TESTING OF SALIVA AS *ANTE*-MORTEM SCREENING FOR ANTIMICROBIALS IN PIGS

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Introduction

- Antimicrobials are often used on pig farms

Study about the use of antimicrobials in 222 BE, FR, DE and SE pig farms: BE second largest use (Postma, 2016)

- The use of antimicrobials is resulting in non-compliant pig meat

leading to recalls or other actions (RASFF)

→ economic losses

→ image damage

- Solutions:

⇒ fast screening: *ante*-mortem or post-mortem (before sales)

⇒ sensitising the sector: less and more careful use of antimicrobials

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Step 1
Choice of antibiotics to be monitored

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Choice of antibiotics

- Based on the use (AMCRA)
  - a) Oral antibiotics (in volume the largest group)
    - amoxicillin - β-lactam
    - colistin - polymyxins
    - sulfonamides & trimethoprim - diaminopyrimidine derivatives
    - doxycycline - tetracyclines
    - tylosin - macrolides
  - b) Injected antibiotics (smaller group)
    - tulathromycin - macrolides
    - ceftiofur - β-lactam
    - amoxicillin - β-lactam

- Based on monitoring data

Choice of antibiotics

- Based on the use
- Based on monitoring data: RASFF notifications

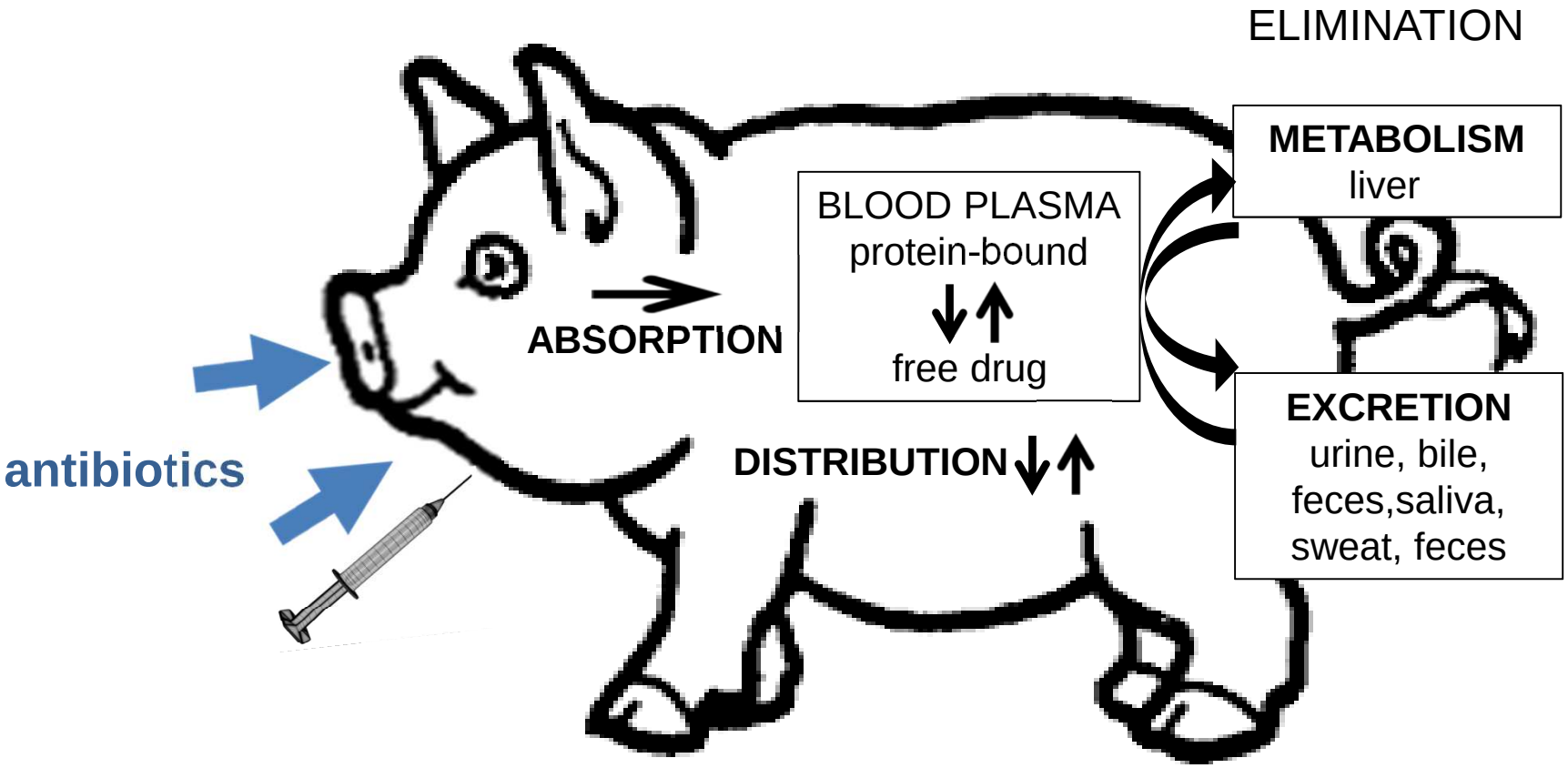
Substance	Concentration (µg/kg)	Date
oxytetracycline	300	20/06/2014
sulfadiazine	280	28/04/2014
sulfadiazine	170	04/12/2013
sulfadiazine	375	13/11/2013
levamisole	>50	04/07/2013
doxycycline	230	04/07/2013
sulfadimethoxine	200	08/03/2013
sulfadiazine	360	15/01/2013
sulfadiazine	140	18/09/2012
sulfadiazine	>200	18/04/2011

