

Molecular epidemiology of *Salmonella* and *Campylobacter* contamination of poultry during transport and slaughter

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Promotor: Prof. Dr. De Zutter



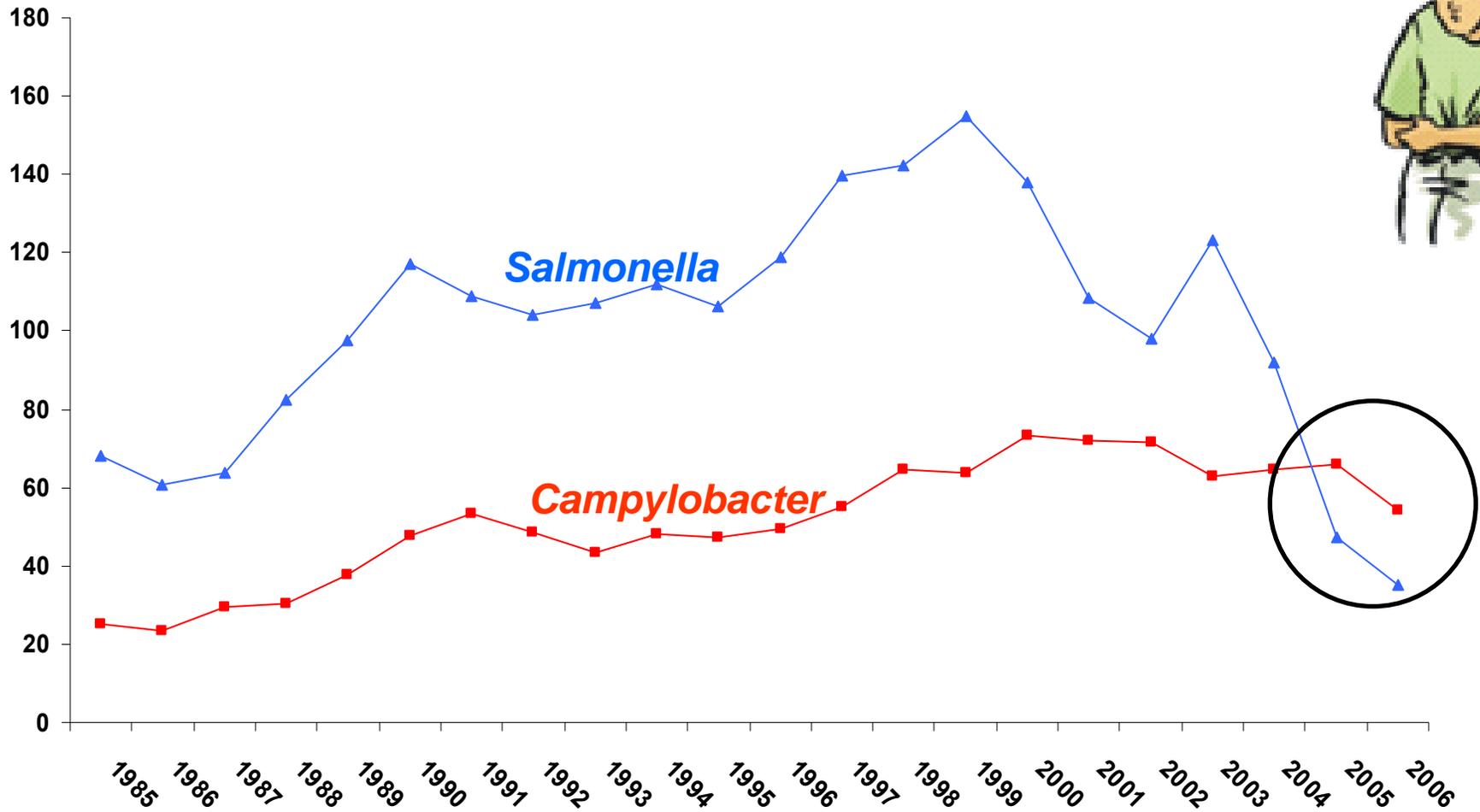
OVERVIEW

1. Situation of the problem
2. Characteristics of *Salmonella* and *Campylobacter*
3. Clinical aspects of *Salmonella* and *Campylobacter* infections
4. Poultry flocks
5. Aim of the study
6. Molecular discrimination of *Salmonella* isolates at serotype level
7. Materials and Methods
8. Contribution of gastrointestinal colonization and cross-contamination to carcass contamination during slaughter
9. *Salmonella*: impact of the slaughter line contamination on carcass contamination
10. *Campylobacter*: contamination or colonization of poultry flocks after transport
11. Conclusions



