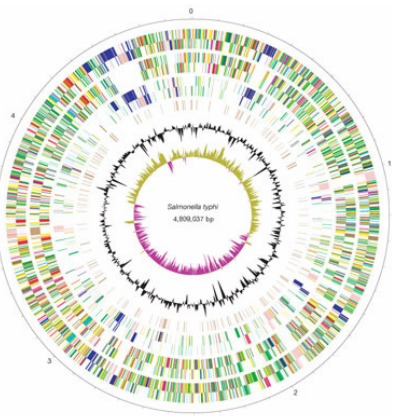
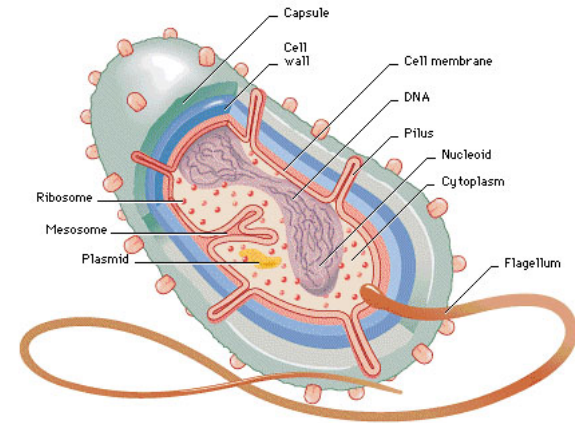
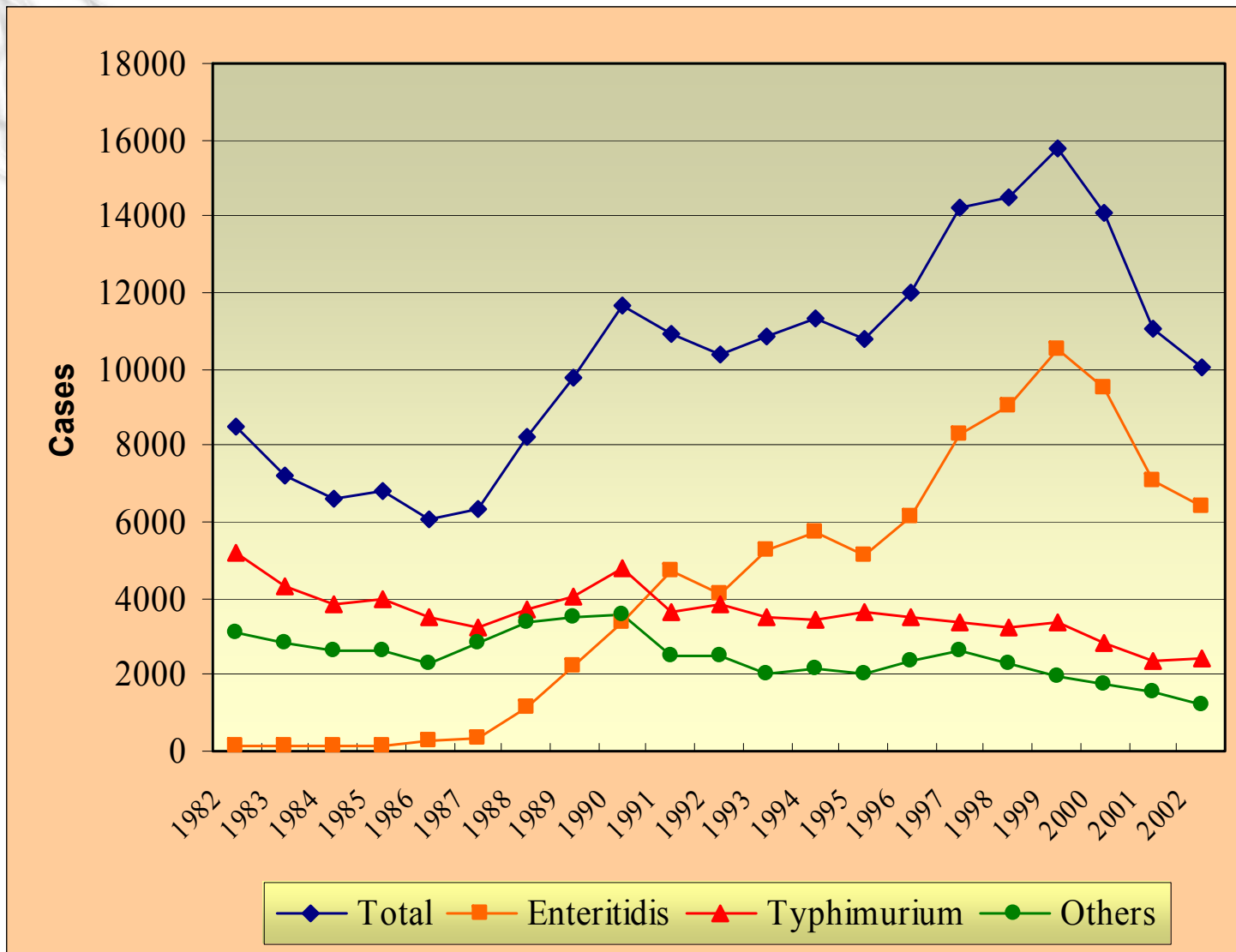


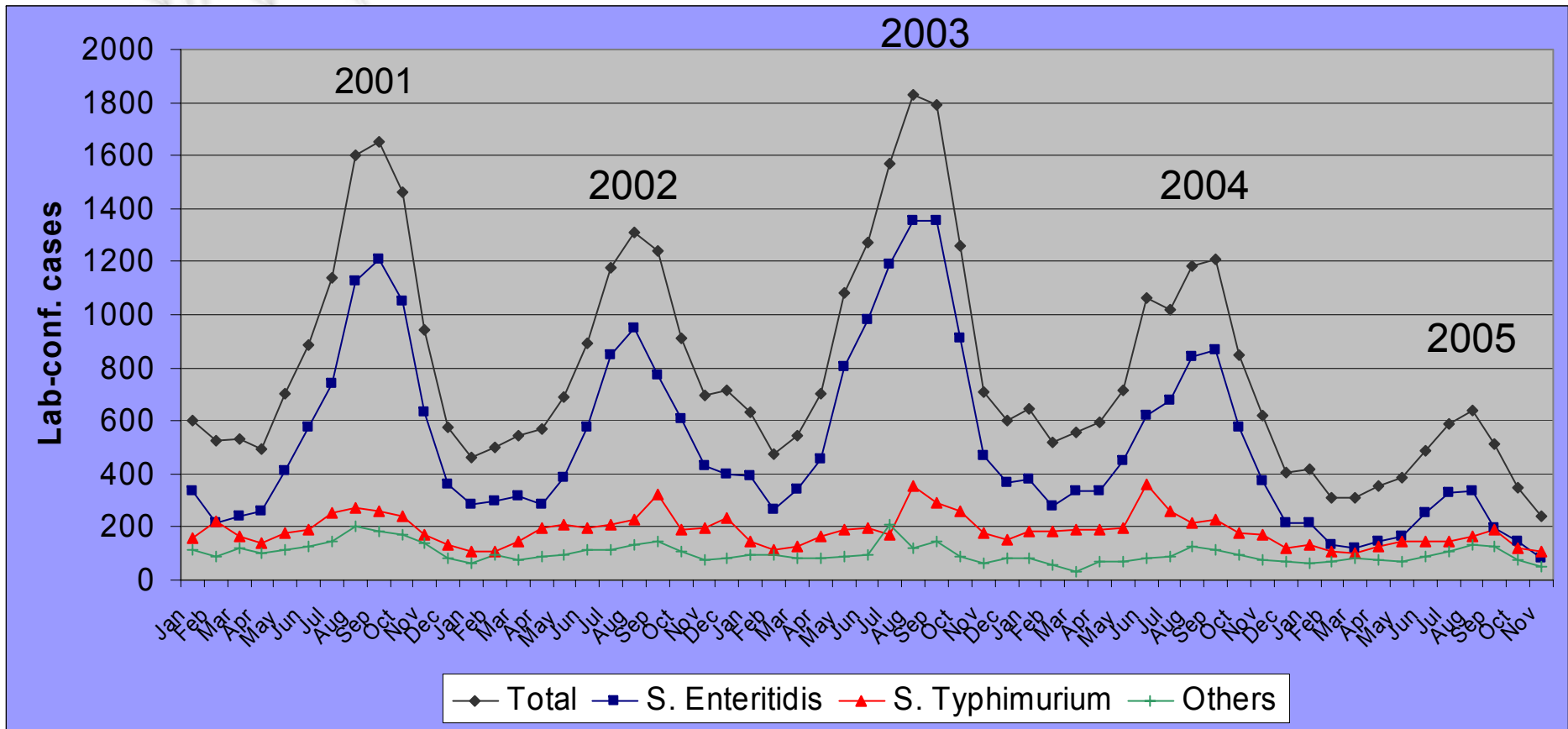
Salmonellosis: a recurrent problem or the end of a zoonosis ?



