ROSS UNIVERSITY
SCHOOL OF VETERINARY MEDICINE

Salmonella, from farm to fork

Patrick Butaye
Ross University, School of Veterinary Medicine
Ghent University, Faculty of Veterinary Medicine

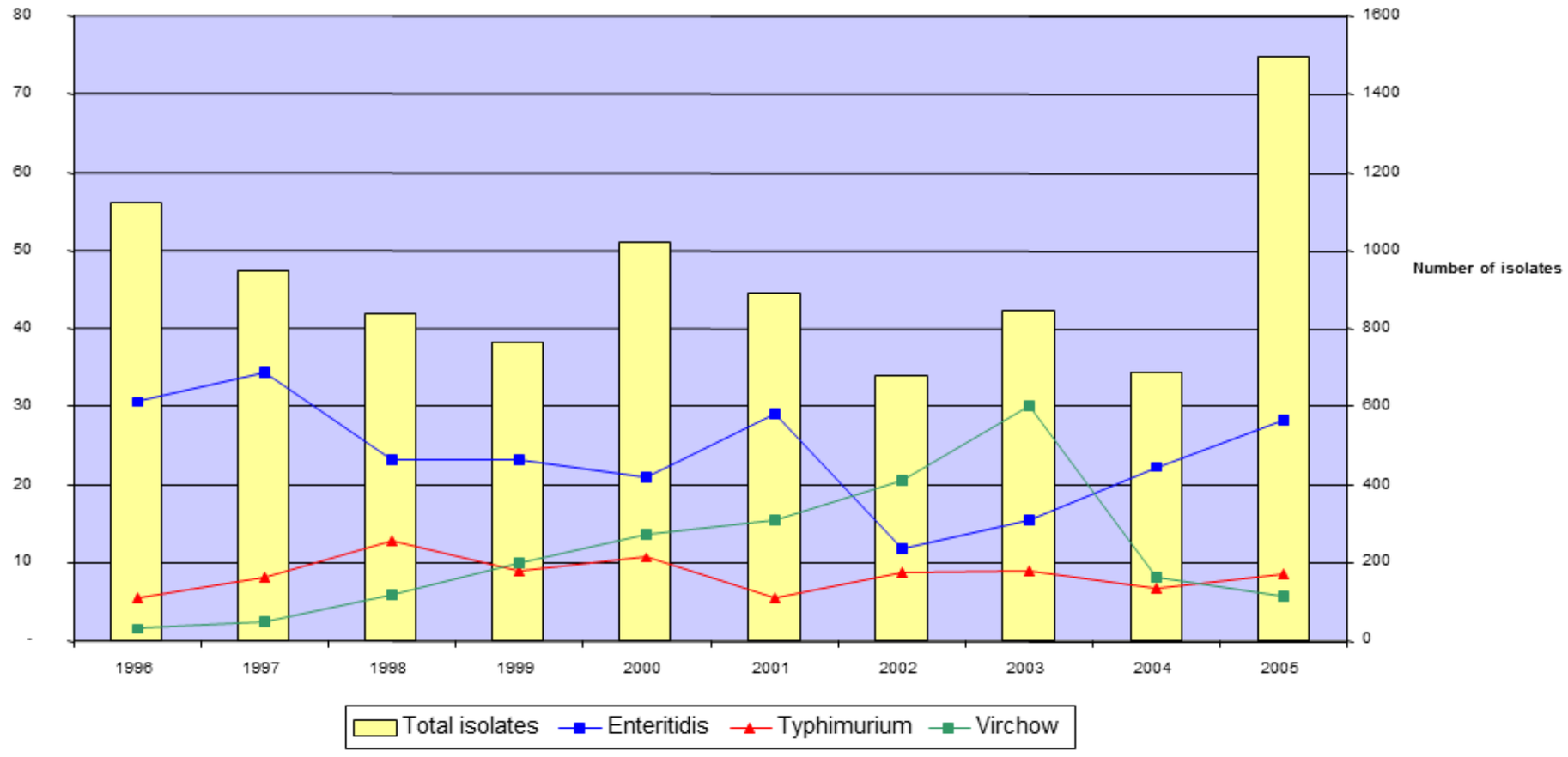
- Introduction
- Prevalence of *Salmonella* serotypes
- Antimicrobial resistance in *Salmonella*
- Epidemiology of *Salmonella*
- Conclusions

Introduction

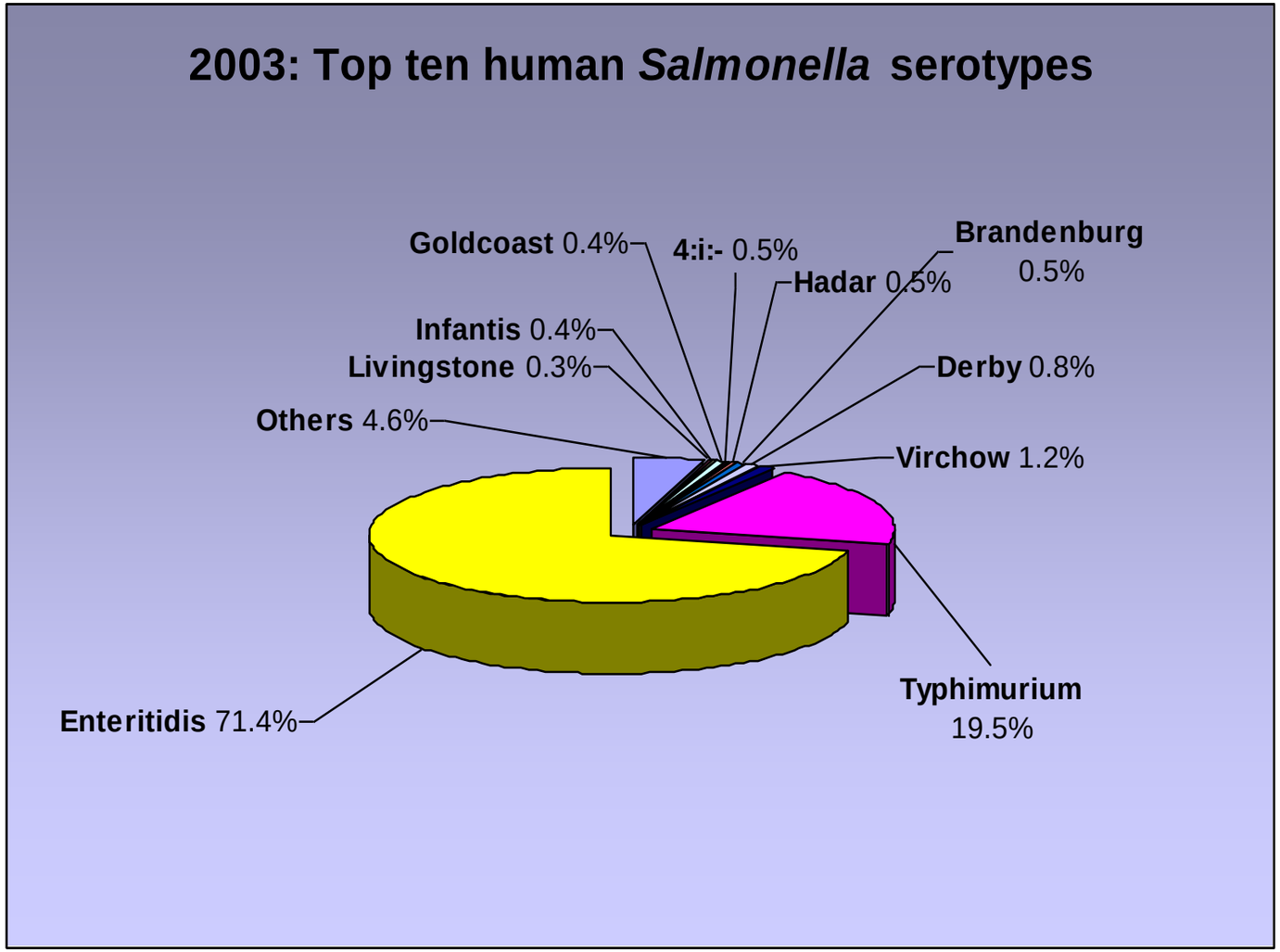
- Zoonotic/foodborne *Salmonella* belong to the species: *Salmonella enterica* subspecies *enterica*
- Over 2600 serotypes
- Some serotypes are of Zoonotic importance: Non-typhoid *Salmonella*
  - Not really host specific
  - Cause diarrhea and eventually paratyphoid fever
- Virulence: complex