- + results Belgian National Residue plan (FASFC)

⇒ Option (FEBEV): β-lactam, tetracyclines, sulfonamides, tylosin

Step 2
Choice of matrix to be sampled

Pharmacokinetics of veterinary drugs - AMDE



AMDE: absorption, metabolisation, distribution and excretion

Choice of matrix

Ante-mortem screening

- urine
- saliva
- blood (serum)
- feces

Post-mortem screening

- meat
- kidney

Testing of sampling of saliva (oral fluids)

TEGO - Swine Oral Fluids ropes or similar

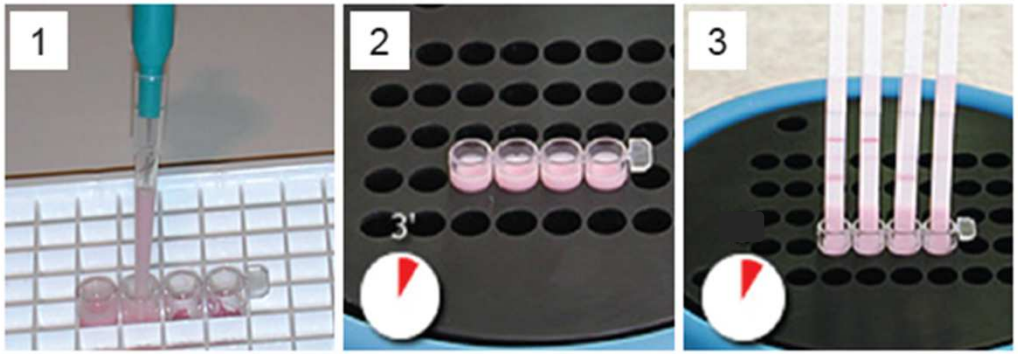
Step 3
Choice of screening tests

Choice of tests + development of sample preparation protocols

Charm Quad Kiwi	β-lactam, tetracyclines, sulfonamides, tylosin	5 min
Trisensor Milk	β-lactam, tetracyclines, sulfonamides	6 min
Tylosensor Milk	tylosin	6 min

Milk tests → sample pretreatment for other matrices (meat, saliva, feed,…)

TriSensor Milk & Tylosensor Milk



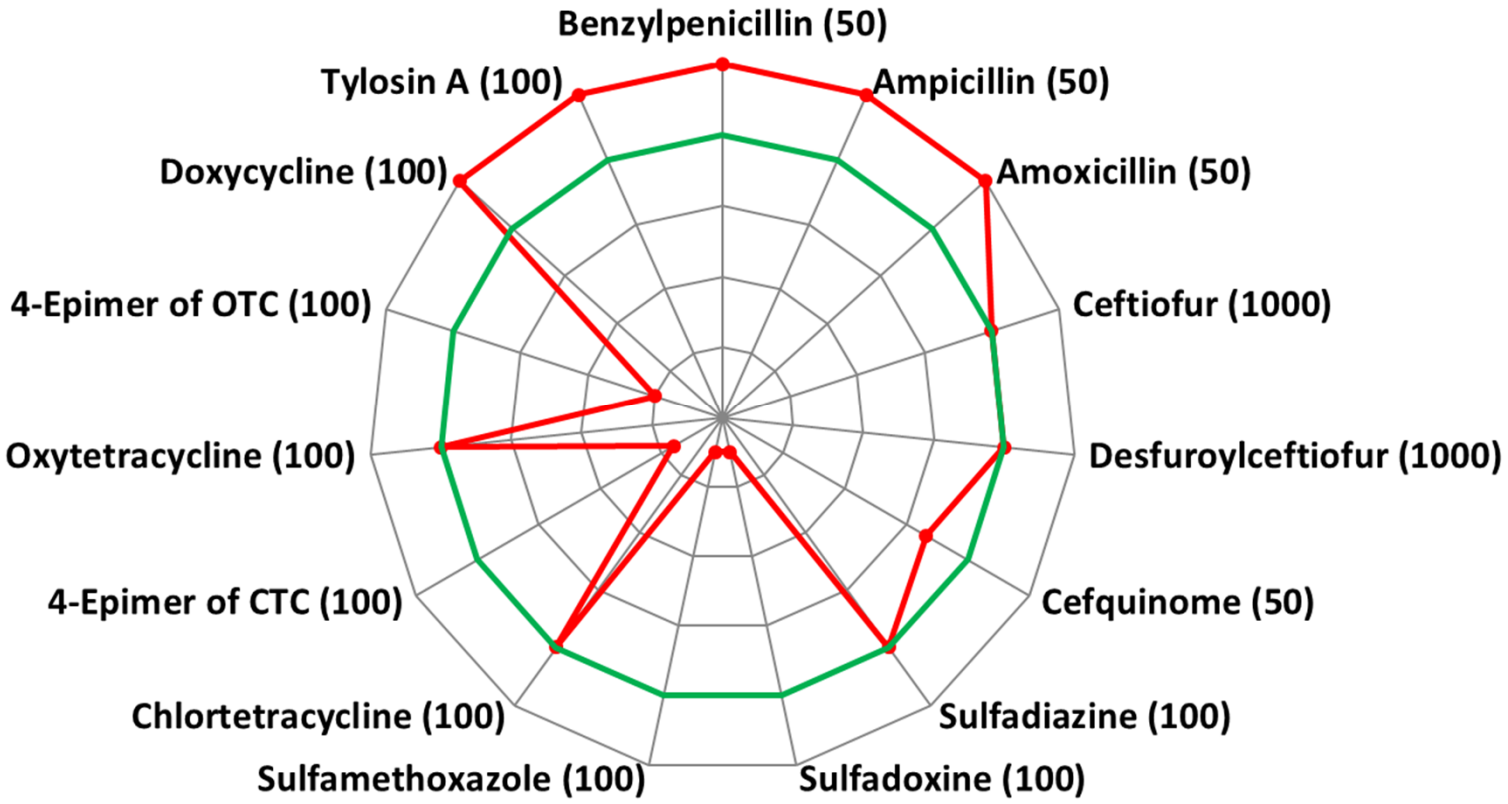
200 µl of extract **3 minutes at 40°C** **4 minutes at 40°C**