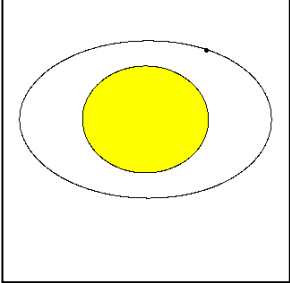
Dr Collard Jean-Marc
National Reference Center for *Salmonella* & *Shigella*
Bacteriology Division, IPH, Belgium
<http://www.iph.fgov.be/bacterio>







Estimated sources and health impact of human salmonellosis



- Denmark (report 2002)
 - Table eggs (~30%); travel (~25%); imported poultry (8-15%); DK poultry (~5%); pork (~3%); unknown (~17%)

- The Netherlands (data RIVM, 2001 – in past 5 years)
 - 39% eggs; 25% pigs; 21% chicken; 11% cattle; 4% other sources



Foodborne outbreaks in humans in Belgium in 2003

Source:
Data compiled by
Dr K. Dierick, IPH



Causative agent	Outbreaks	ill	died	hospitalised	Sources
Salmonella	63	696	-	83	Preparations with raw eggs, meat
<i>B.cereus</i>	2	43	1	5	Pasta salad, beef stew
<i>S. aureus</i>	3	15	-	10	Candy, milk
<i>B.cereus</i> and <i>S.aureus</i>	2	9	-	4	Pita, pasta salad
<i>B.cereus</i> and <i>Cl.perfringens</i>	1	61	-	2	Beef stew
Campylobacter	1	40	-	-	Barbecue meat
Other	7	39	-	1	Giardia, shigella, chemical substances, toxins, heat strike
Unknown	22	390	-	37	
Total	101	1293	1	142	

62 % of the outbreaks were due to *Salmonella*, with *Salmonella* Enteritidis as predominant serotype
Other serotypes : Dublin, Derby, Typhimurium, Virchow and 9: -: -.

Comparison Man – food

	IPH	1999	2000	2001	2002
MAN	Enteritidis		67.45%	64.2%	63.68%
	Typhimurium		19.87%	21.5%	24.15%
	Brandenburg		2.29%	1.8%	1.5%
	Total cases		14 088	10 783	9 718
Food agency	ULg-UG-IPH				
Hen	Enteritidis		86%	86%	92%
(20-26%)	Virchow		4%	0%	0%
Broiler filet	Paratyphi B	9%	16%	20%	10%
(12-15%)	Hadar		14%	9%	10%
	Enteritidis	55%	3%	14%	0%
Pork	Derby		28.4%	15%	23%
(15-20%)	Typhimurium		25.2%	43%	46%
Beef	Enteritidis		50%	25%	0%
(6 → 3%)	Brandenburg		3.3%	6%	0%

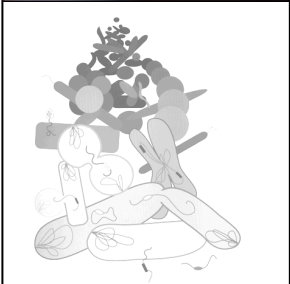


Salmonella prevalence in eggs

Very low frequency :

0 - 62 on 10.000 eggs in commercial circuit

Infected flock : 1.1% tot 7.4% of eggs



0.005 - 0.0264 % in the USA (Henzler et al., 1998, Ebel & Schlosser, 2000)

0.3% of the eggs produced in the UK (Elson & Little, 2003)

5.1% and 6.7% in two UK surveys of eggs

imported from Spain (Elson & Little, 2003)

7.7% in other imports where the country of

origin was unknown (Elson & Little, 2003)

0 - 0.03% of eggs produced in NL (Van Pelt & Valkenburgh, 2001)

0.15 - 0.77% Belgium (DVK-CLO, 1999)

Very high contamination load is possible:

Up to 10^8 cfu/g yolk (t° and time dependent)

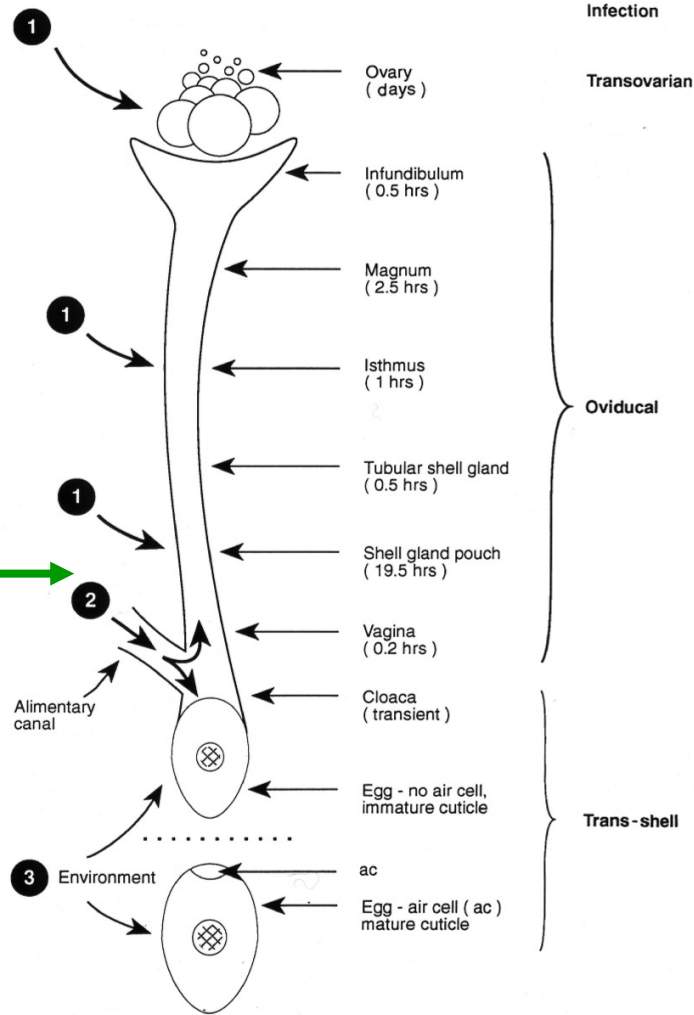
Enteritidis: « tropism » for the reproductive tract of laying hens



Via
blood
stream

From
intestines

From
environment



Infection

Transovarian

Oviducal

Trans-shell

vaccination

~~Vertical
transmission~~

Horizontal
transmission



Control measures

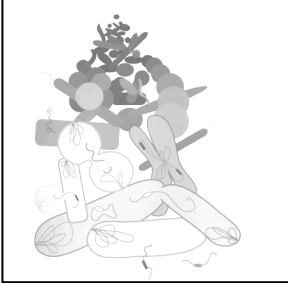
Directive 92/117/EEC

- Monitoring of zoonoses in feed, animals, food and man
- National general measures on zoonoses
- Rules on salmonella control in breeding flocks (hen)



Directive 2003/99/EC

- monitoring in feed, animals, food + AR
- Food-borne outbreak investigations
- Reporting on trends and sources