Prevalence of *Salmonella* serotypes

Figure 1. Evolution of *Salmonella* serotypes among poultry



Prevalence of *Salmonella* serotypes



Prevalence of *Salmonella* serotypes

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Drastic decrease of *Salmonella* Enteritidis isolated from humans in Belgium in 2005, shift in phage types and influence on foodborne outbreaks

J. M. COLLARD[1]*, S. BERTRAND[1], K. DIERICK[2], C. GODARD[3], C. WILDEMAUWE[3], K. VERMEERSCH[4], J. DUCULOT[4], F. VAN IMMERSEEL[5], F. PASMANS[5], H. IMBERECHTS[6] AND C. QUINET[7]

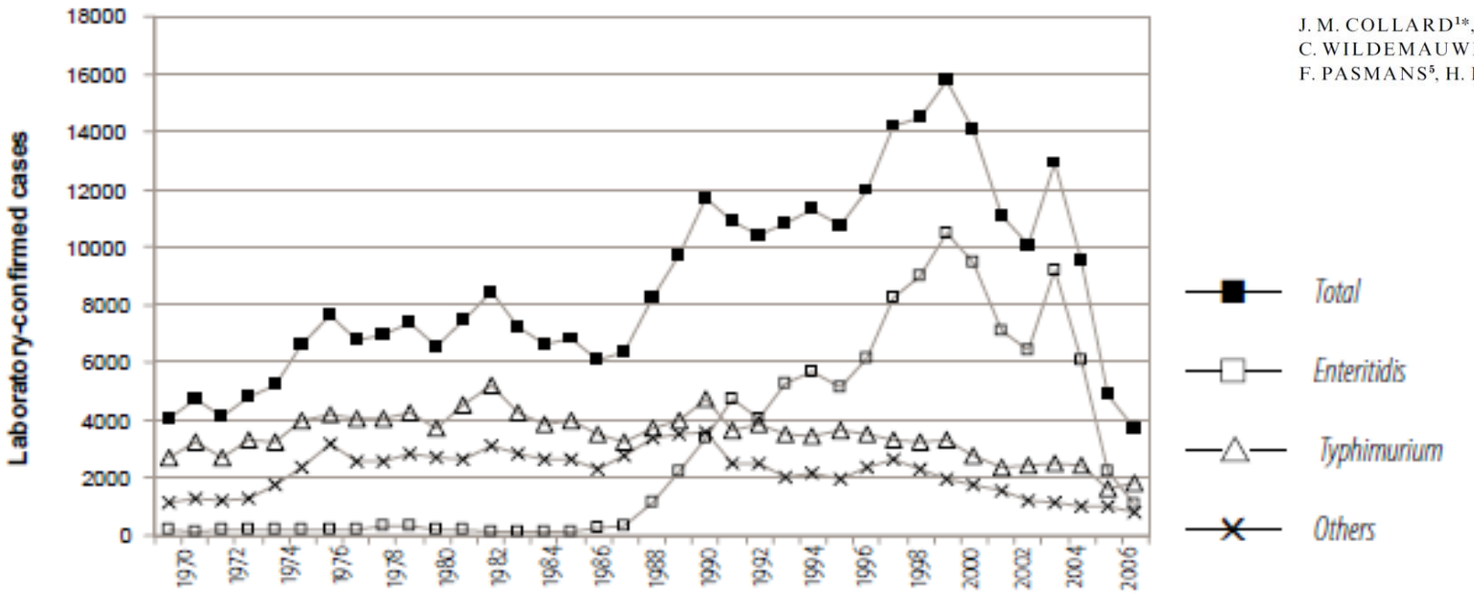


Figure 15. *Trend of the human Salmonella isolates and of the two major serotypes Enteritidis and Typhimurium over the last thirty six years in Belgium: number of laboratory confirmed cases*