results

Result: ratio test line / reference line

Cut-off = 1.10 (ratio ≤1.10 positive; ratio> 1.10: negative)

Detection capability of Trisensor Milk and Tylosensor Milk in saliva related to the MRL (µg/kg) in pork meat



Inner circle = 10 × MRL; circle 2 = 5 × MRL circle 3 = 2 × MRL; circle 4 = MRL; circle 5 = 0.5 × MRL.

Results obtained with ReadSensor and cut-off = 1.10.

Step 4
Development of a monitoring protocol

Present testing strategy

Post-mortem screening of meat

Sampling of meat in slaughterhouse

Monitoring of at least one carcass of each tome of pigs

Screening for β-lactams, tetracyclines, sulfonamides with TriSensor Milk and tylosin with TyloSensor Milk

Positive screening result:

- all carcasses of suspect tome are kept in quarantaine
- residues identified and quantified by LC-MS/MS at ILVO
- based on LC-MS/MS result: decision about destination

Testing strategy for the future

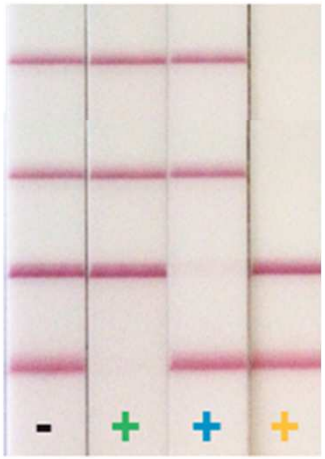
Ante-mortem screening of saliva at the farm

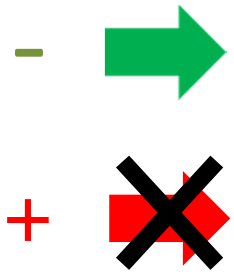
Sampling of saliva of each tome at the pig farm by truck driver

Screening for β-lactam, tetracyclines, sulfonamides with TriSensor Milk and tylosin with TyloSensor Milk

Positive screening result:

- no loading of pigs, slaughtering postponed
- sampling of feed and drinking water





Step 5
Proof of functionality of testing saliva

Sampling at 101 farms with no antimicrobial treatment

At 13 farms the saliva tested positive (screening)

- 6× sulfonamides
- 3× tetracyclines
- 3× tylosin
- 1× sulfonamides & tylosin

LC-MS/MS analysis confirmed these data

- 1× sulfamethazine (10 ppb)
- 4× sulfadiazine (63-340 ppb) & sulfadoxine (2-35 ppb),
- 1× sulfadiazine (102 ppb) & sulfamethoxazole (4 ppb)
- 3× doxycycline (123-618 ppb)
- 3× tylosin (spores – 41 ppb)
- 1× sulfadiazine (264 ppb) & tylosin (21 ppb)

14 Positive feed samples (carry-over feed)

- 11× β-lactams
- 2× sulfonamides
- 1× tetracyclines

6 Positive water samples (error by farmer)

- 4× β-lactams
- 2× tetracyclines (doxycline, 65 & 350ppb)

Sampling at 7 farms with antimicrobial treatment

Treatment with sulfadiazine, tylosin or doxycycline →
positive saliva samples were found for the respective antibiotic group

Oral treatment with amoxicillin: not detected in the saliva (enzymes)
but saliva was positive for sulfonamides (152 ppb sulfadiazine)
(contamination via drinking water)

Both samplings:
Some false positive results for Tylosensor in saliva and feed

Step 6
Relation between residues in the saliva and residues in the meat (and other matrices)