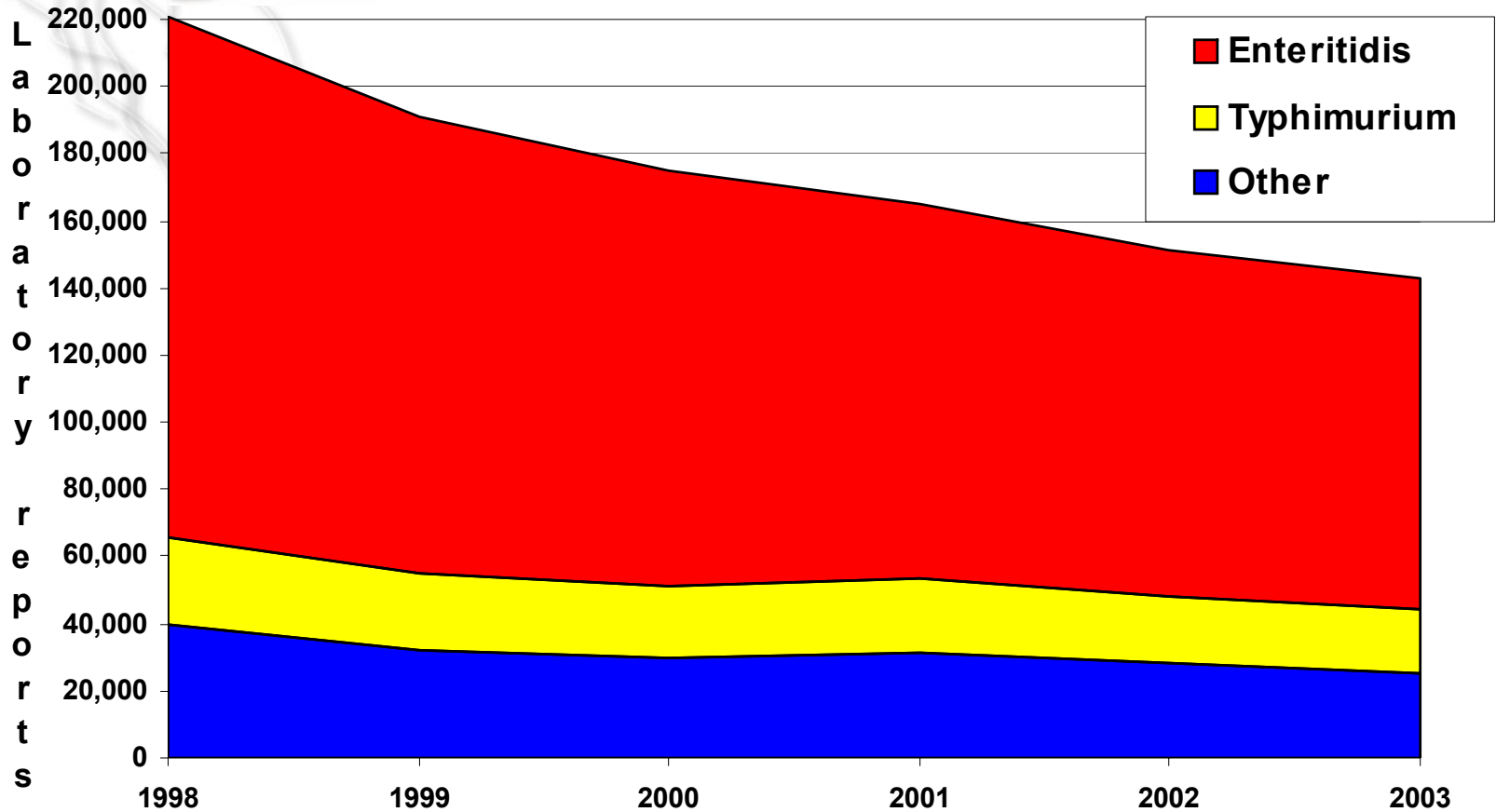
Regulation 2160/2003 on control on Salmonella

- Community targets
- Control programmes and methods (MS)
- Predefined specific measures

Certification in intra-Community trade



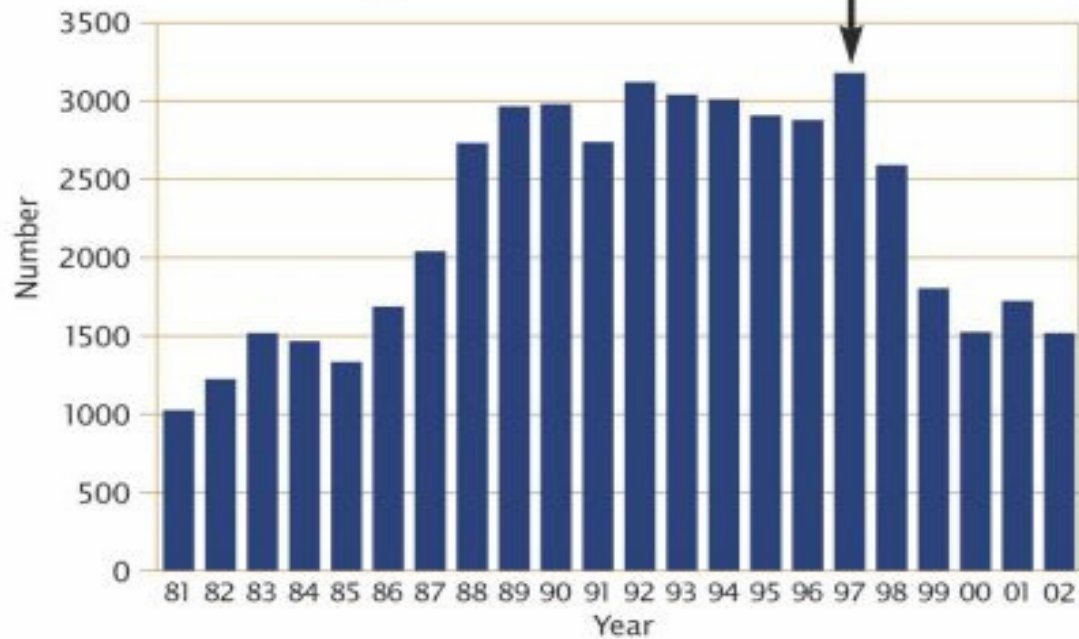
Total human salmonella 1998-2003



International trends in salmonella serotypes 1998-2003 – a surveillance report from the Enter-net international surveillance network. Ian ST Fisher on behalf on the Enter-net participants. Eurosurveillance. 9(11):9-11



Salmonella in humans
England & Wales 1981-2002



Control measures

Directive 92/117/EEC

- Monitoring of zoonoses in feed, animals, food and man
- National general measures on zoonoses
- Rules on salmonella control in breeding flocks (hen)

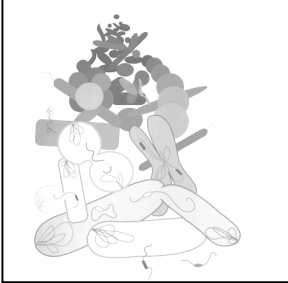
Directive 2003/99/EC

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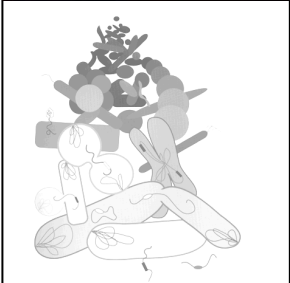