Implementation of vaccination of layers against *S.* Enteriditis in 2004

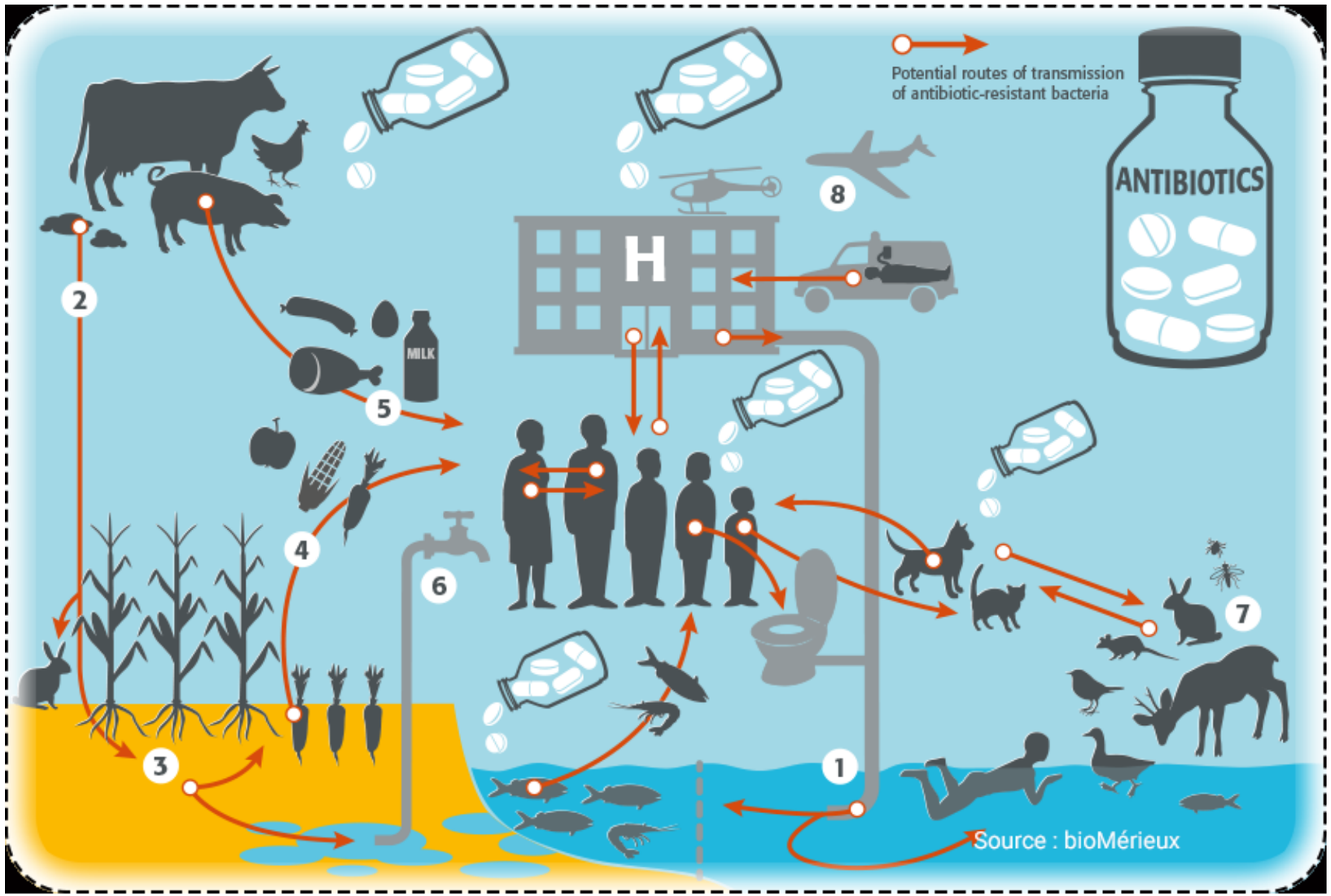
Prevalence of *Salmonella* serotypes

- Salmonella control programs (EU)
  - Focus on control in breeders
  - Initial hygiene and sanitation measures did not help
  - Vaccination: success
- Salmonella control (US)
  - Carcass decontamination with bleach (not allowed in EU)

Antimicrobial resistance in *Salmonella*

- Antibiotic resistance problem
  - Direct problem in *Salmonella*
    - Resistance is transferred directly from the animal to humans, causing a direct treatment problem (when Salmonella has to be treated)
  - Indirect resistance problem in *Salmonella*
    - Resistance is transferred from Salmonella to human bacteria and eventually to human pathogens
      - Transfer:
        - » Plasmids
        - » Transposons
        - » Genomic Islands
      - Transfer of multiple resistances at one time

Antimicrobial resistance transmission



Antimicrobial resistance in *Salmonella*

- *Salmonella* and resistance seems to be quite heterogeneous
- Some serotypes (e.g. Enteritidis) seem little susceptible to acquire resistance
- Other serotypes seem to easily gather resistances
- The reason for this remains unclear
- AMR in *Salmonella* should be looked upon from a serotype specific way rather than all *Salmonella* together

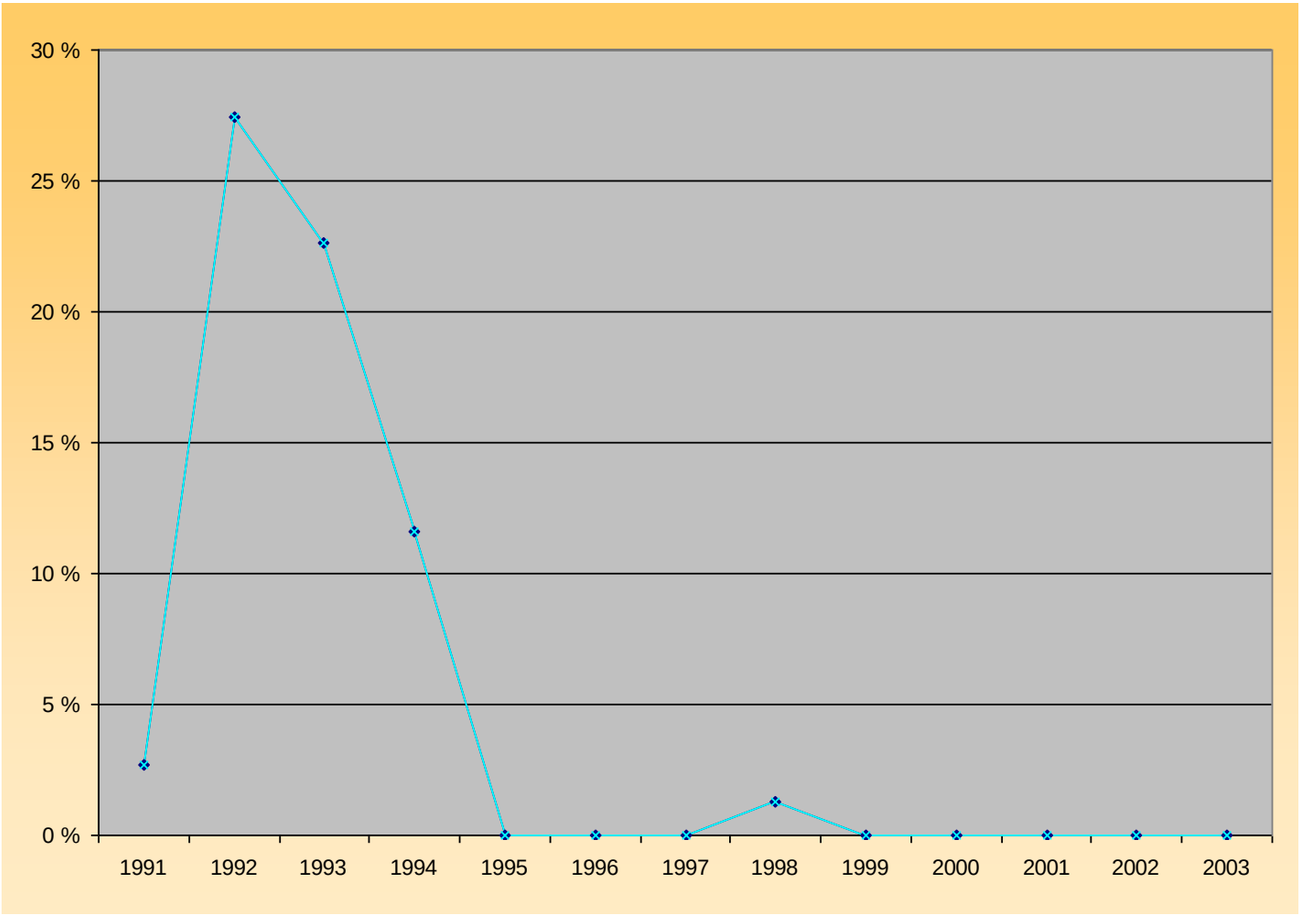
Epidemiology of *Salmonella*

- Serotype specific epidemiology
  - *S.* Enteriditis ≠ *S.* Typhimurium
- Need for Serotype specific investigations and needs inclusion of host pathogen interaction for full understanding
- Some episodes are characterized by the spread of a specific antimicrobial resistant serotype
- Methods for typing:
  - PFGE
  - Other
  - WGS

Fluoroquinolone resistant *S.* Typhimurium

- Fluoroquinolone resistance is rare or absent in most *Salmonella*.
- Some serotypes however are almost « naturally » resistant to quinolones (nalidixic acid): *S.* Virchow et *S.* Hadar.
- Nature of the resistance:
  - One mutation: resistance to quinolones/low level fluoroquinolone resistance
  - Two mutations: high level resistance to fluoroquinolones
  - Plasmid mediated Quinolone resistance (low level FQ resistance)
- Mostly mutation mediated: implicates that resistance spreads clonally (non-transferable resistance)
- Example from Belgium: From 1991 to 1995: « The rise and fall of fluoroquinolone resistant bovine *Salmonella* Typhimurium»