Sampling at 23 farms (26 compartments) with pigs under antimicrobial treatment

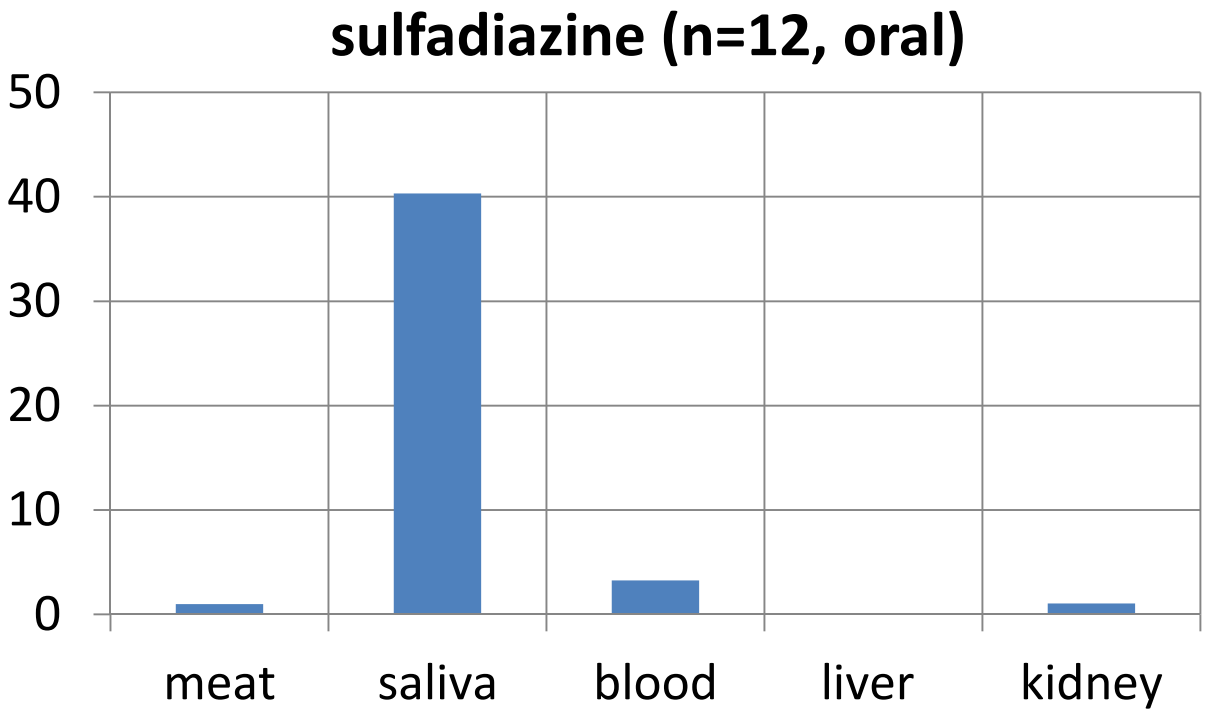
Applied antibiotics: (sometimes combination)
- β-lactam: amoxicillin, benzylpenicillin
- tetracyclines: doxycycline
- sulfonamides: sulfadiazine, sulfadoxine
- macrolides: tylosin
- other families: lincomycin, spectinomycin, tilmicosin

Sampling
- saliva
- drinking water
- feed
- pig (blood, meat, kidney, liver)

Analysis:
- rapid receptor tests (Trisensor Milk & Tylosensor Milk)
- LC-MS/MS

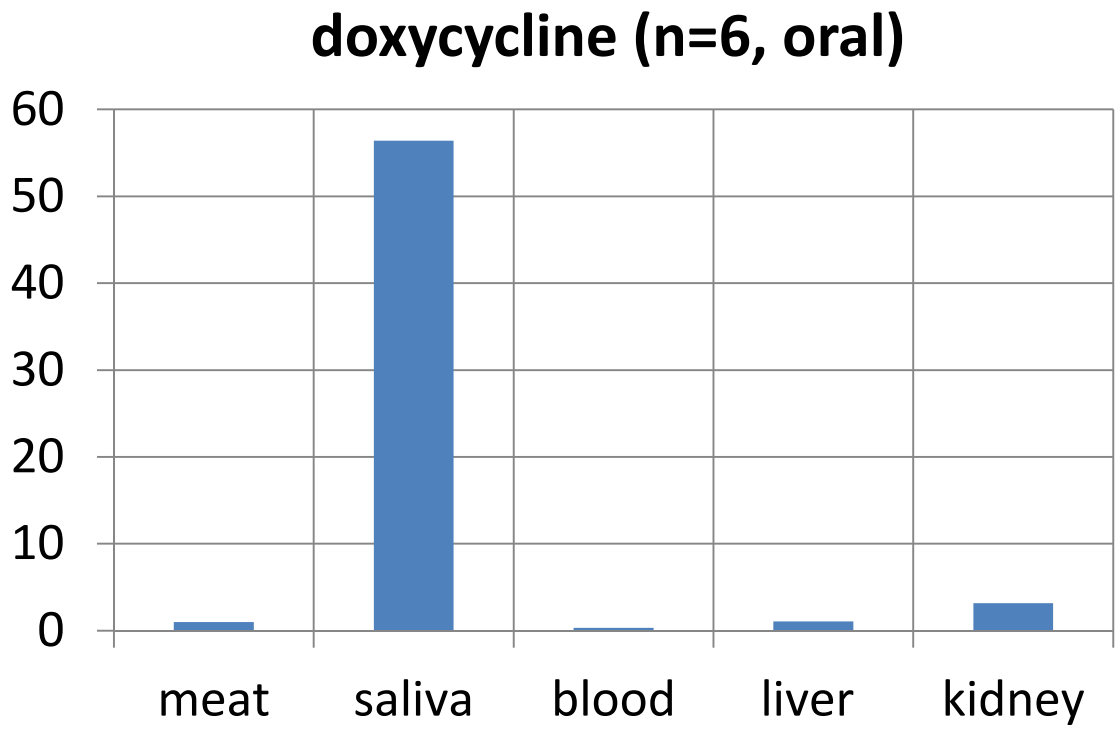
Concentration in meat *versus* concentration in saliva and other matrices: results for sulfadiazine