Community Salmonella reduction targets

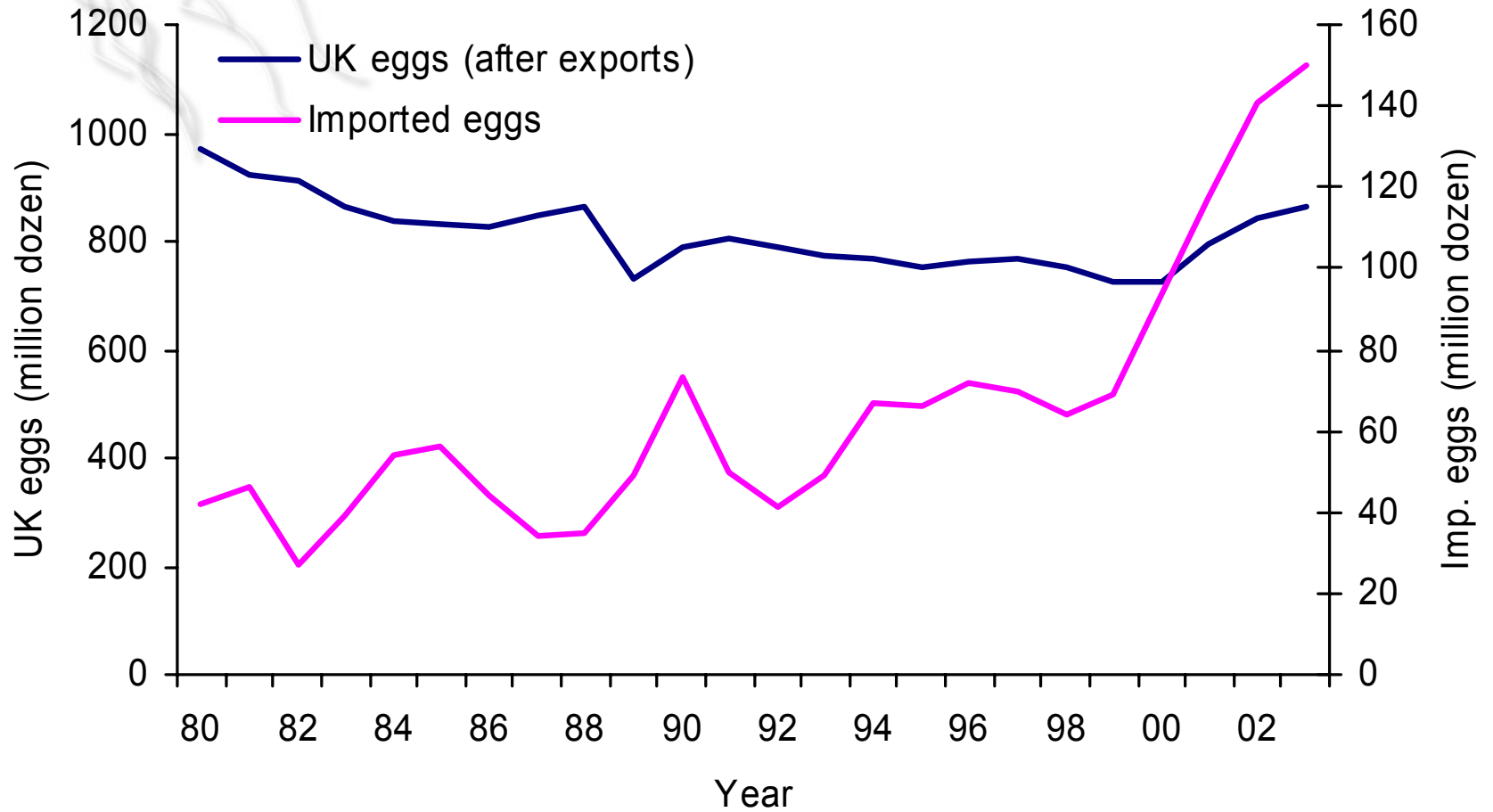
- Breeders : in process
- Layers: end 2005
- Broilers: end 2006
- Turkeys and pigs: en 2007

Predefined specific measures

- Fowl breeding flocks infected with SE/ST: slaughter/heat treatment/destruction
- Tables eggs: have to originate from negative flocks (end 2009)
- Poultry meat: criterion of absence of salmonella in 25g or industrial heat treatment (end 2010)



The UK egg market: 1980 to 2003



Press Release



14th October 2004

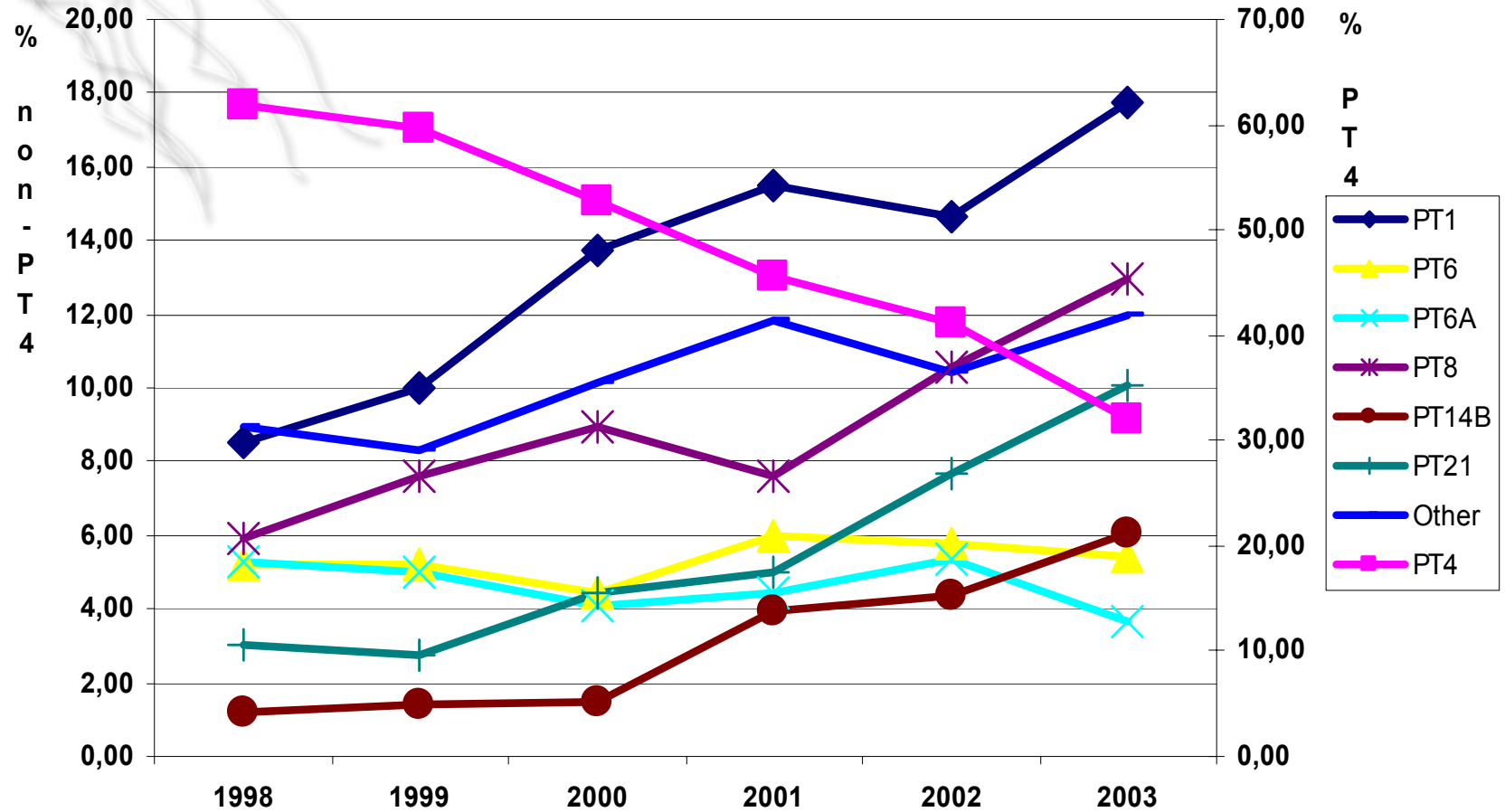
Agencies step up action on salmonella outbreaks linked to Spanish eggs

The Health Protection Agency (HPA) and the Food Standards Agency (FSA) are stepping up action to protect the public's health following continued outbreaks of Salmonella Enteritidis (other than phage type 4) since 2002, many of which have been linked to Spanish eggs used in the catering trade.