Fluoroquinolone resistant *S.* Typhimurium



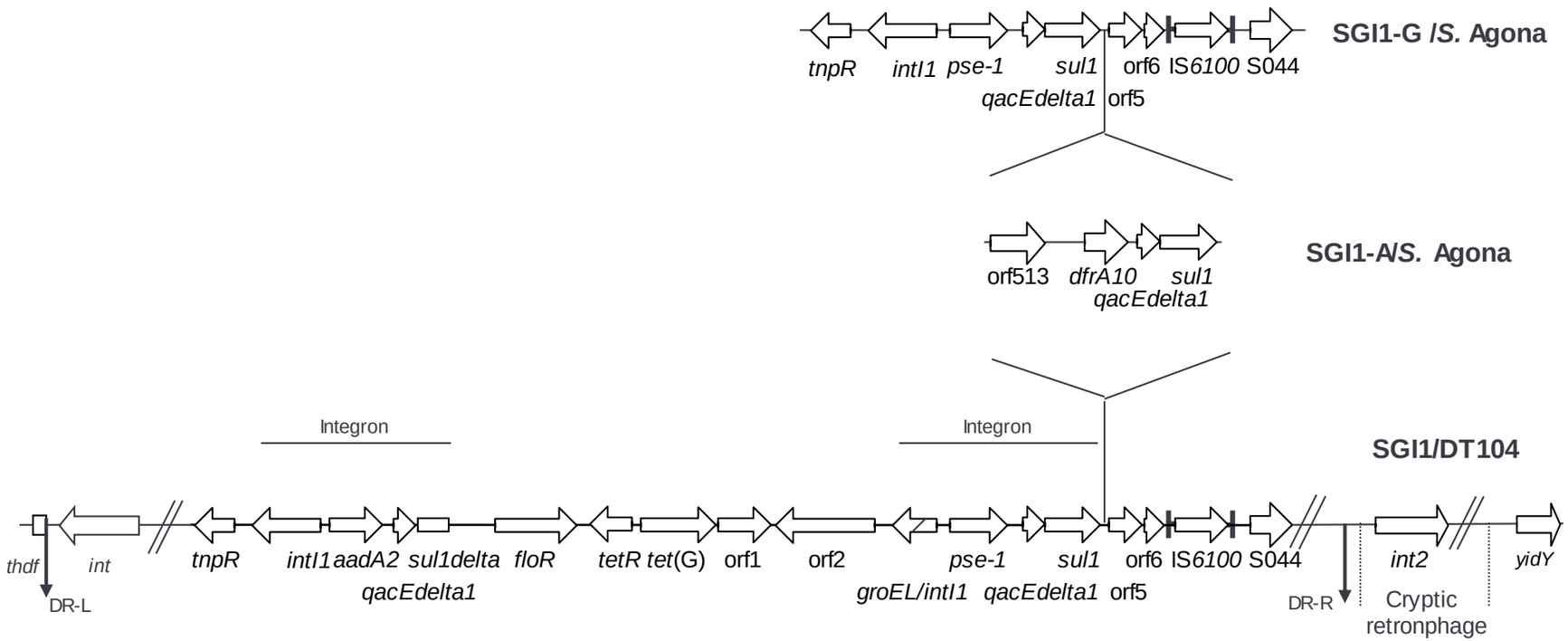
Multi-resistant *S.* Agona

- A serotype most frequently associated with poultry
- The resistance profile was similar to the worldwide spreading S. Typhimurium DT104: ApCmFfSmSpSuTc
- An more in depth study on
  - Phenotypic follow up
  - Resistance genes involved, localisation and mobility
  - Characterisation of the genetic environment of the resistance cluster
  - Characterisation of the genetic background

Multi-resistant *S.* Agona

- This multi-resistance is only detected in *S.* Agona from poultry.
- The resistance genes are localised in or between integrons on a genomic island on the chromosome.
- The complex is mobile.
- The resistance profile associated with this genomic island may differ (8 different have been found until now of which 5 in Agona). Several other subtypes (deletions and the right end) have also been described. This means that the SGI1 is a dynamic unity.
- The genomic island was also described in *S.* Typhimurium DT104, Paratyphi B, Albany,…

Multi-resistant *S.* Agona



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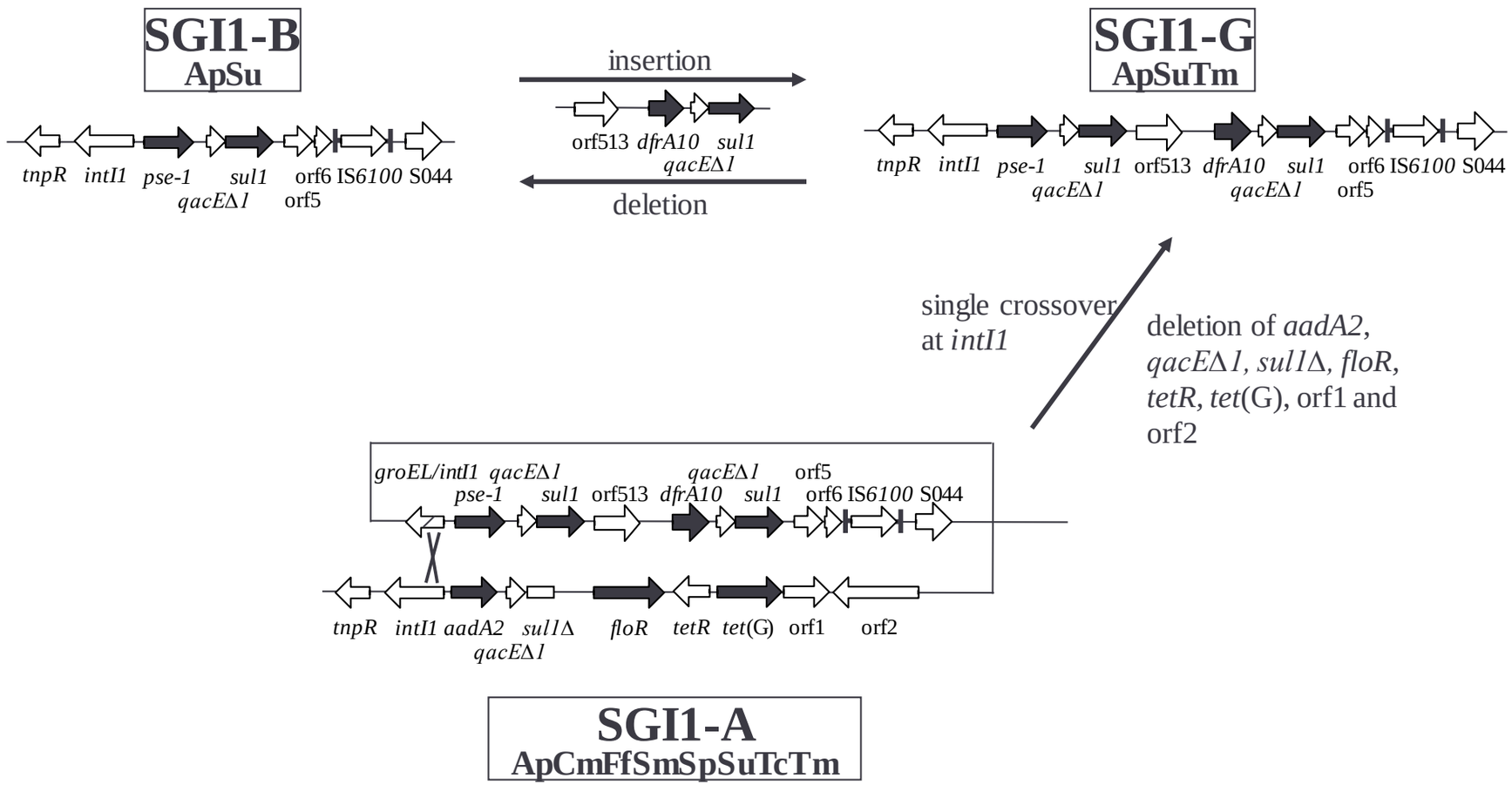