	meat	saliva	blood	liver	kidney
mean conc. (ppb)	814	6452	3163	17	1442
MRL (ppb)	100	--	--	100	100
ratio (matrix : meat)	1.0	40.3	3.2	0.1	1.0



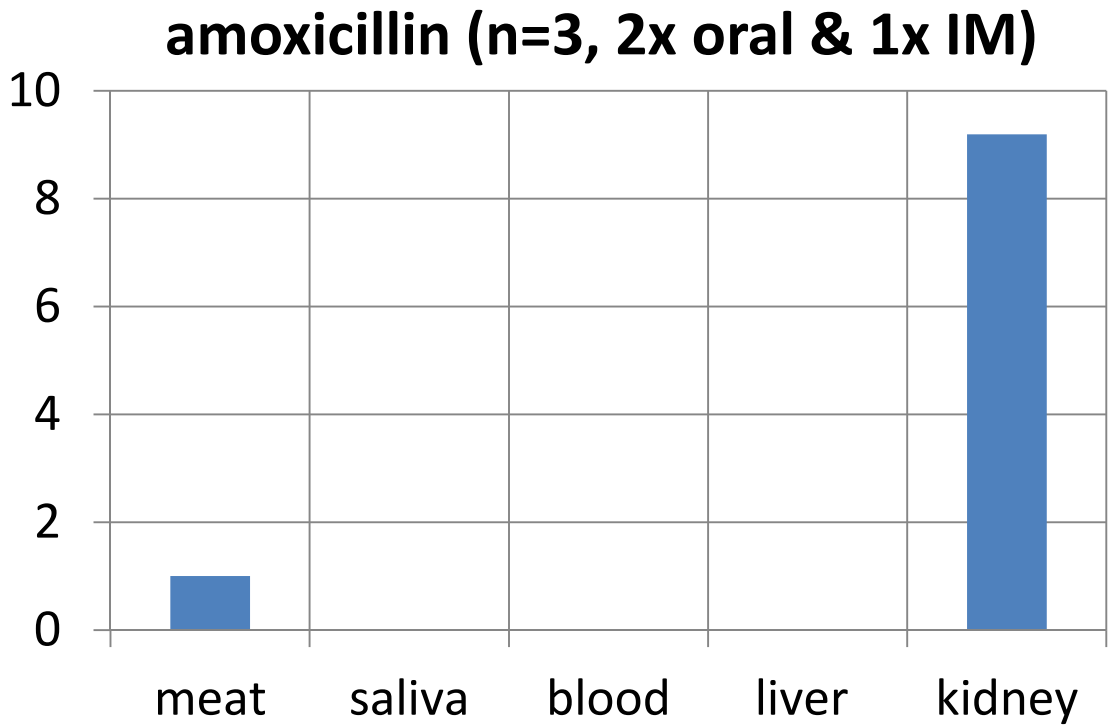
Concentration in meat *versus* concentration in saliva and other matrices: results for doxycycline

	meat	saliva	blood	liver	kidney
mean conc. (ppb)	110	3469	58	191	404
MRL (ppb)	100	--	--	300	600
ratio (matrix : meat)	1.0	56.4	0.3	1.0	3.1



