



Contribution of mass analytical methods to identification of antibiotic residues in meat – Application to antibiotic control in France



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OUTLINE

Strategy of control of antibiotics in meat

Analysis of antibiotics

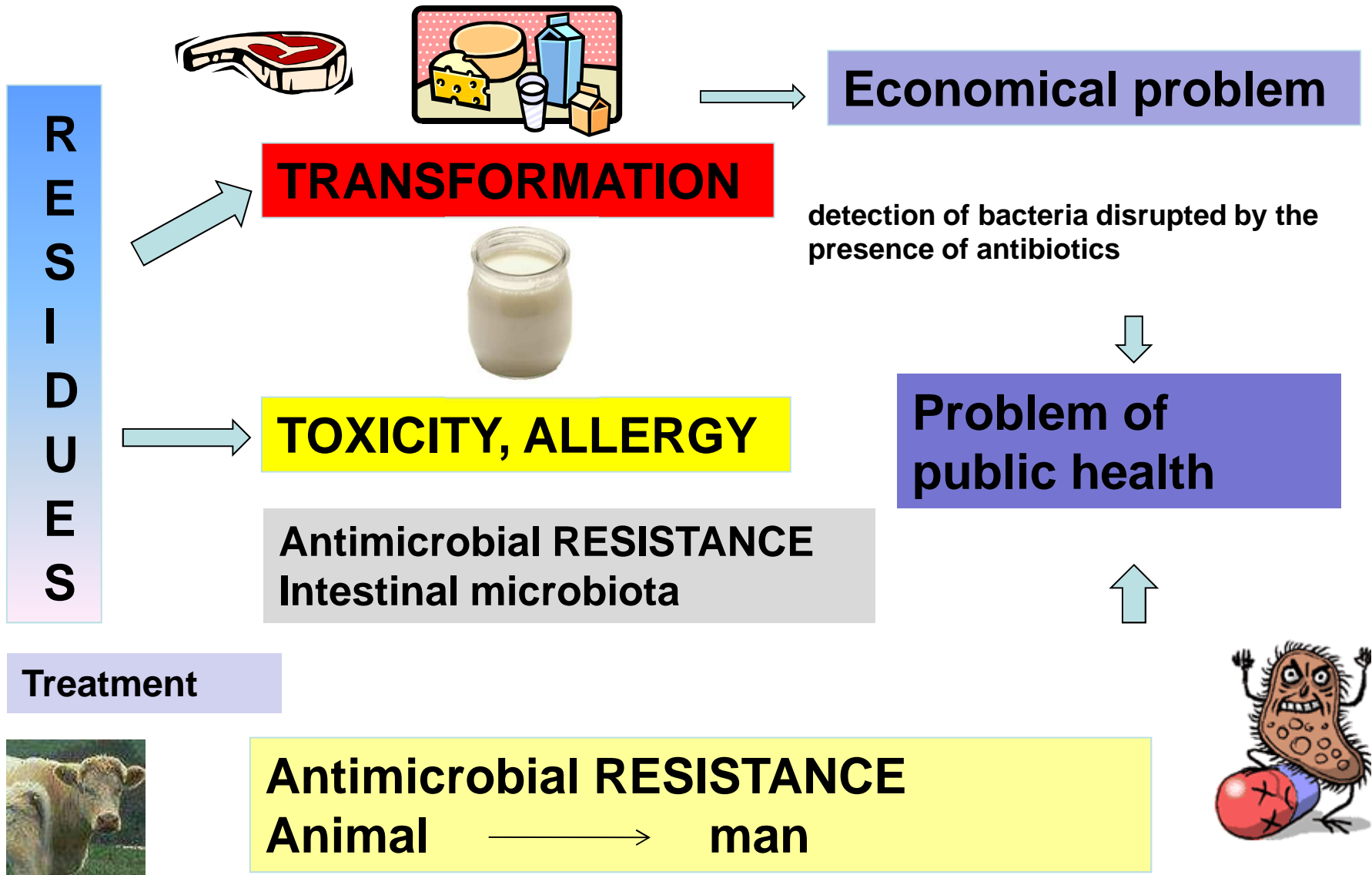
Results : overview 2011- 2014

Comparison inter-species

Link with actions initiated to decrease antibiotic consumption

Conclusion

Use of antibiotics : what risks ?

