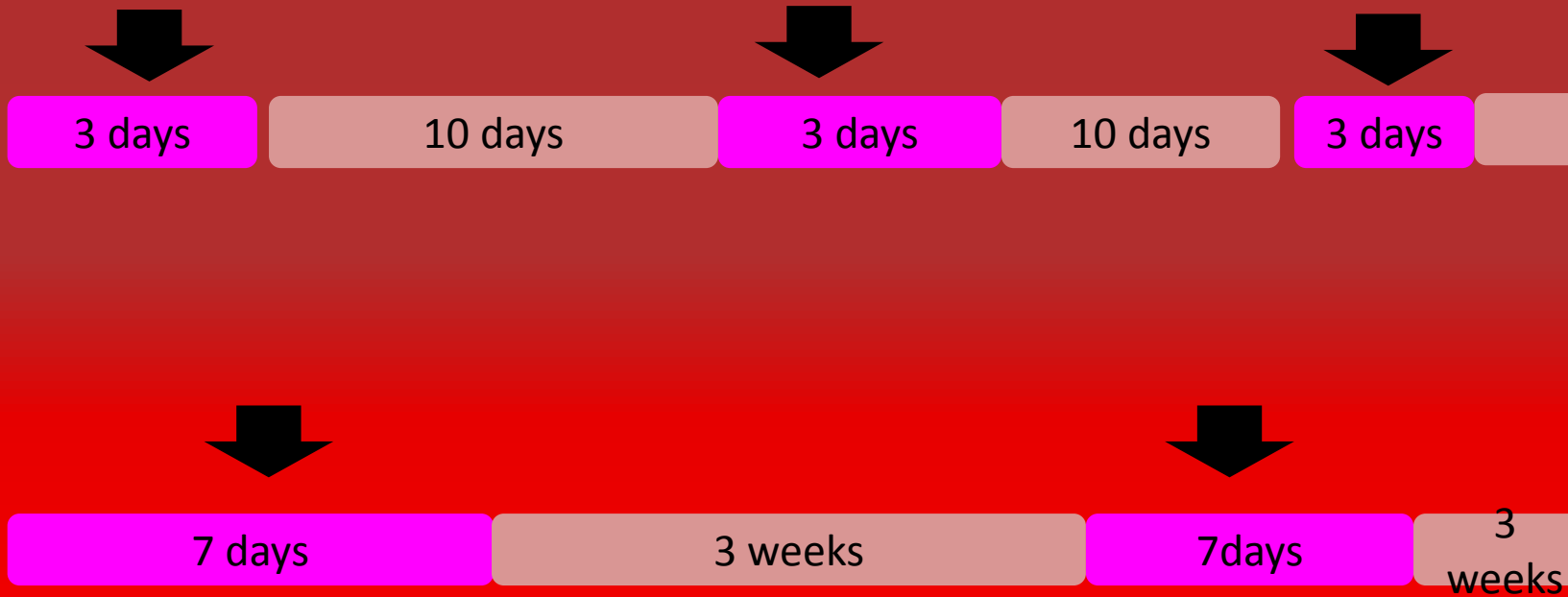
- Tylosin treatment improved:
 - ☐ egg weight
 - ☐ eggshell quality

Dosage = +/- 50mg/kg BW



Macrolide treatment strategy in breeders

Longer treatment durations=> longer intervals



Key message

- Treat sufficiently long !!
 - 3-5 days minimal
 - Good experience with 7-10 days treatment period, followed by quite long intervals (+/- 3 weeks)