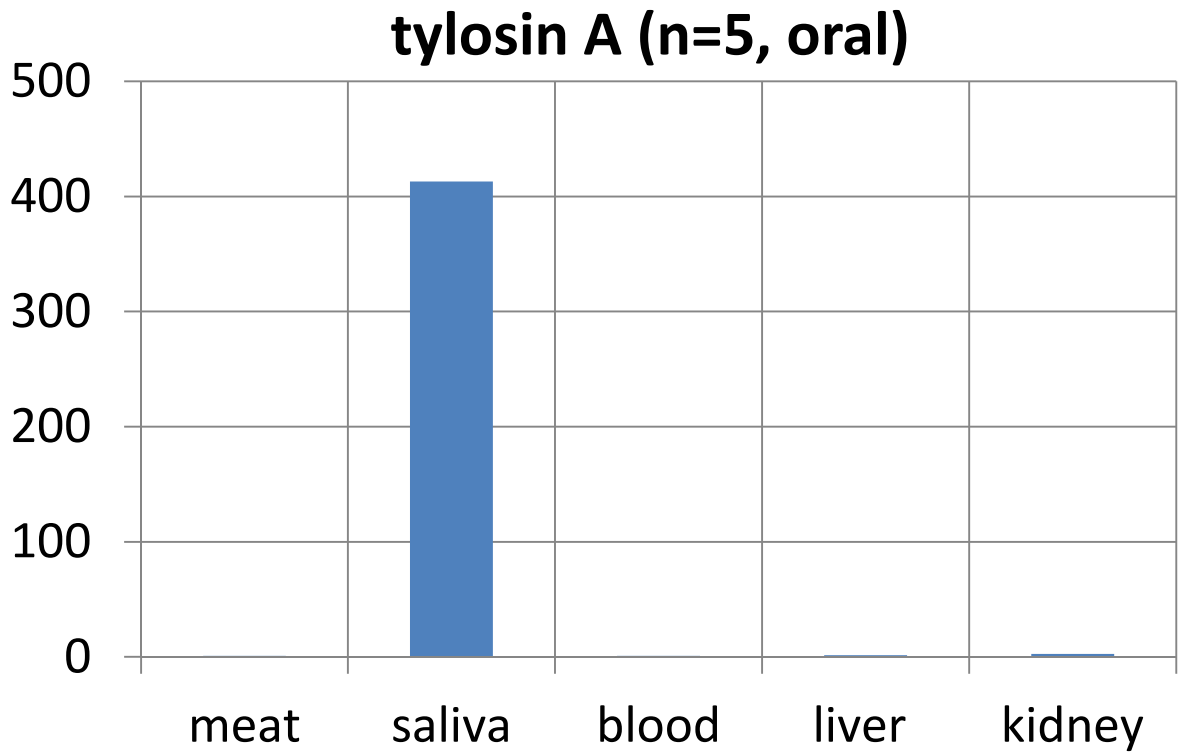
Concentration in meat *versus* concentration in saliva and other matrices: results for amoxicillin

	meat	saliva	blood	liver	kidney
mean conc. (ppb)	28	0	0	0	247
MRL (ppb)	50	--	--	50	50
ratio (matrix : meat)	1.0	--	--	--	9.2



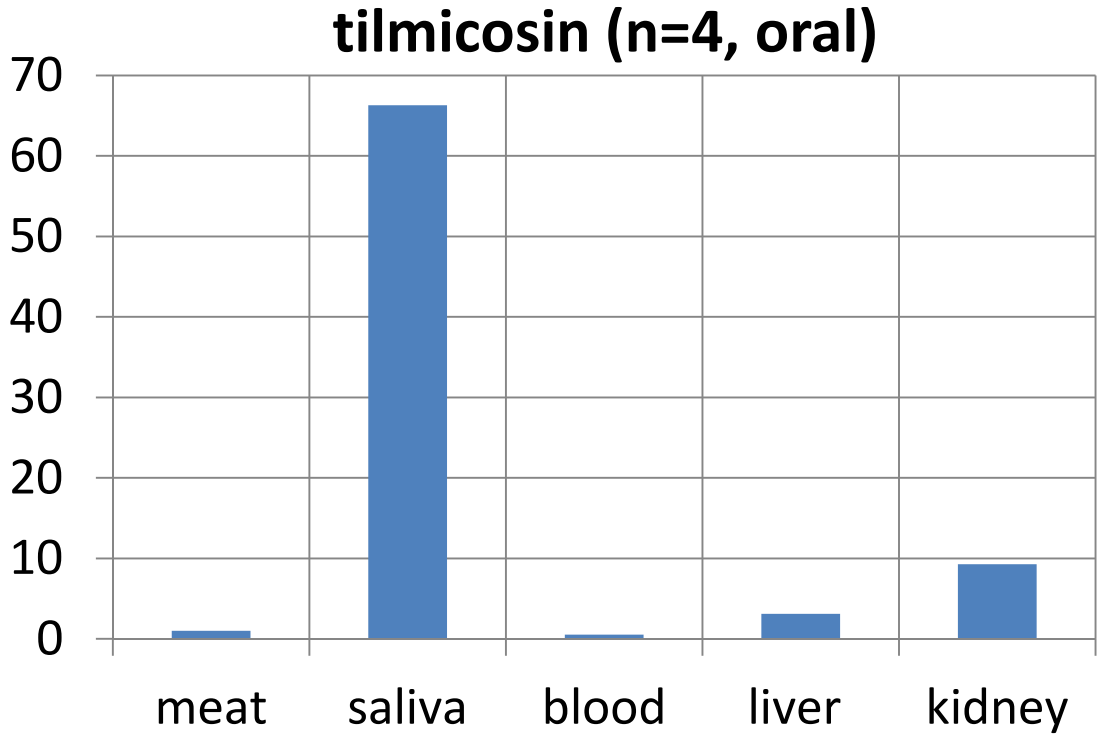
Concentration in meat *versus* concentration in saliva and other matrices: results for tylosin A

	meat	saliva	blood	liver	kidney
mean conc. (ppb)	19	3064	19	45	110
MRL (ppb)	100	--	--	100	100
ratio (matrix : meat)	1.0	412.8	0.9	1.5	2.6