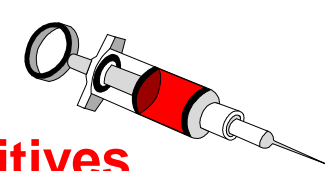


Regulatory framework in the EU

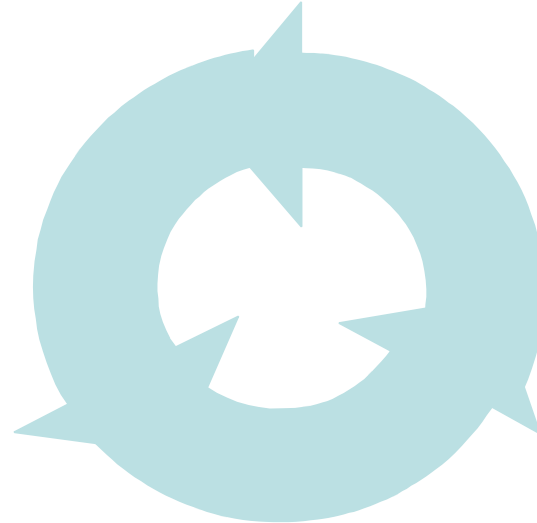
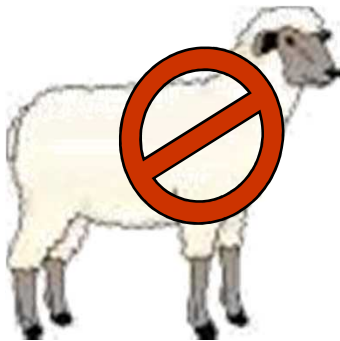
Additives

R.1831/2003

2006 : **Ban of antibiotic additives**



Ban of growth promoters
96/22/CE

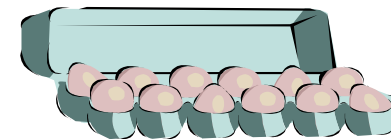
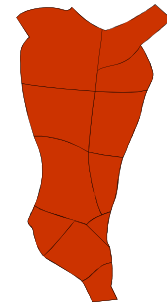


Use of veterinary drugs
2001/82/CE

MRL

R.470/2009

R.37/2010



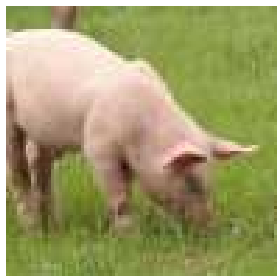
Veterinary drug residue monitoring

Directive 96/23/CE

Food Law 178/2002

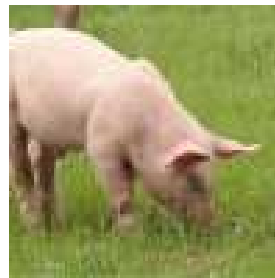
Food monitoring

882/2004



French analytical strategy used for control of antibiotics in meat

Authorised substances
2011 - 2014



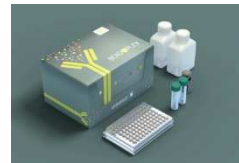
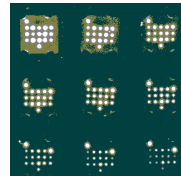
Strategies of control of antibiotics in meat

Screening step

- Effect based screening
 - Inhibition of growth
 - Premitest, Explorer
 - 4 Plate test



- Biosensors
 - Chemiluminescence
 - Bead-based flow cytometric IA



No identification of each analyte



- Cheap, quick
- Sensitive (< 5 % of false negative results)
- Wide spectrum detection depending on the target analytes
- High throughput of samples

- Chemical methods

LC-MS/MS

- Accurate analysis
- More expensive
- Capacity of detection



Identification of each analyte and metabolites



Analytical method

Extraction : 2 procedures

TCA 5%

Aminoglycosides (8)
Tetracyclines (4)
Quinolones (10)
Lincosamide (1)

47 transitions

ACN

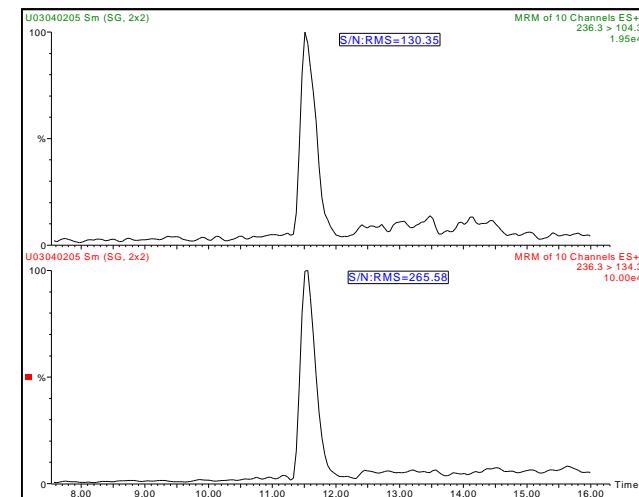
Penicillins +
Cephalosporines (14)
Macrolides (6)
Sulphonamides (9)