1. Situation of the problem

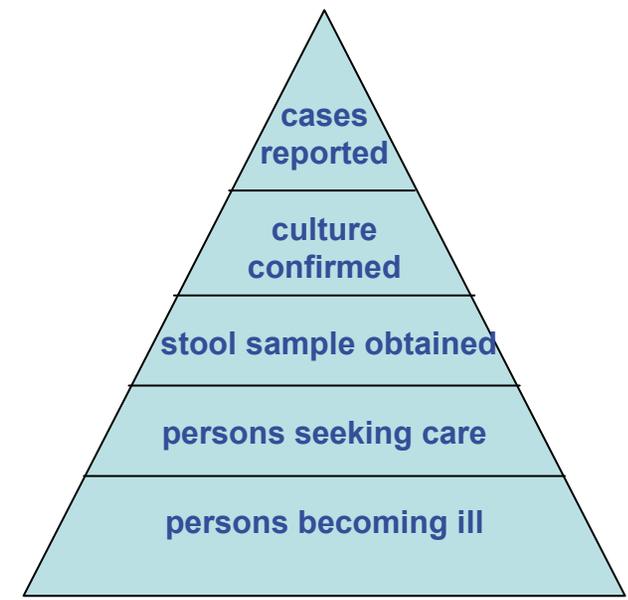
Incidence of *Salmonella* and *Campylobacter* infections per 100 000 Belgian habitants



35 *Salmonella* infections/100 000
54 *Campylobacter* infections/100 000

} reported cases in 2006

↓
true incidence is much higher !
ca. x 10 !



↙
socio-economic impact:
- employment costs,
- GP care,
- medicines,
- laboratory costs,
- hospital care

↘
complications
mortality

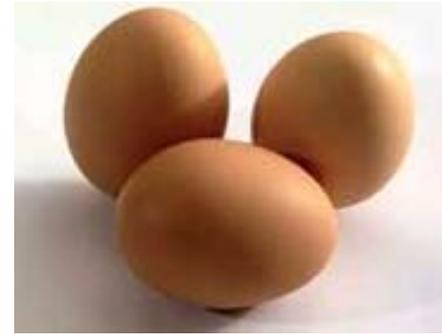
→ e.g. 27 million euro in Belgium for *Campylobacter*



Sources of *Salmonella* infection

- eggs & egg products : 39%
- pork : 25%
- poultry meat : 21%
- beef : 10%

(van Pelt *et al.*, 1999)

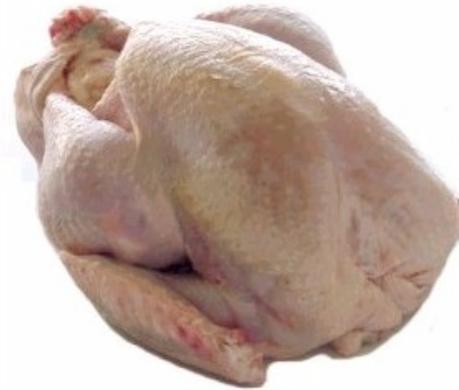


Outbreak: > 2000 persons eating pre-cooked chicken from a particular brand (Spain, 2005)



Sources of *Campylobacter* infection

- barbecue
- contact with pets or other animals
- overseas travel
- consumption of poultry meat
- cross-contamination in kitchen



- 40 % reduction in *Campylobacter* infections during dioxin crisis
- **outbreaks:** 12 persons eating stir-fried chicken in a restaurant (UK, 1997)
 - > 80 employees eating chicken salad in a canteen (Denmark, 2005)