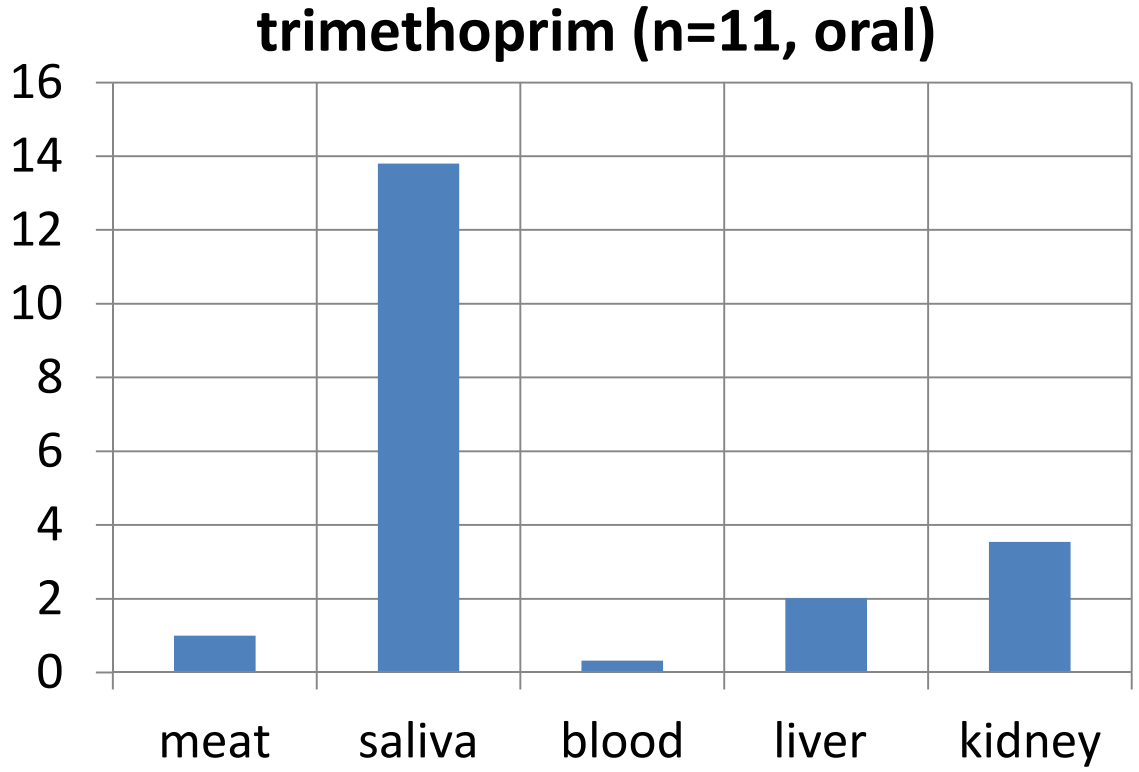
Concentration in meat *versus* concentration in saliva and other matrices: results for tilmicosin

	meat	saliva	blood	liver	kidney
mean conc. (ppb)	108	6887	53	359	988
MRL (ppb)	50	--	--	1000	1000
ratio (matrix : meat)	1.0	66.3	0.5	3.1	9.3



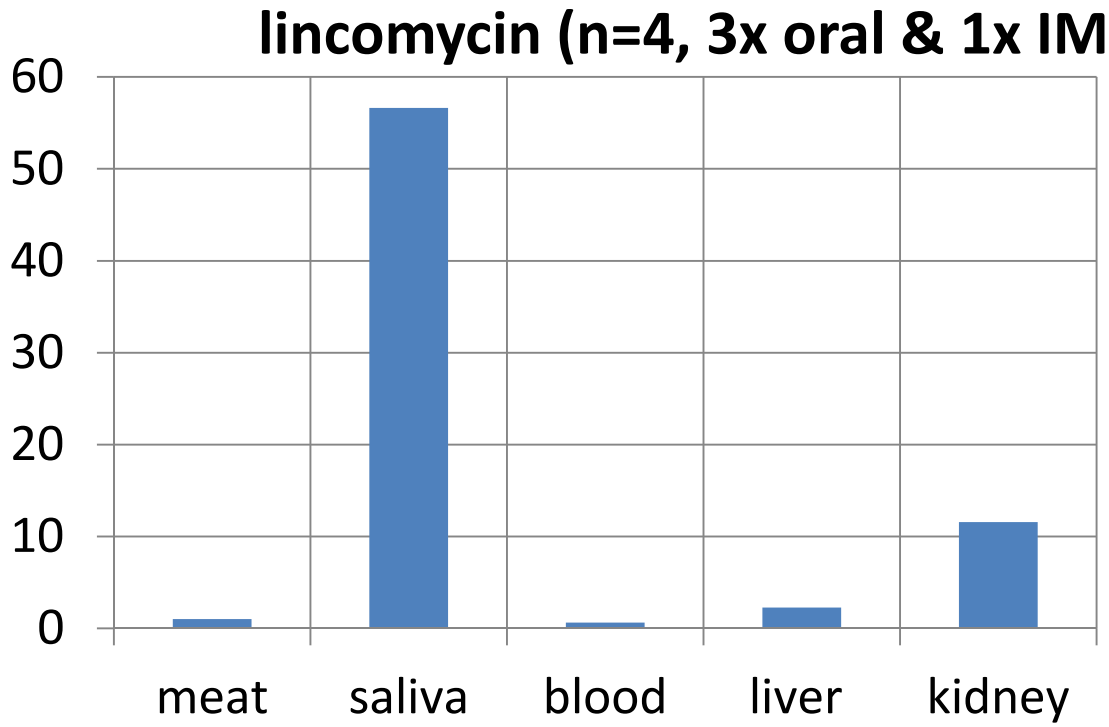
Concentration in meat *versus* concentration in saliva and other matrices: results for trimethoprim