The HPA has investigated over 80 outbreaks of these strains of salmonella in the past two years, with at least 2,000 confirmed cases, and our evidence shows that the use by the catering trade of Spanish eggs is a major source of this infection.

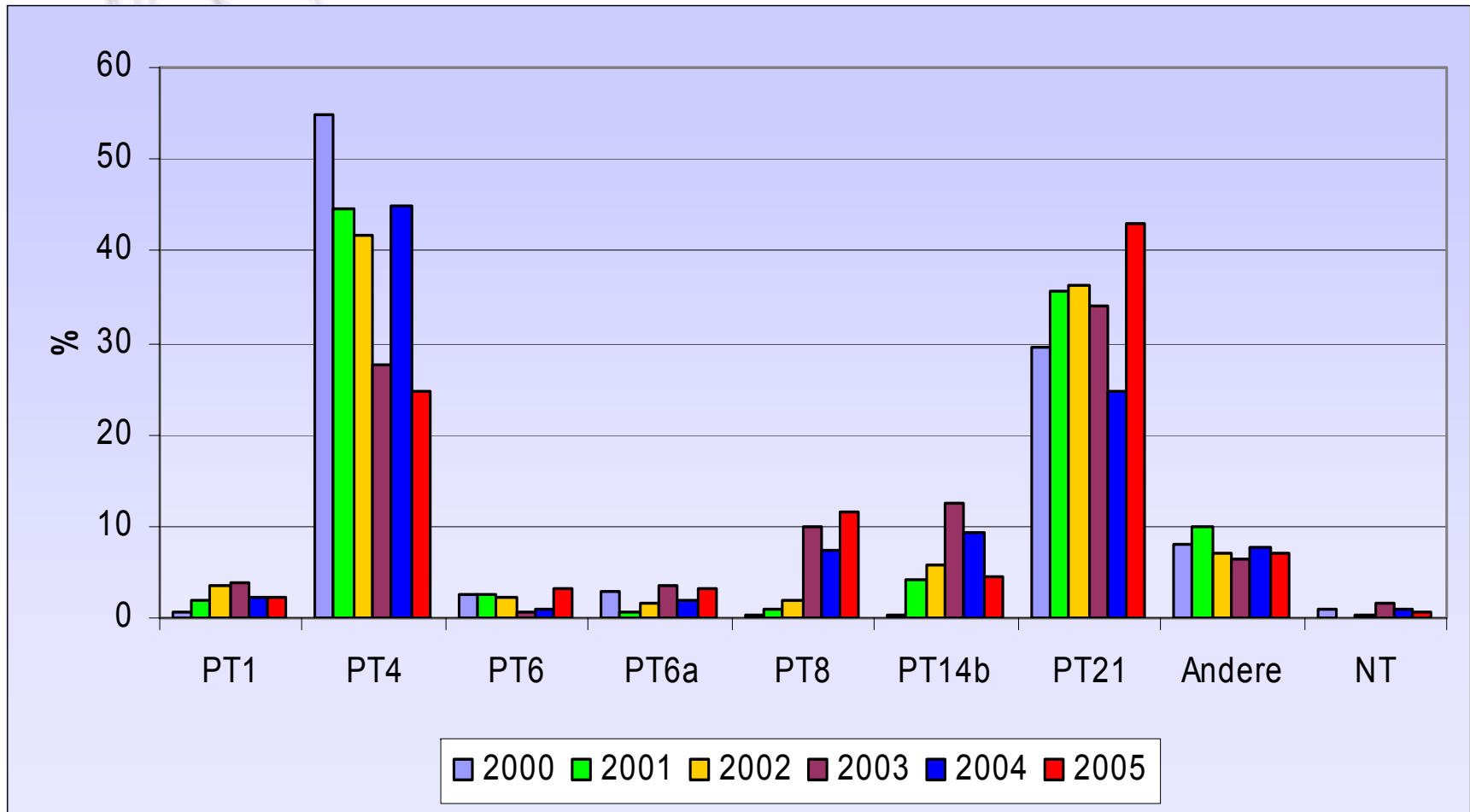
A national outbreak control team, which includes the FSA, was convened by the HPA to look at this problem, and recommended that various actions should be taken, in the UK and in Europe, to prevent further people from becoming ill, this includes alerting caterers to the risks to health that are clearly associated with some non-UK eggs.

S. Enteritidis Phage-type trends % of total 1998-2003



Dramatic shift in the epidemiology of *S. Enteritidis* phage-types in Western Europe 1998-2003 – results from the Enter-net international salmonella database. Ian ST Fisher on behalf on the Enter-net participants. Eurosurveillance.

Phage type distribution in *Salmonella* Enteritidis



Eieren van pluimvee, in de schaal, vers, verduurzaamd of gekookt België

Import

Land	2002		2003	
	Totaal Kg		Totaal Kg	
China	17.920	0,1%		
Polen	516.747	1,7%	352.202	2,3%
Tsjechie	1.204.765	3,9%	367.201	2,4%
Verenigde Staten van Amerika	1.048.045	3,4%	2.274.752	14,7%
Denemarken	3.773	0,0%	20.500	0,1%
Duitsland	1.225.426	4,0%	796.140	5,1%
Frankrijk	8.722.367	28,5%	4.098.983	26,5%
Italie	2	0,0%	824.427	5,3%
Luxemburg	90.442	0,3%	12.636	0,1%
Nederland	17.721.358	58,0%	6.647.368	43,0%
Spanje			74.817	0,5%
Verenigd Koninkrijk	14.780	0,0%		