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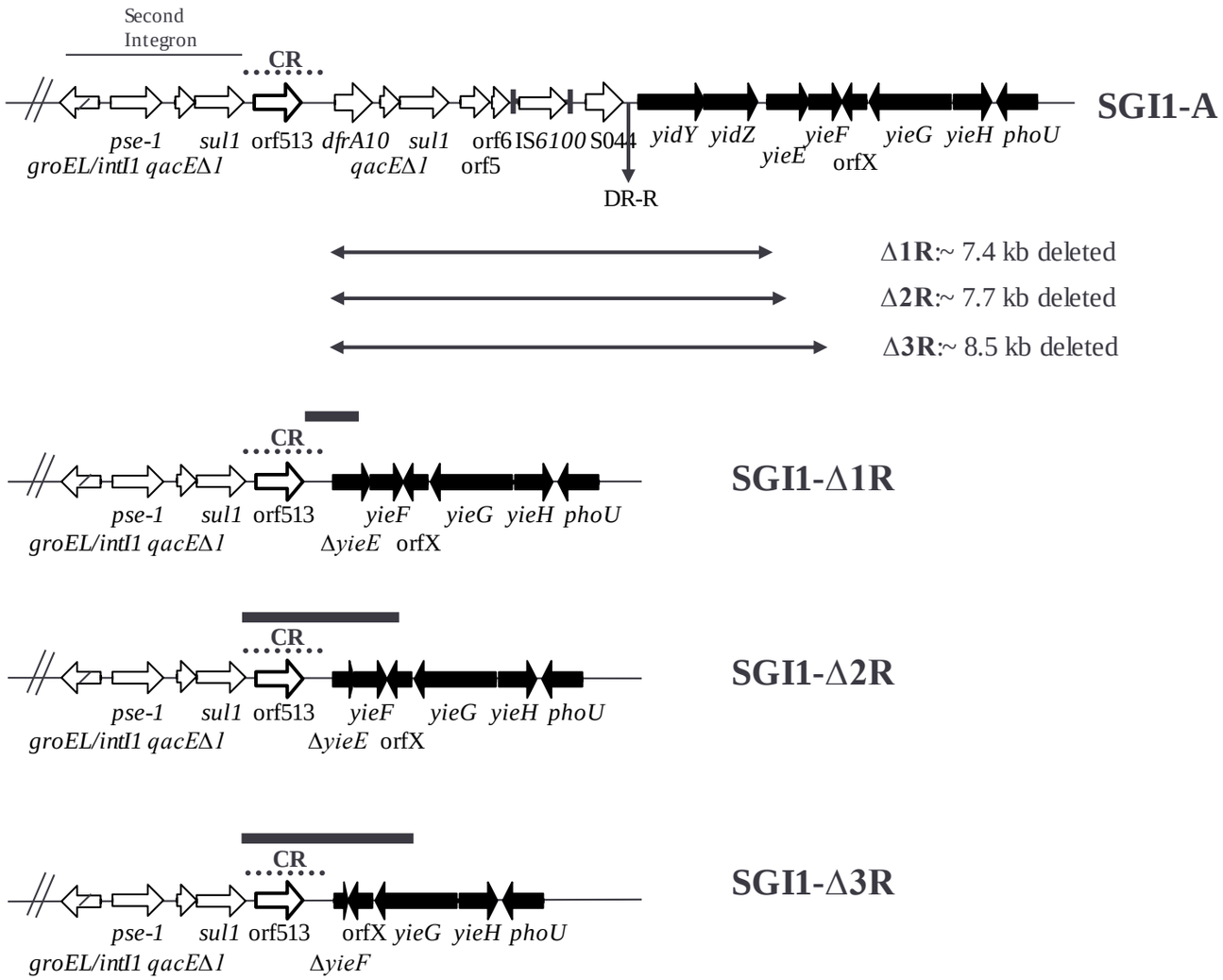
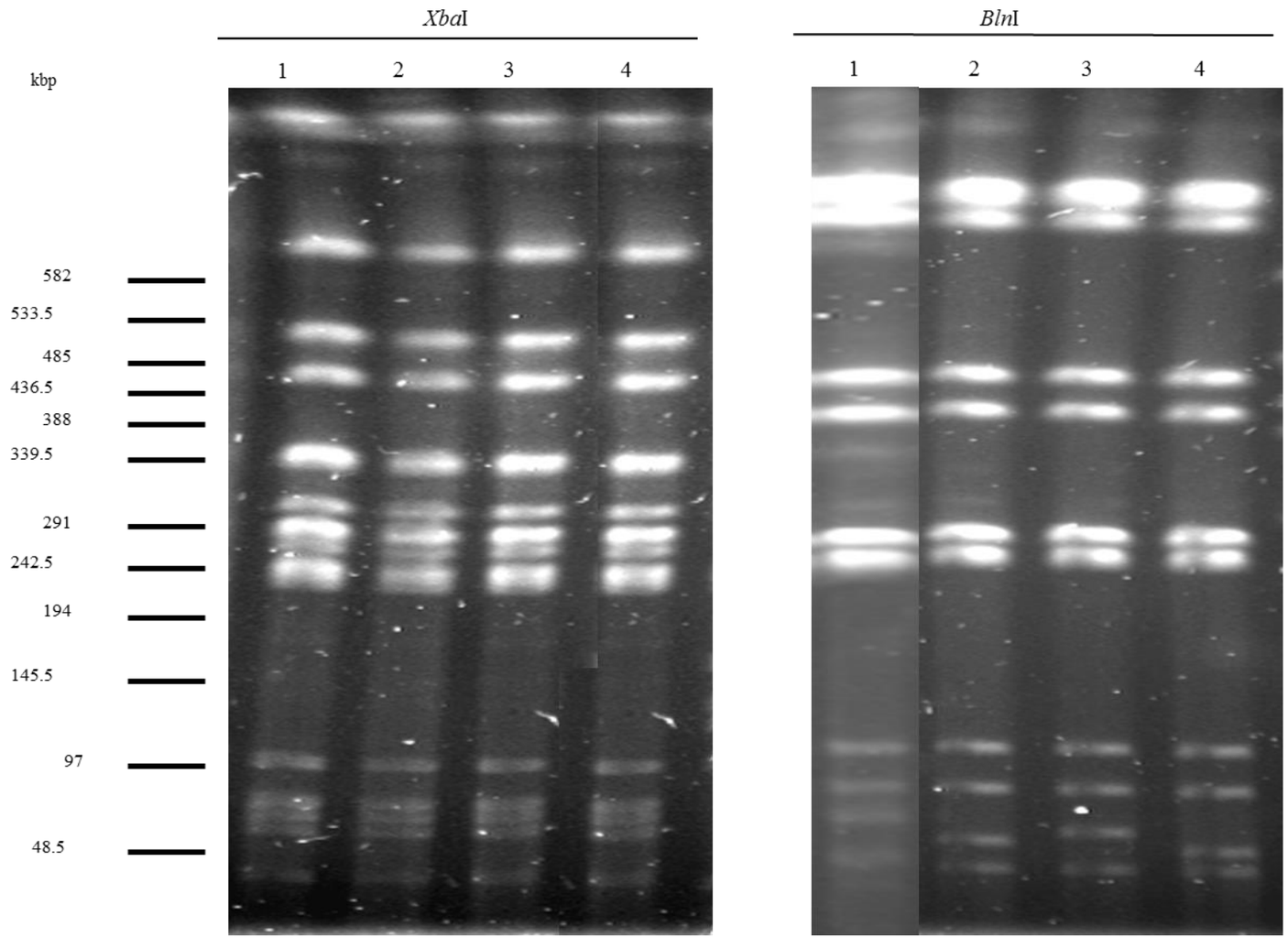


Multi-resistant *S.* Agona



Multi-resistant *S.* Agona

- This multi resistance is only detected in *S.* Agona from poultry.
- Characterisation of the strains (PFGE with XbaI and BlnI) showed that all SGI1 positive strains represented one clone
- This one multi resistant *S.* Agona clone circulates from 1992-2004

- Only once, an identical strain has been found back in a case of human diarrhoea

Multi-resistant *S.* Agona

- One strain that underwent modifications in its major resistance region was circulating for more than 10 years in Belgium
- This strain has most probably not disappeard, though the resistance in Agona has diminished
- Why?