	meat	saliva	blood	liver	kidney
mean conc. (ppb)	183	260	121	583	759
MRL (ppb)	50	--	--	50	50
ratio (matrix : meat)	1.0	13.8	0.3	2.0	3.5



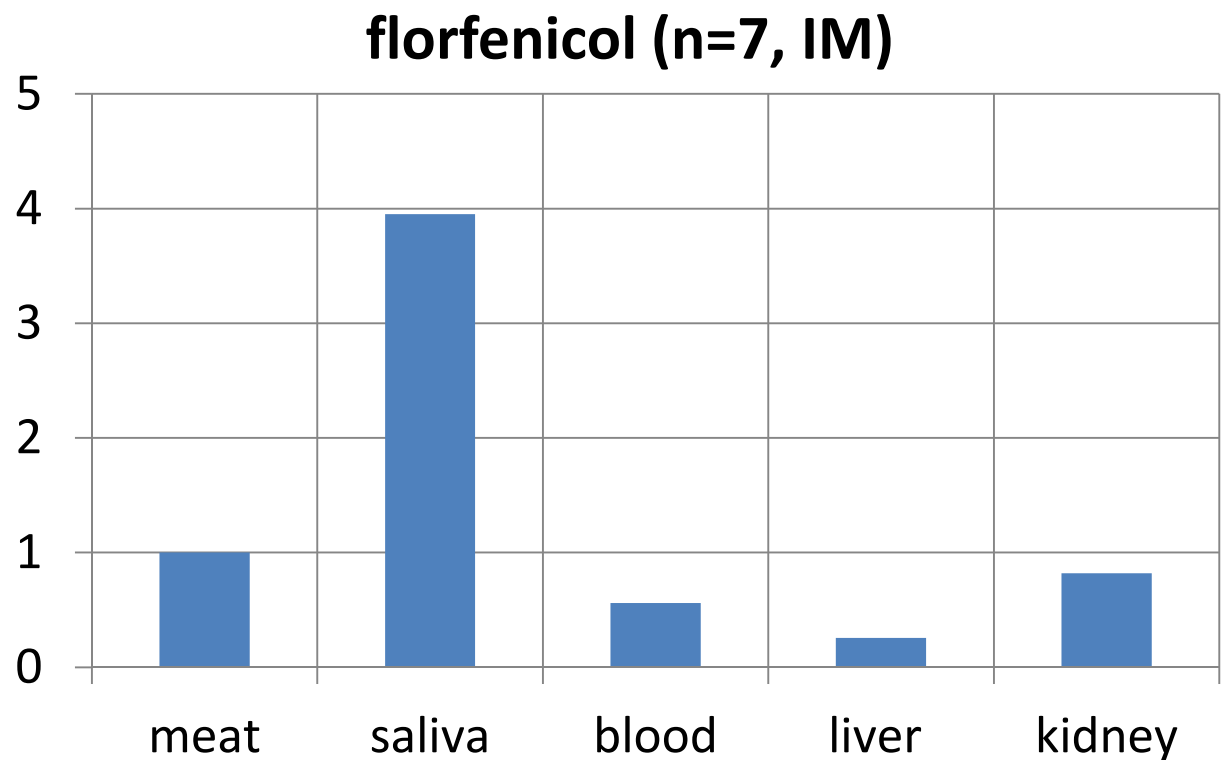
Concentration in meat *versus* concentration in saliva and other matrices: results for lincomycin

	meat	saliva	blood	liver	kidney
mean conc. (ppb)	160	1554	152	196	1238
MRL (ppb)	100	--	--	500	1500
ratio (matrix : meat)	1.0	56.6	0.6	2.3	11.6



Concentration in meat *versus* concentration in saliva and other matrices: results for florfenicol

	meat	saliva	blood	liver	kidney
mean conc. (ppb)	1628	2219	2216	662	2252
MRL (ppb)	300	--	--	2000	500
ratio (matrix : meat)	1.0	4.0	0.6	0.3	0.8



Sampling at 23 farms (26 compartments) with pigs under antimicrobial treatment: summary

Oral treatment: concentration in the saliva >>> in the meat

Only in two cases of IM (florfenicol and lincomycin):
concentration in the meat > concentration in the saliva

No amoxicillin found in the saliva, blood and liver

Very large variations due to interfering factors

- quantity and the time of consumption
- resorption
- size and health status of the animal, …

Again many cross-contaminations, even the presence of antibiotics (e.g. thiamphenicol) claimed to be never used on the farm

End conclusions

The testing of saliva is feasible for *ante*-mortem monitoring of pigs on antimicrobial residues

Such an approach would result in a low rate of positive meat samples

Thank you

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