30.565.625

15.469.026

Export

81.167.716

35.083.602

Vera Cantaert



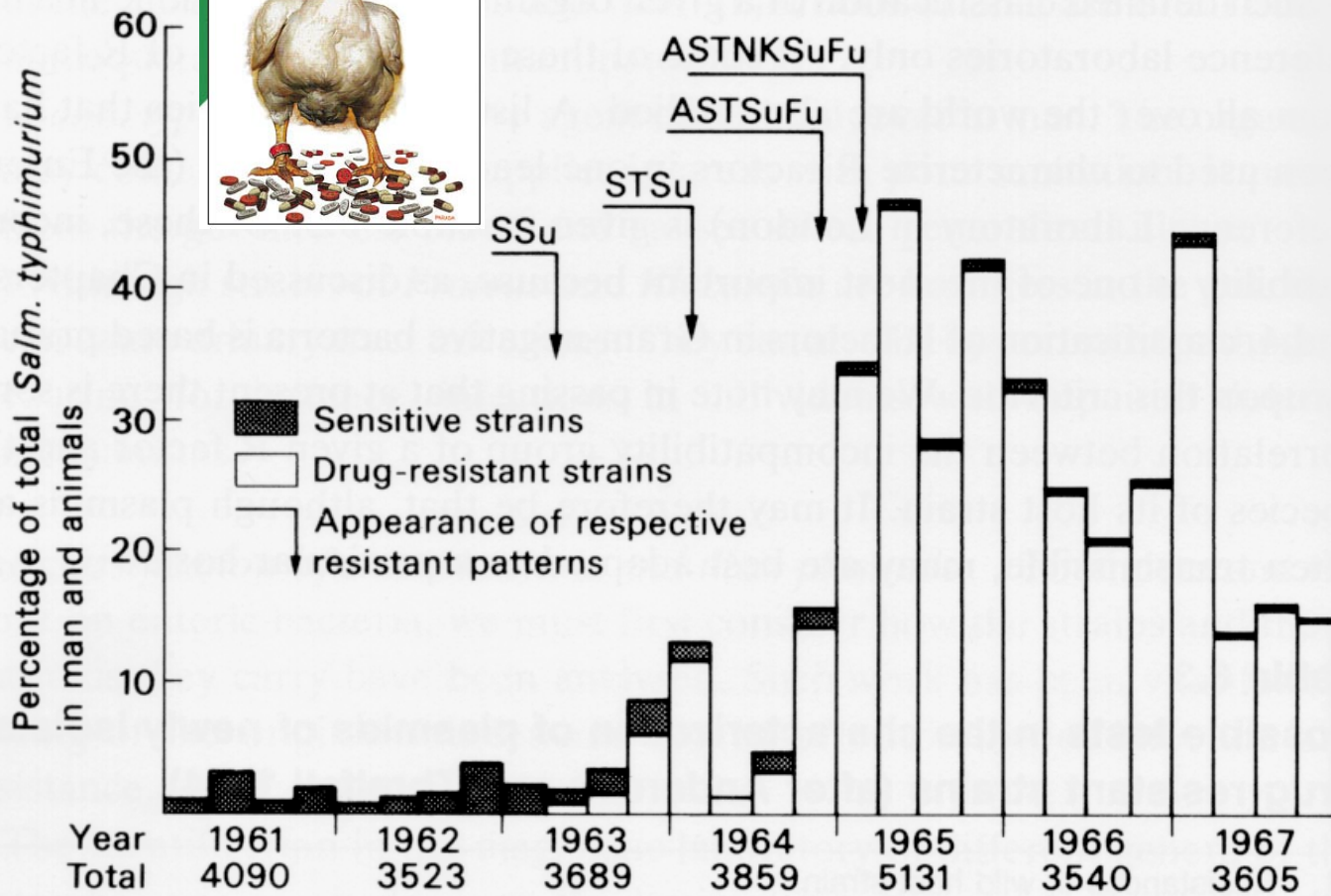
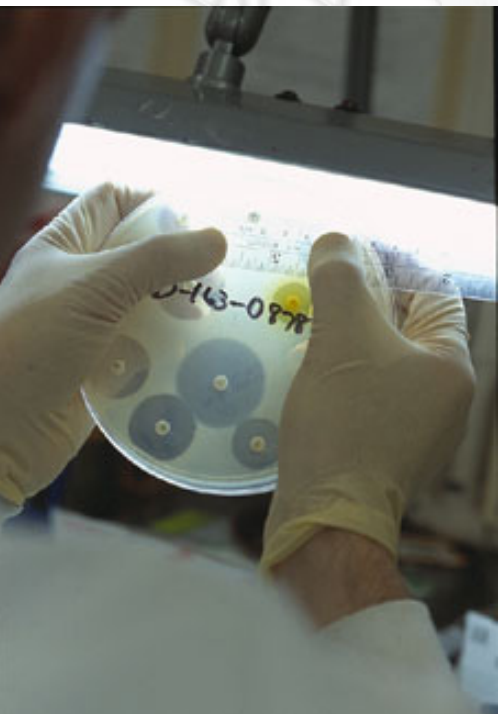
Oeufs d'oiseaux et de volaille de basse cour (en coquille, frais, conservés ou cuits) Statistiques du commerce extérieur – Banque Nationale de Belgique

Pays	2002		2003	
	Quantité (1000 pièces)	%	Quantité (1000 pièces)	%
Allemagne	22.974	0,8%	37.201	0,4%
Chine (Rép. Populaire)	275	0,0%	0	0,0%
Danemark	519	0,0%	318	0,0%
Espagne	0	0,0%	2.512	0,0%
Etats-Unis d'Amérique	402	0,0%	0	0,0%
Finlande	0	0,0%	297	0,0%
France	2.446.516	83,1%	9.245.934	92,6%
Hongrie	0	0,0%	302	0,0%
Iran	0	0,0%	302	0,0%
Italie	82.245	2,8%	272.832	2,7%
Luxembourg	6.801	0,2%	2.971	0,0%
Pays-Bas	357.154	12,1%	382.779	3,8%
Pologne	9.435	0,3%	26.574	0,3%
Royaume-Uni	18.223	0,6%	34.391	0,3%
Tchèque (République)	23.558	0,8%	17.300	0,2%
	2.945.128		9.986.512	



<http://www.nbb.be/>

Exportation	1.681.862		1.475.050	
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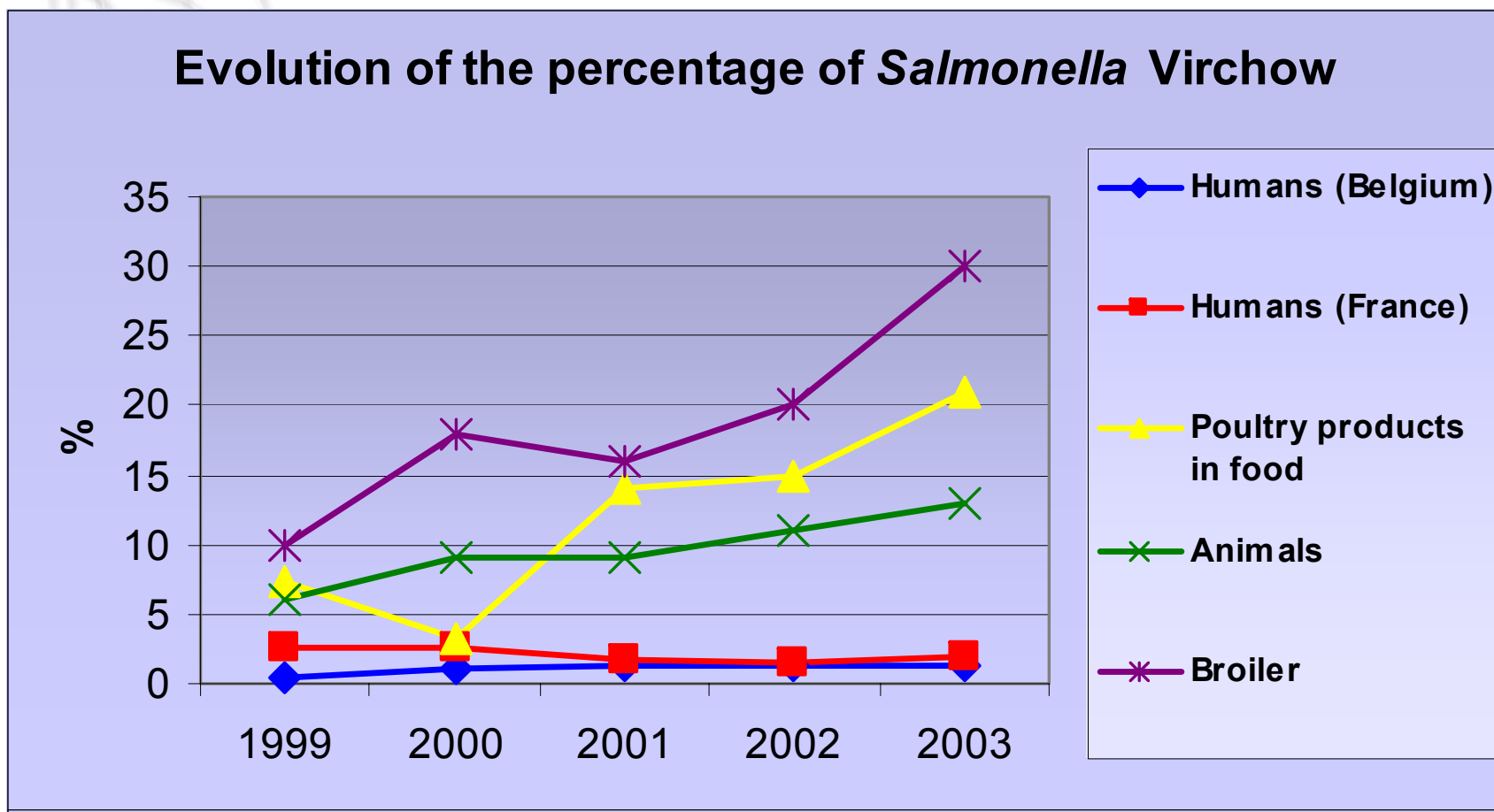
Salm. typhimurium (Man and animals)
 A-Ampicillin N-Neomycin Su-Sulphonamides Fu-Furazolidone
 K-Kanamycin S-Streptomycin T-Tetracycline