ESBls in *S.* Virchow

- ESBL: extend spectrum β-lactamase
  - Resistance against third generation cephalosporines (ceftiofur)
- At a certain moment the prevalence of *S.* Virchow increased dramatically in poultry
- Associated with a new antimicrobial resistance fenotype:

  ACefTTsuNal
- It seemed that an ESBL had inserted in the fenotype:

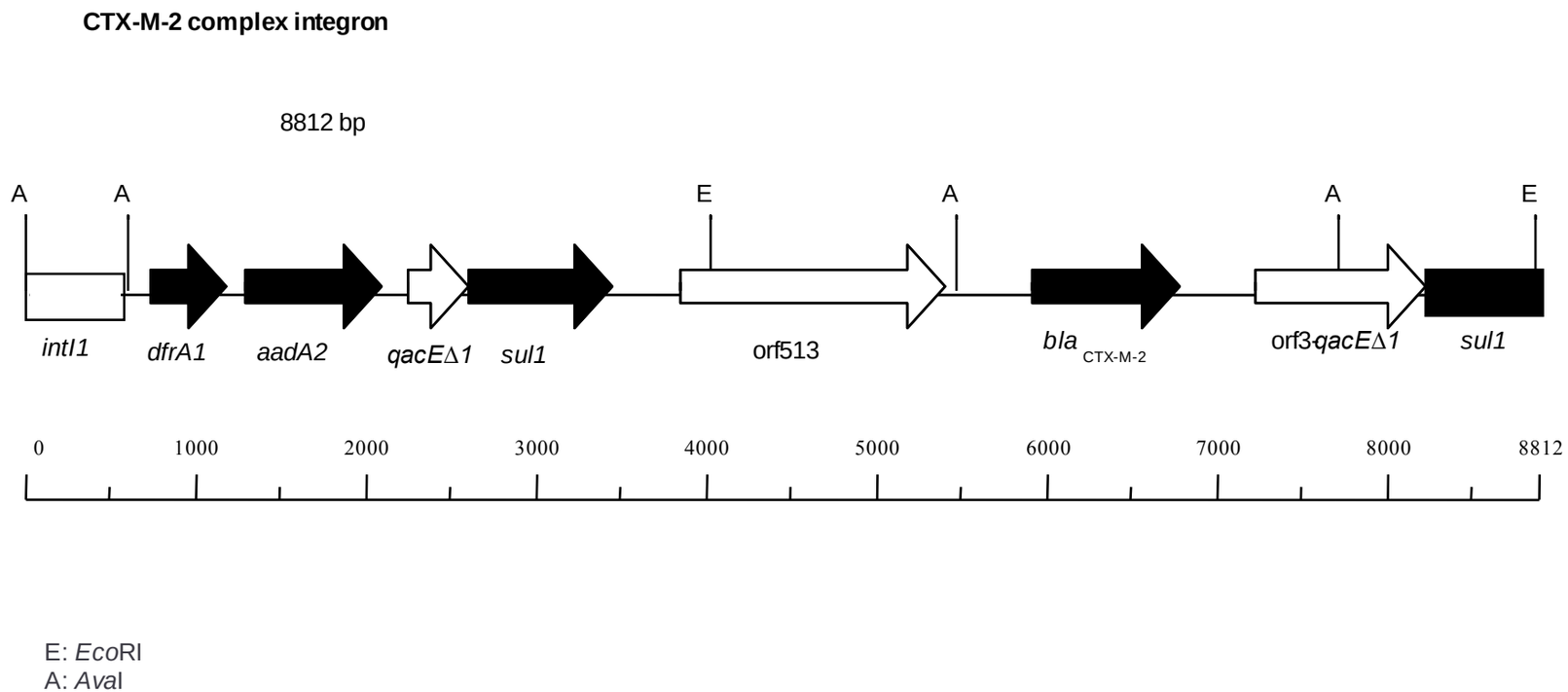
  ATTsuNal

CETRX
AM+CL
AZTRM
CFEPM
CEZDI

ESBIs in *S.* Virchow

- Transferable resistance
- Plasmid localisation
- Localisation in a complex integron
- ESBL: CTX-M2