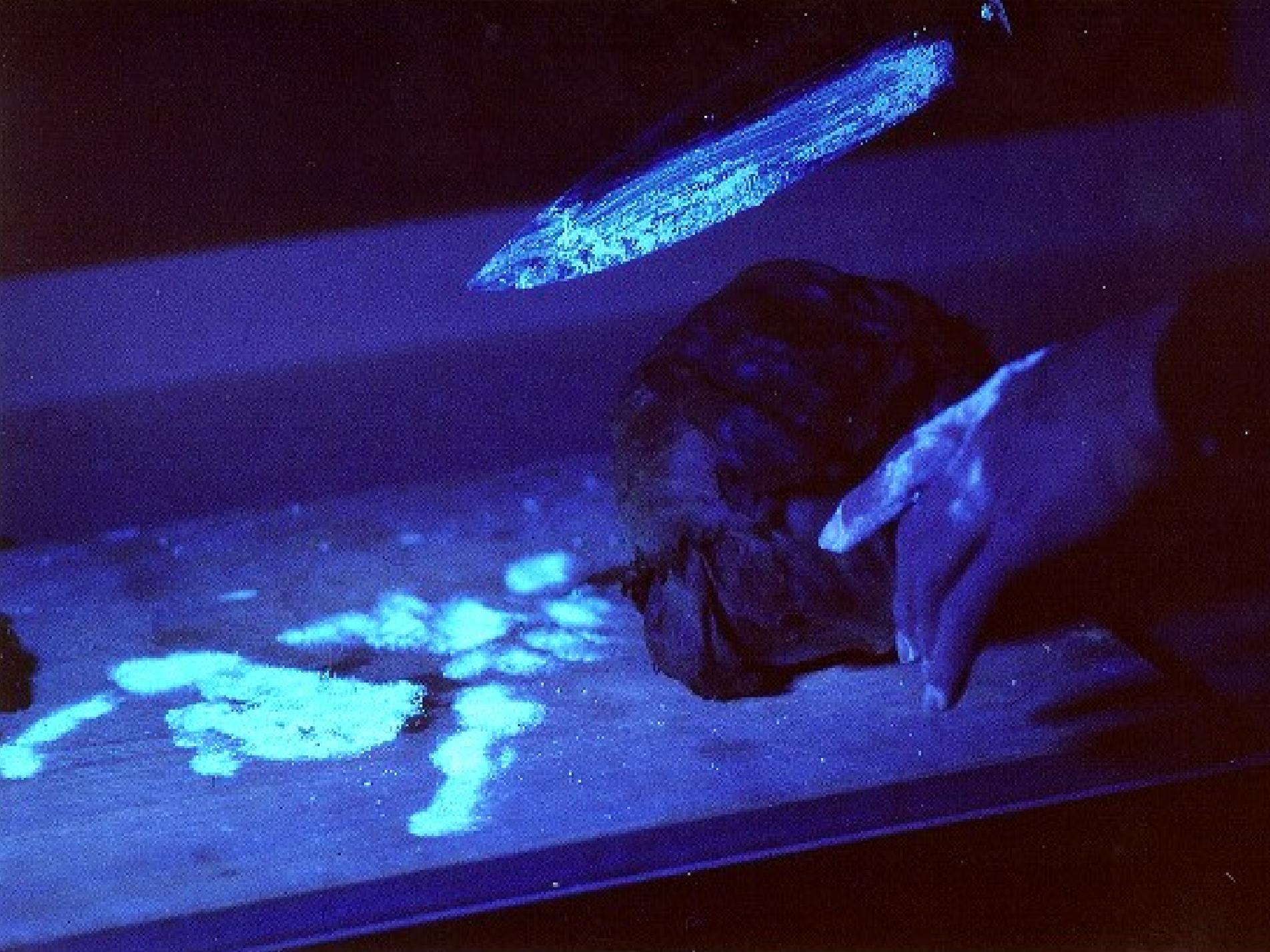
Dr. Henk van der Zee, Food Inspectorate, the Netherlands