Macrolide treatment strategy in broilers

Longer treatment durations=> longer intervals

3 days

10 days

3 days

10 days

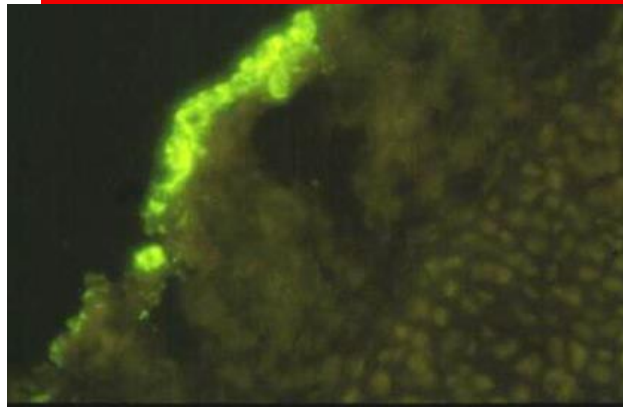
3 days

7 days

3 weeks

7 days

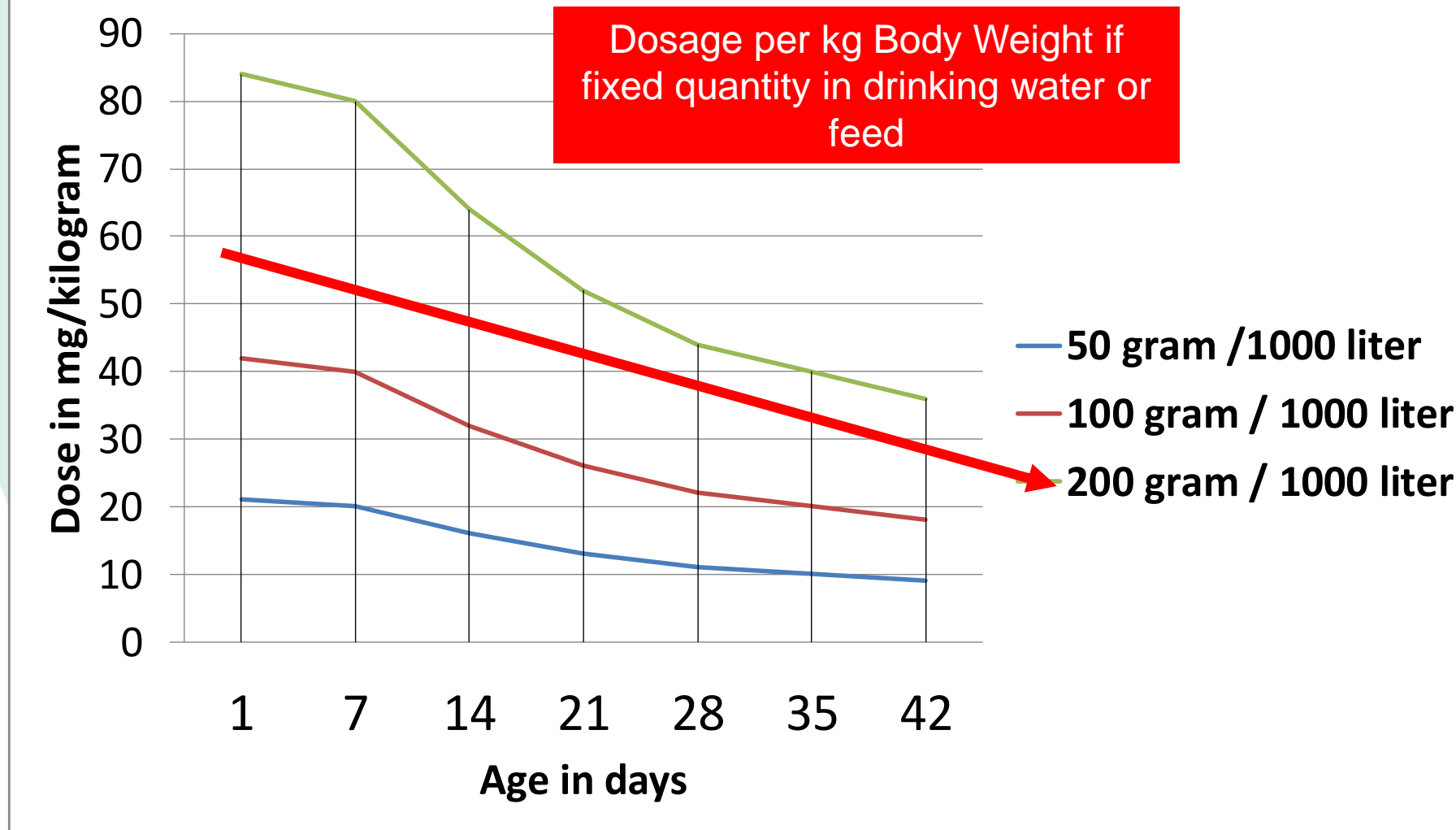
3 weeks



Key messages

- **Treat sufficiently long !!**
 - 3-5 days minimal
 - Good experience with 7-10 days treatment period, followed by repeating at around 24 days of life
- **Treat always first days of life !!**
 - To inhibit as much as possible the horizontal spread to negative day one old chicks and to inhibit the early onset of mucosal damage

Broiler: Concentration in the water versus dose in mg/kg bodyweight



Agenda

- What are we talking about?
- What do 'they' expect from the Animal Health industry?
 - Consumers, authorities
 - Poultry producers
- What are we doing today?
- Where to go from here?

Food for thoughts

- Extreme scrutiny on antibiotics in live stock production
- However, antibiotics represent a direct and indirect cost (image), so producers will not use them when not needed (disease, animal welfare).
- Distinction to be made between human important antibiotics (shared-class) and others.
- Evidence based science. Always ?

Food for thoughts

Clinical Infectious Diseases Advance Access published April 24, 2015

Marc J. M. Bonten¹ and Dik Mevius^{2,3}

¹Department of Medical Microbiology, Julius Center for Health Sciences and Primary Care University Medical Center Utrecht, ²Department of Bacteriology and Transmissible Spongiform Encephalitis, Central Veterinary Institute of Wageningen University and Research Centre, Lelystad, and ³Department of Infectious Diseases and Immunology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Less Evidence for an Important Role of Food-Producing Animals as Source of Antibiotic Resistance in Humans

Facts and figures, no more
decisions by decibels

Food for thoughts

- Alternatives ? No track record, limited data
- Antibiotics
 - There is room for improvement !
 - Good Veterinary (medication) Practice is too often still Terribly Inconsistent Practice
 - Dramatically different doses for same (branded) products across different (neighbouring) countries
 - Dramatically 'bad' recommendations for duration of treatment programs

Low Hanging Fruit

- Use narrow spectrum antibiotics where possible ...often the best ! (tylosin)
- Refrain from excessive shared-class antibiotic use (fluoroquinolones)
- Implement correct treatment regimes (duration and dosage)
- Improve management (husbandry) practices
- Develop more (and better) vaccines...

Industry (Animal Health) must take its responsibility

- Good training/education/instructions
- Withdraw 'prehistoric' claims and registrations
- Withdraw excessive (bazooka) claims
- Harmonize registrations and strategies across countries
 - Dosage ! Duration of treatment
 - Based on science
- Decently research the Best Practices for its molecules (Finally...)

General Conclusions

- Poultry industry remains dependent (too much) on damage control
- Antibiotics still needed from a global perspective
 - Still a lot to improve in knowledge and application of antibiotics
- SO... JUST GET IT RIGHT !
 - Antibiotics
 - Vaccines