ESBIs in *S.* Virchow

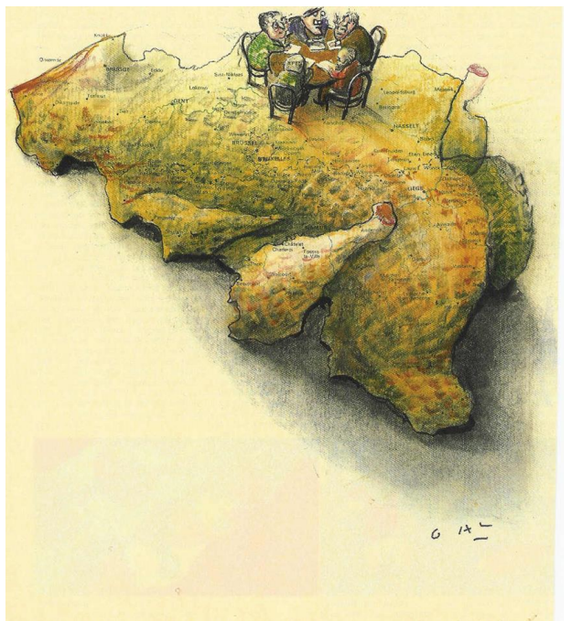


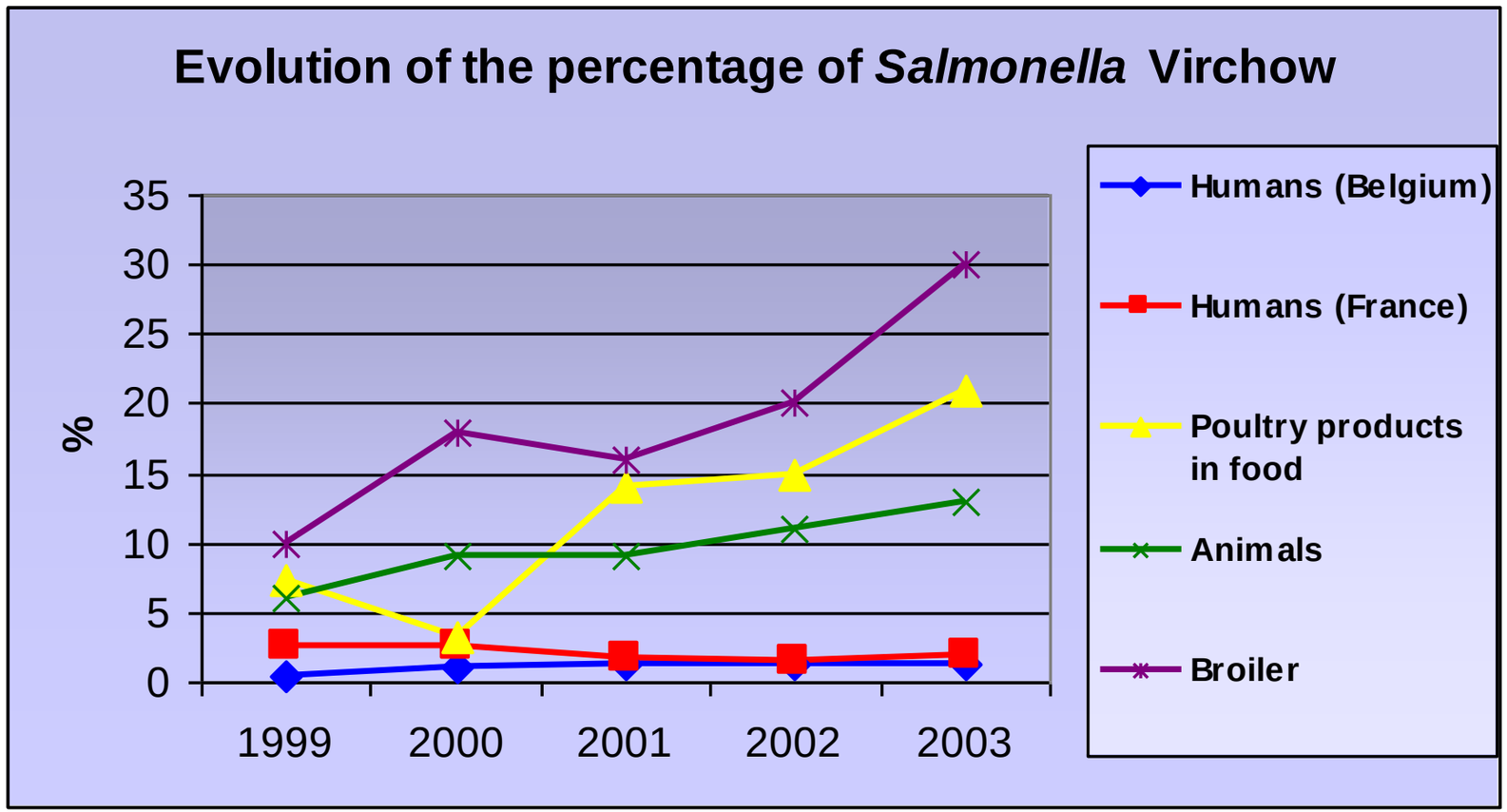
E: *Eco*RI
A: *Ava*I

ORF513: Insertion sequence

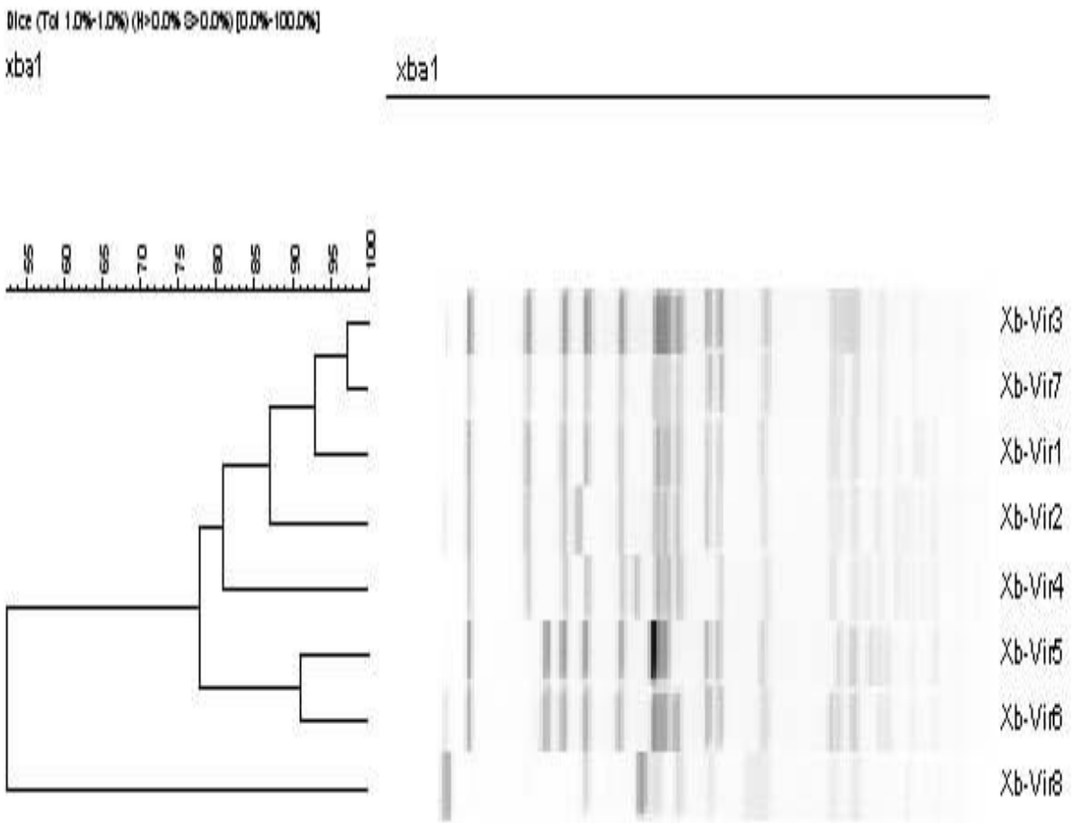
ESBIs in *S.* Virchow

- Several human cases were reported, geographically clustered





ESBIs in *S.* Virchow



PFGE Type	N	Phage Type	*bla* gene
Xb-Vir3	1	PT34	*bla* $_{tem}$
Xb-Vir7	1	NT	NT
Xb-Vir1	31	PT4, PT37	*ctx-m2* and *tem-1*
Xb-Vir2	1	PT31	*ctx-m2* and *tem-1*
Xb-Vir4	1	PT4	*ctx-m2* and *tem-1*
Xb-Vir5	1	PT26	*ctx-m3* and *tem-1*
Xb-Vir6	1	NT	NT
Xb-Vir8	1	NT	NT

A single clone was circulating

Clone has disappeared

Molecular epidemiology of cephalosporin resistant *S.* Infantis

- In 2004 an new episode of resistance came: *S.* Infantis.
- In 2003: no cephalosporin resistance was detected. Only 2 strains out of 49 were resistant to Amp-Str-tet
- Third most isolated from humans in 2004

Molecular epidemiology of cephalosporin resistant *S.* Infantis

Resistance profile	N strains	% R strains
Susceptible	82	55,4
AP	1	0,7
AP CEF	46	31,1
AP CEF CM NE SU TSU	1	0,7
AP CEF NAL	2	1,4
AP CEF SU	1	0,7
AP CEF TC	1	0,7
AP NAL SU TC TSU	2	1,4
AP ST SU TSU	2	1,4
AP SU	1	0,7
CM	1	0,7
CM ST SU TSU	1	0,7
CM SU TSU	5	3,4
NE	1	0,7