59 transitions

Chromatography : 1 system (API 4000)

ESI +

PFPA 0,1 % /ACN



Benefits : specificity, unequivocal identification, quickness of the qualitative analysis

Drawbacks : possible matrix effects, need of appropriate internal standard for quantification with specific methods

Analytical method



- ✓ Identification by LC/MS-MS following **2 transitions MRM** for each analyte (46 + 70) + retention time.
- ✓ Use of an internal standard similar for the two extractions : **sulfaphenazole**
- ✓ Use of incurred samples for the Quality Control (QC) to provide reliable results
- ✓ Network of laboratories use different brands of LC/MS/MS : Agilent 6460, Sciex API 3000, API 3200 Qtrap, API 4000 (2), API 4000 Qtrap (2), Thermo Ultra and Waters Xevo TQ MS



Control of antibiotics in the EU

- In the European Union : targeted samples
- In France :
 - Main targeting criteria for control of bovines
 - Calves and young cattle : infiltration of the collar
 - Cull cows : medium visual state of the carcass
 - Lactating cows: information package on food chain (FCI)
 - Other criteria : myositis, pleurisy, injuries, pneumonia,...
 - and sometimes, withdrawal of the carcass

Results of targeted control antibiotics

2011 - 2014

Results of targeted control - antibiotics

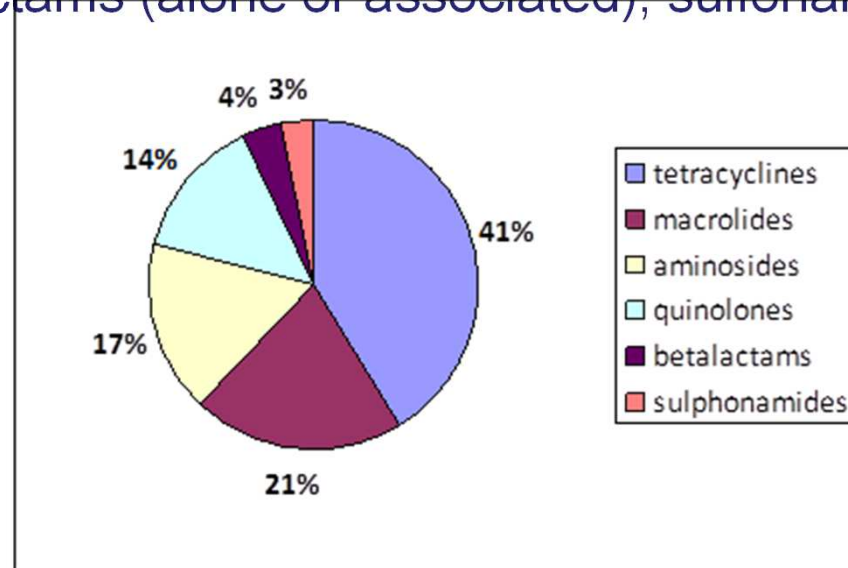
Year	Non-compliant rates (%) CL-SM/SM screening			Non-compliant rates (%) Microbiological screening		
	bovine	pig	ovine	bovine	pig	ovine
2014	1.61 <i>497</i>	0.41 <i>484</i>	0.80 <i>497</i>	0.26 <i>1135</i>	0.13 <i>794</i>	0 <i>42</i>
2013	1.71 <i>293</i>	/	0.72 <i>279</i>	0.90 <i>1891</i>	0.16 <i>1921</i>	0.5 <i>200</i>
2012	3.33 <i>299</i>	0 <i>299</i>	/	0.35 <i>2128</i>	0.10 <i>1943</i>	0.4 <i>750</i>
2011	1.36 <i>295</i>	0 <i>283</i>	/	0.93 <i>2146</i>	0.10 <i>2020</i>	/
Mean	2.00 <i>1384</i>	/ <i>1066</i>	0.76 <i>776</i>	0.61 <i>7300</i>	0.12 <i>6678</i>	0.40 <i>992</i>

non compliant samples : 2 to 3 times higher after screening by LC-MS/MS than after microbiological method (4 plate test)