From Anderson, 1969

Antimicrobial resistance in Salmonella

- Salmonella and resistance seems to be quite heterogeneous: Some serotypes (e.g. Enteritidis) seem less susceptible to acquire resistances than other serotypes (e.g. Typhimurium)
The reason for this remains unclear
- Different outbreaks of multidrug-resistant salmonella have been described worldwide (e.g. Tm DT104), but also in Belgium: some of these outbreaks have been documented
 - Fluoroquinolone resistance in *S. Typhimurium*
(bovine Salmonella 91-94 + 2000 – Baucheron et al. MDR 2002 8:281-9; Imberechts et al. Vet Rec 2000 147:76-7)
 - Multi-resistant *S. Agona* (poultry + 1 human case)
Doublet et al. AAC 2004 48:756-8; Doublet et al. EID 2004 10:756-8
 - Extended spectrum β -lactamases in *S. Virchow*
Bertrand et al. submitted to JCM

With the diminishment of *S. Agona*, *S. Virchow* took over the tradition of multi-resistances in poultry



ESBL-producing *Salmonella enterica* serovar Virchow

Between 2000-2003, ESBL-producing Virchow strains were isolated from:

- chicken (68)
- food (broiler meat) (22)
- human (13)

It seemed that ESBLs have appeared in the phenotype: **AmpTeTmpSuNal**

To form the new phenotype **AmpCtxTeTmpSuNal**

Ser83 → Phe

	*	240	*	260	*	280	
gyrase-A :	gattCgcgagtgatgacaccatcgttcgtatggcgcagccattctcgctgcgttac						: 174
03-664 :	gatttcgcgagtgatgacaccatcgttcgtatggcgcagccattctcgctgcgttac						: 285
	GATT CGCAGTGTATGACACCATCGTTCGTATGGCGCAGCCATTCTCGCTGCGTTAC						

ESBL-producing *Salmonella enterica* serovar Virchow

Transferable resistance

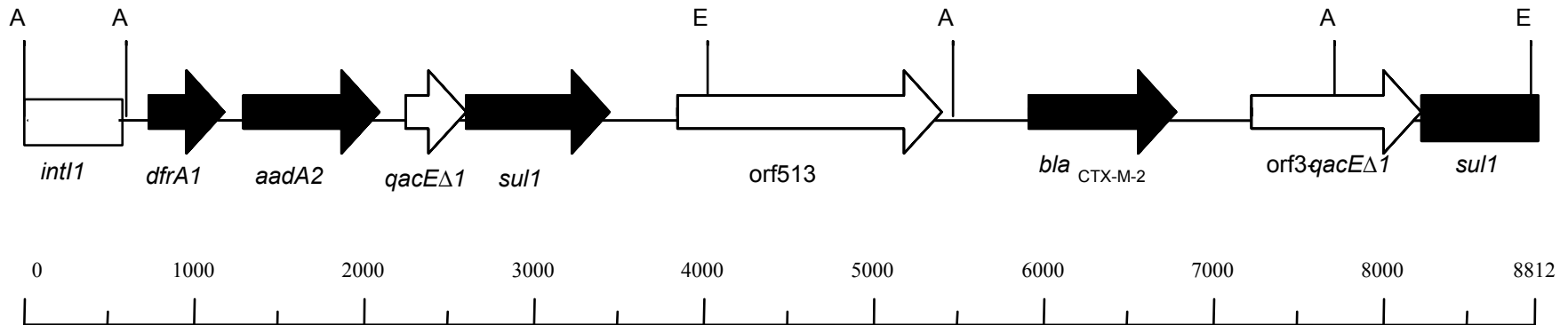
Plasmid localisation

Localisation in a complex integron

ESBL: CTX-M2

CTX-M-2 complex integron

8812 bp



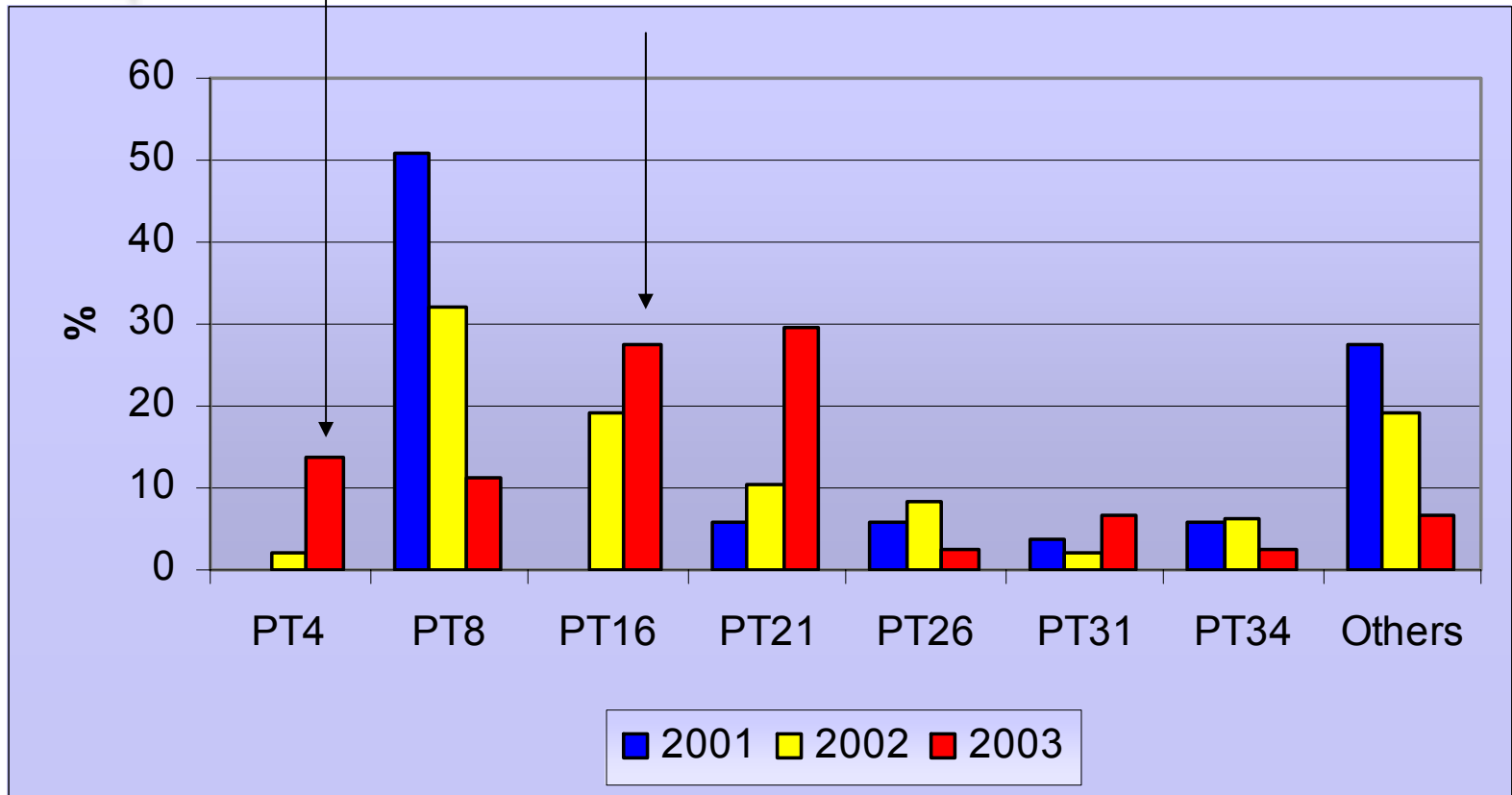
E: *EcoRI*
A: *AvaI*

Courtesy of A. Cloekaert and P. Butaye

Virchow of human origin: Phage Type distribution

ESBL producing strains (Amp Ctx Tet Sxt Nal + Cip_{LowR})