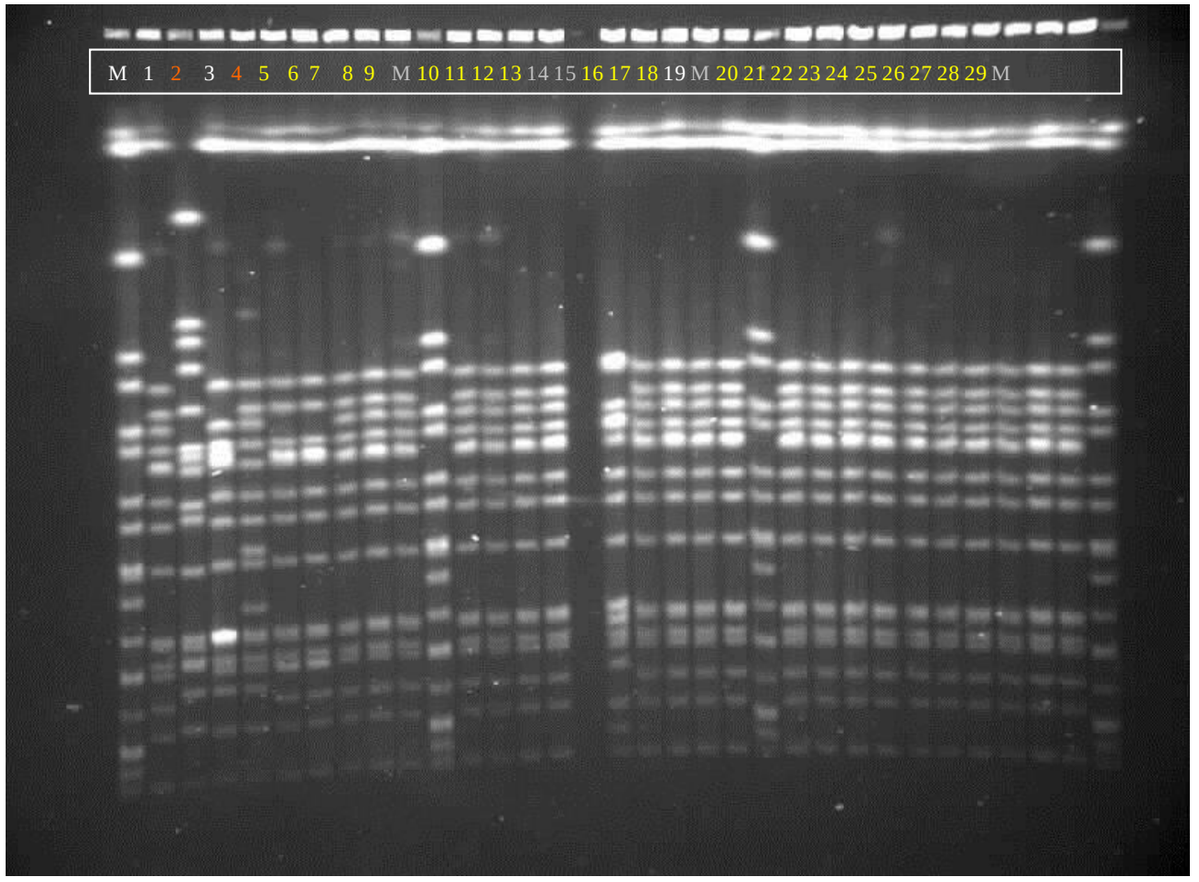
Total number of strains:
149

Molecular epidemiology of cephalosporin resistant *S.* Infantis

- Striking resistance to Amp-Cef
- Few strains: other resistance profiles
- Resistance: transferable, though at low frequencies and with difficulties
- Resistance gene: TEM-52
- On a plasmid, similar to a plasmid circulating in *Salmonella* in France
- Genetic background of the resistance: monoclonal for the same resistoprofile

Molecular epidemiology of cephalosporin resistant *S.* Infantis

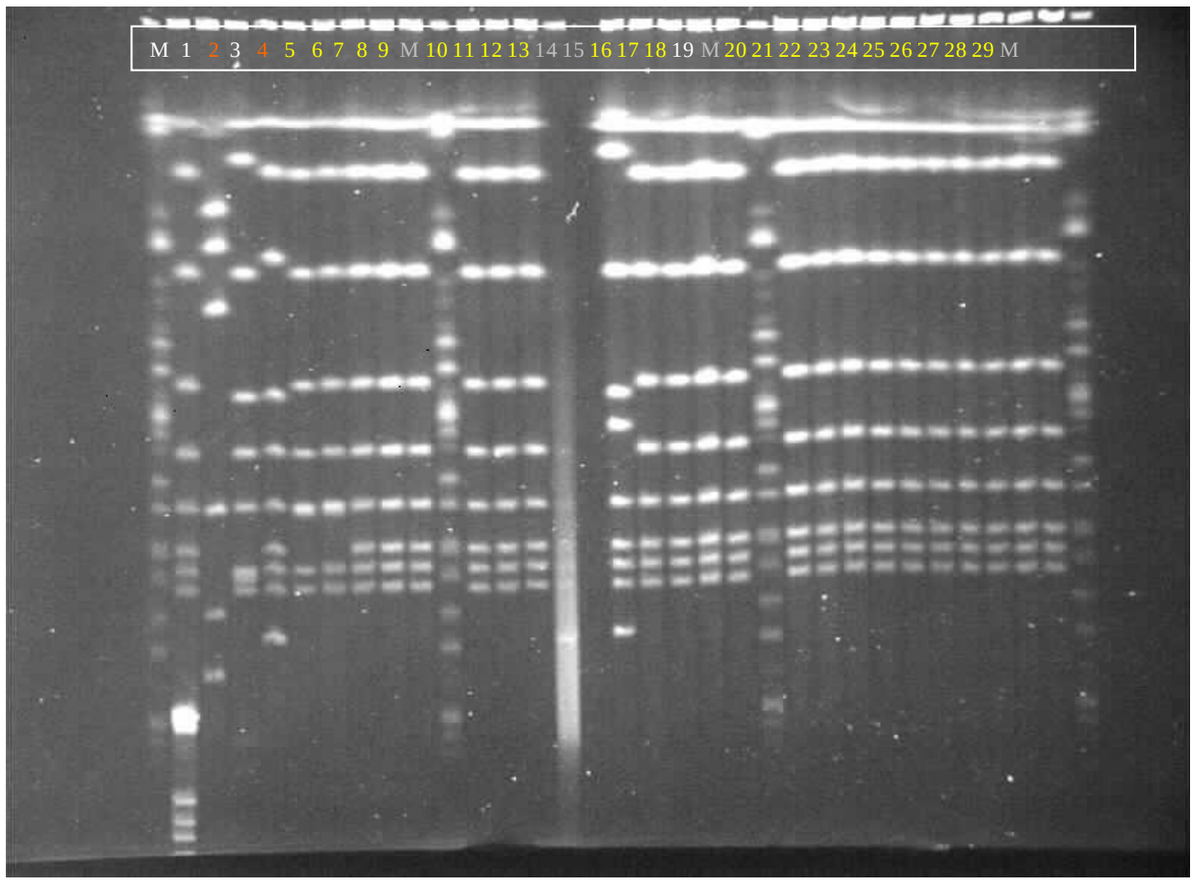
PFGE
XbaI



White: susceptible to Cephalosporins
Red Cephalosporin R and other resistances
Yellow: predominant resistotype: AmpCef

Molecular epidemiology of cephalosporin resistant *S.* Infantis

PFGE
BlnI



White: susceptible to Cephalosporins
Red Cephalosporin R and other resistances
Yellow: predominant resistotype: AmpCef

Conclusions

- Serotypes involved in foodborne infections: variation in time
- Control by vaccination is possible, though other strategies are also implemented

- Several episodes of multi-resistance: not always clear why increase or decrease
- Similar episodes (with other serotypes and other resistance genes) have been reported worldwide
- Within serotype spread is rather monoclonal: what is favoring these clones?

- The use of antibiotics and the subsequent appearance of resistance needs to be further investigated in *Salmonella* to understand their dynamics

THANK YOU FOR YOUR ATTENTION!

ANY QUESTIONS?

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