Results of targeted control - antibiotics

Screening and confirmation of muscles by LC-MS/MS 2011-2014

- Bovine : 1387 **2.0 %** non-compliant samples
 - tetracyclines (alone or associated) **0.87%**
 - aminoglycosides (alone or associated) **0.36 %**
 - macrolides **0.43 %**
 - beta-lactams (alone or associated), sulfonamides, quinolones **0,29 %**



- Veals and reform cows
 - Calves and young cattle : infiltration of the collar (50 %)
 - Reform cows : medium visual state of the carcass (42 %)

Results of targeted control - antibiotics

- Non-compliant samples for pigs

- sulphonamides
- treatments by the oral route (mainly by drinking water)



- Non-compliant samples for sheep and goats

- aminoglycosides, oxytetracycline, sulphonamides
- extra-label uses frequently described in this sector



- Comparison inter-species

- Non-compliance rates in beef sector significantly higher (Khi 2 test) than in the other sectors
 - ✓ Bovine : 2%
 - ✓ Sheep and goats : 0.8 %
 - ✓ Pig : 0.4 %



- Efforts have to be made to reduce antibiotic administration in calves and to respect the dose and withdrawal times in cattle

Link to decrease of antibiotic consumption

Need for reduction of antimicrobial use

A « consumption » of antibiotics in France smaller than the european mean

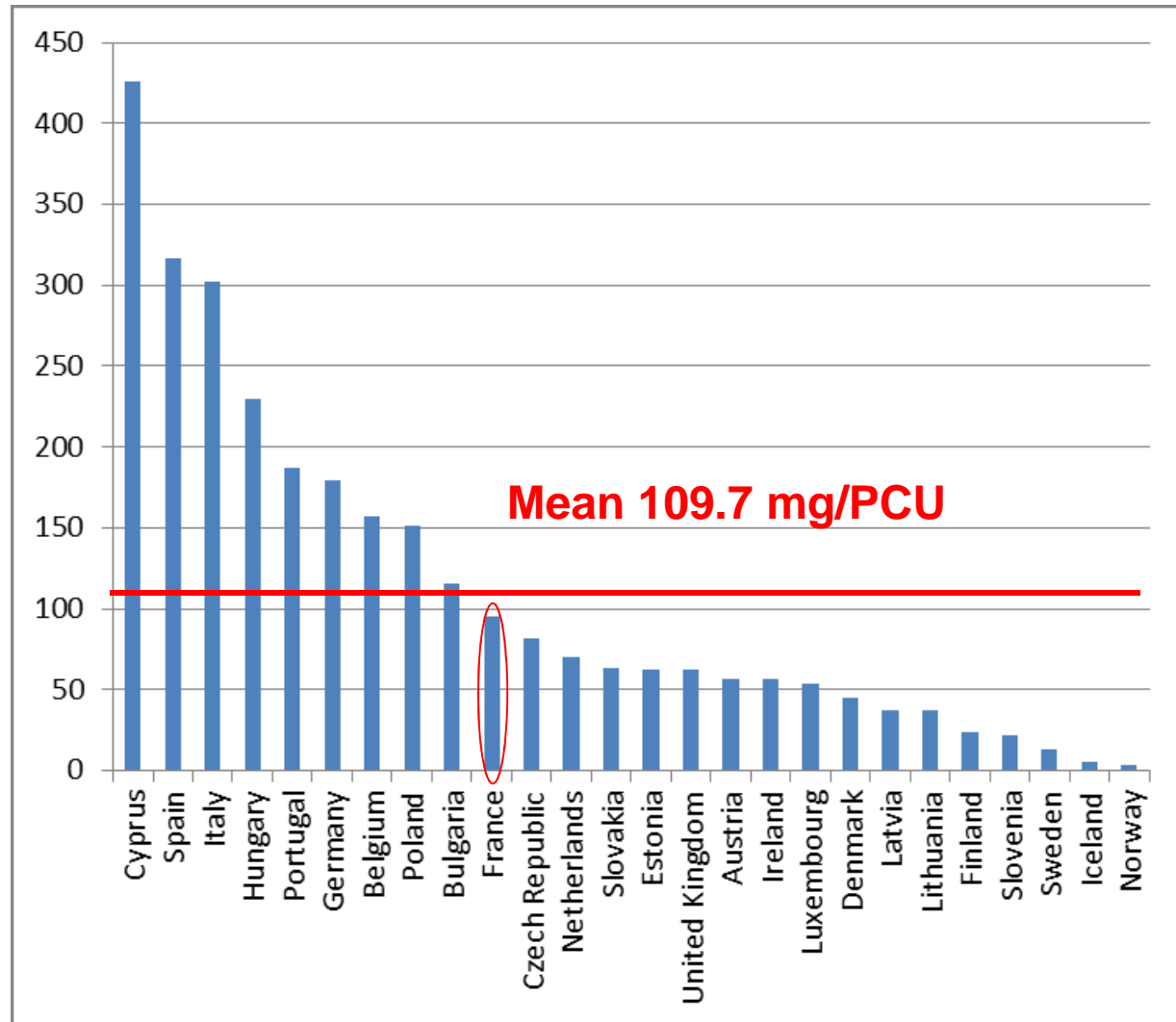
EMA 2015



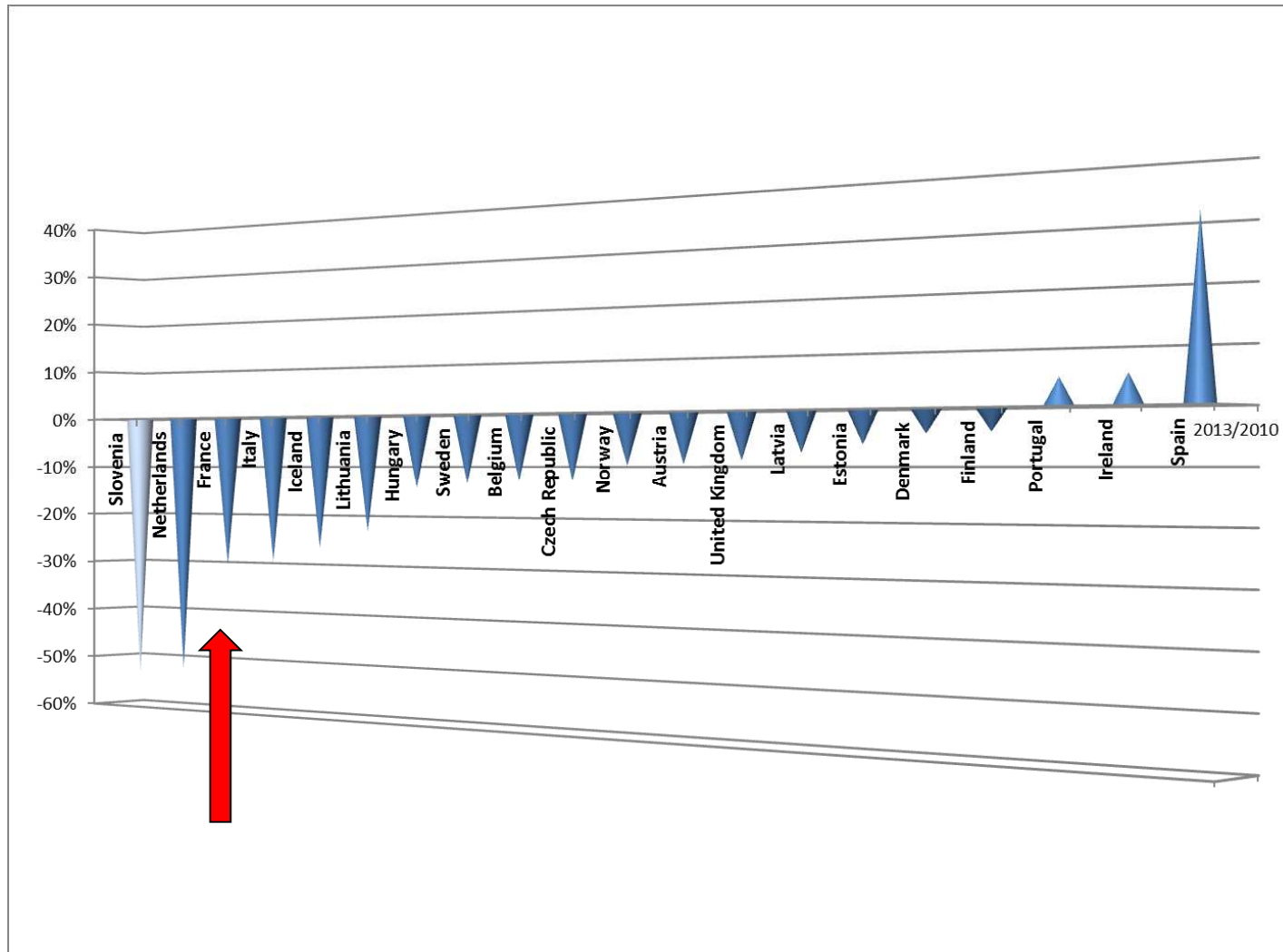
EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