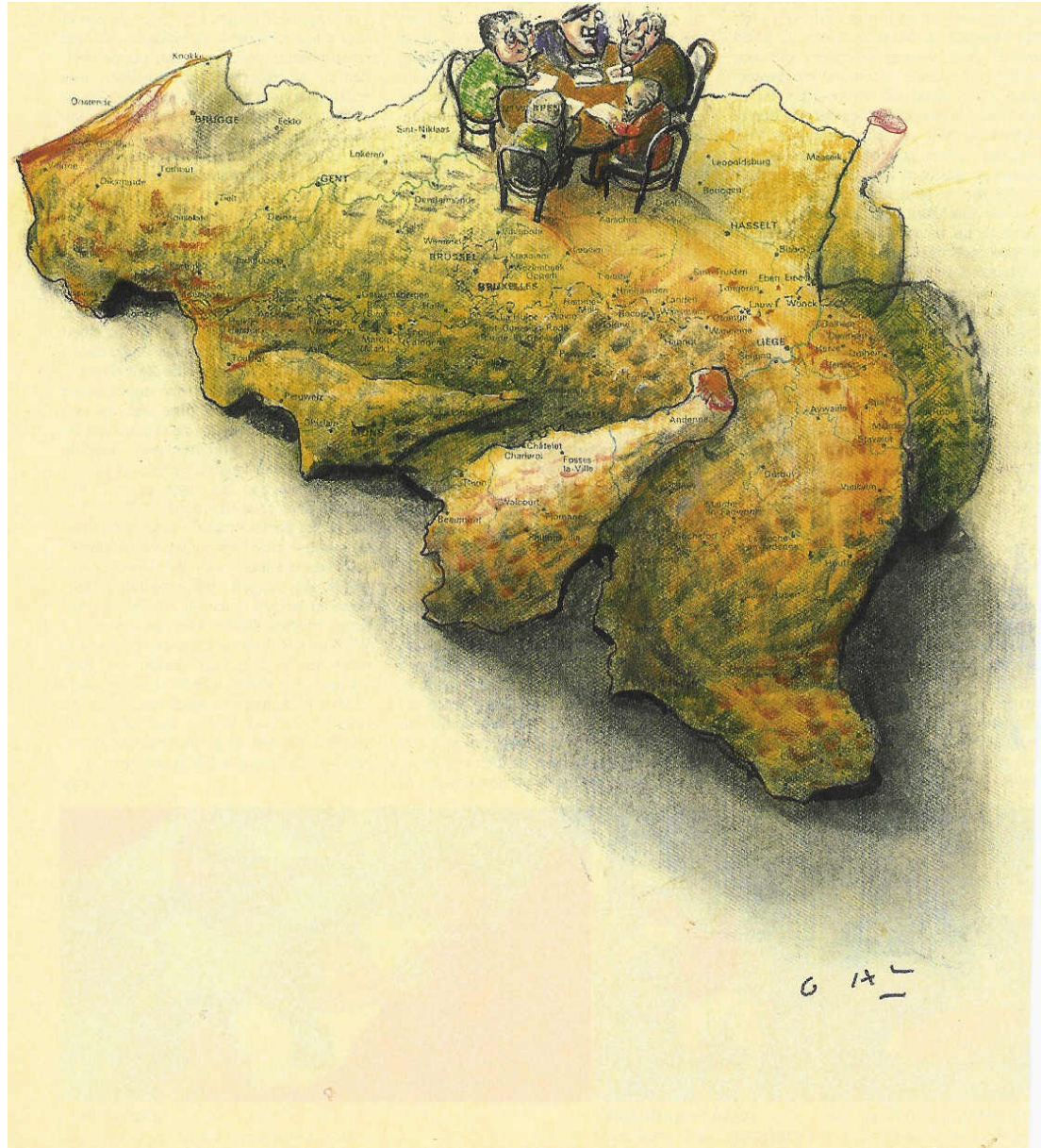
Amp Tet Sxt Nal – Cip_{LowR}



In Belgium

- Geographically clustered

Antwerp-Gent
- Hasselt

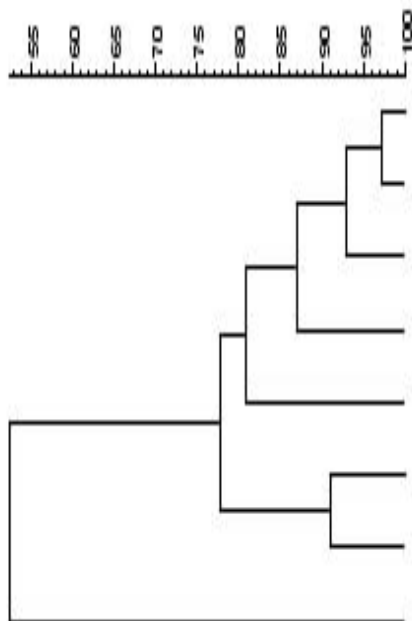


Molecular typing

Dice (Tol 1.0%-1.0%) (H>0.0% $\leq 0.0\%$ [0.0%-100.0%])

xba1

xba1



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

Conclusions

ESBL: CTX-M2 in *S. Virchow*

France: CTX-M9 in *S. Virchow*,

The Netherlands: TEM-52 in *S. Blockey*

South Africa: ESBL – varies from 3% in Ent, to 61.5% in Muenchen and even more 95% in Isangi: TEM-63 and TEM-131

US and Canada: CMY-2 in *S. Newport*

CTX-M2 have also been found in Enterobacteriaceae in Argentina (75% CTX-M2), in *E. coli*, Spain and Belgium (emergence of CTX - mainly CTX-M1 family - in *E. coli*, Rodriguez et al. 2005)

Conclusions

Several episodes of multi-resistance have been detected in other countries as well as in Belgium

Why do they appear/disappear or be maintained?

Role of antibiotic usage in agriculture on the subsequent appearance of resistance and spread to humans

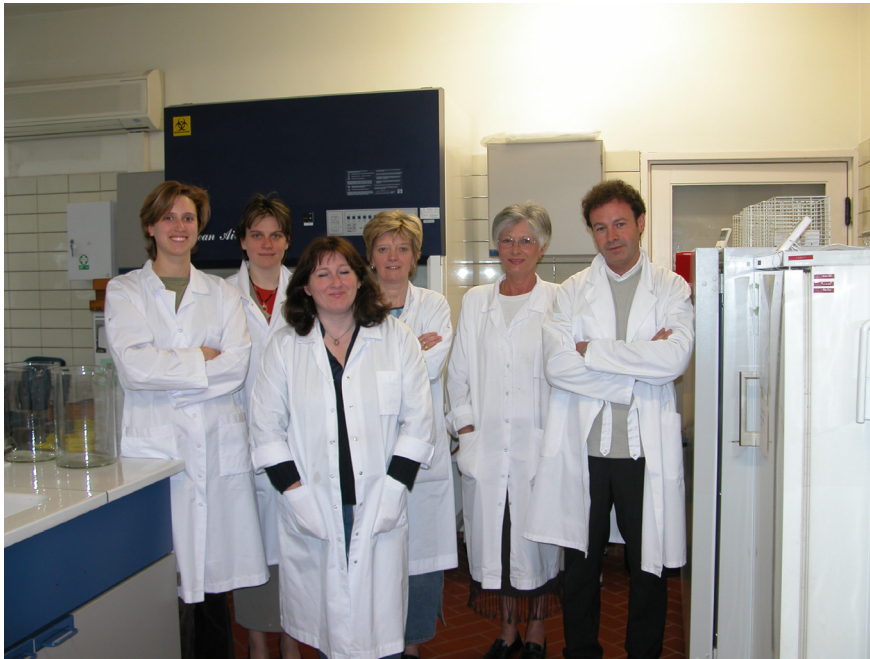
Gene mobility needs to be further investigated to understand the dynamics of MDR in Salmonella

Acknowledgments

All associated laboratories - National Centre for Phage typing – Food agency

F.X. Weill , A. Cloeckaert, K. Dierick, P. Butaye

Team of the National Reference Centre Salmonella/Shigella



Thank you for your attention