Sales of veterinary antimicrobial agents in 26 EU/EEA countries in 2013
Fifth ESVAC report

An agency of the European Union

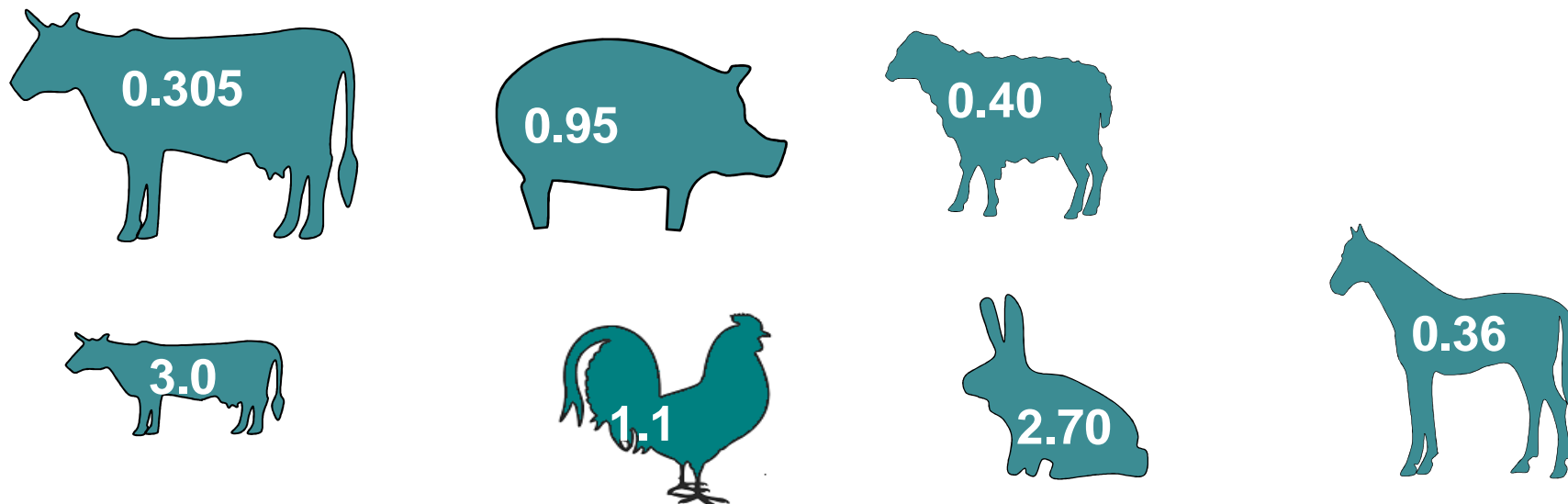


Percentage of decrease of antibiotic sales 2010 - 2013 (mg/PCU)



Exposure indicator by species in 2013

Exposure indicators (ALEA : animal level of exposure to antimicrobials)



- In progress : distinction to calves, poultry uses...
- Residues: depend of the exposition during the last part of breeding
- Animal sectors in which voluntary actions have been initiated show a greater fall in the consumption of antibiotics:
 - Pigs: Inaporc in 2010, Anses/IFIP survey in 2014—2015
 - Calves : Idele in 2014
- EcoAntibio 2017 : reducing use of antibiotics by 25 % in 5 years

Conclusion

Great interest of implementation of LC-MS/MS screening at or below the MRL level and to detect new use of authorised antibiotics (eg : macrolides in bovines)

Where will the future?

- New methods in 2016 to screen more antibiotics in aminoglycosides apart (80 molecules)
- Extension to fish and shrimp, rabbit,...



Better and more cost-effective control

Screening and quantification by full-scan HRMS (untargeted analyses) or CL/SM-SM

Quantification of antibiotics at level below the MRL

Exposition of man by antibiotic residues

Evolution of legislation to risk-based analyses

Revision of official control Regulation 882/2004 & directive

96/23/EC



THANKS



**Department in charge of agriculture
DDPP
Official laboratories (in charge of screening)
Laboratory of Fougères – ARC unit**



THANK you for your attention

THANK
YOU!

The text 'THANK YOU!' is rendered in a playful, hand-drawn style. 'THANK' is in blue, 'YOU!' is in green, and there are three flowers: a red one on the left, a purple one on the right, and a smaller orange one above the 'K'. The letters have a slightly textured, stippled appearance.