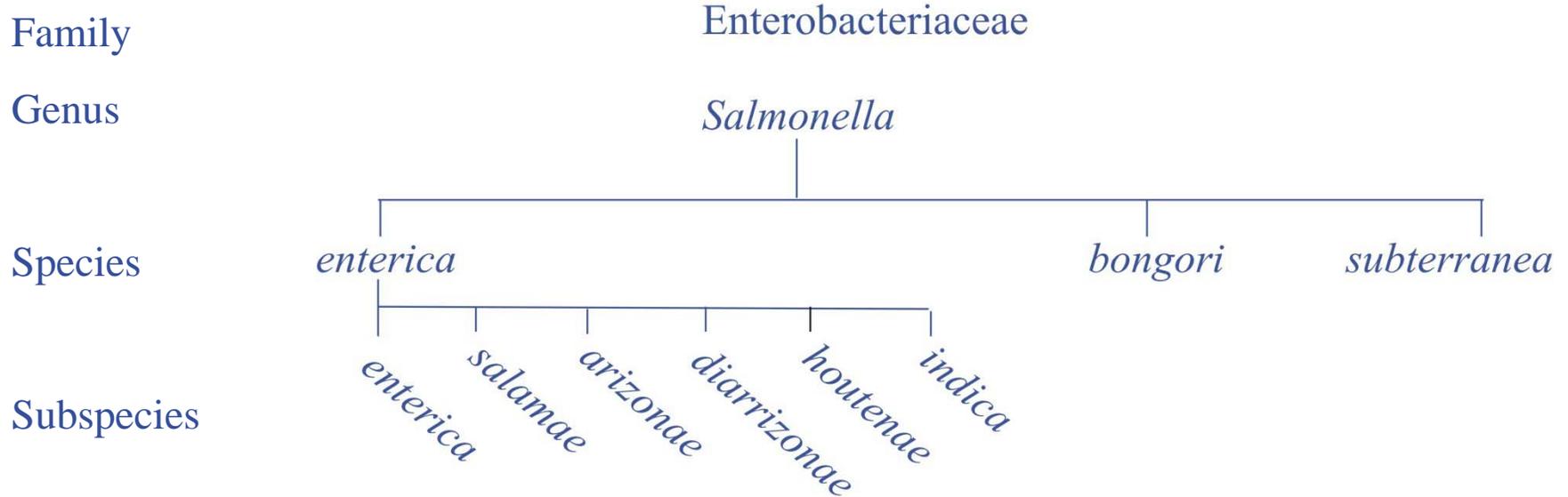
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2. Characteristics of *Salmonella* and *Campylobacter*

Salmonella



> 2500 serotypes – Kauffmann-White scheme

↳ e.g. *Salmonella enterica* subspecies *enterica* serotype Typhimurium
or short *Salmonella* Typhimurium



- Gram-negative rods
- 0.7-1.5 μm – 2.0-5.0 μm
- usually motile – peritrichous flagella
- facultative anaerobic
- temp: 5°C – 46°C (opt. \pm 37°C)
- pH: 3.8 – 9.5 (opt. \pm 7)

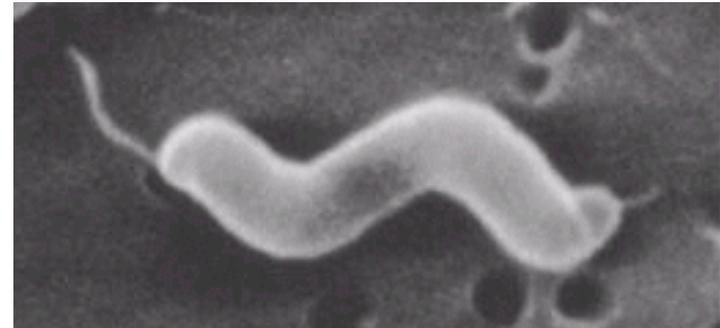


Source: USDA, Science Source, Photo Researchers

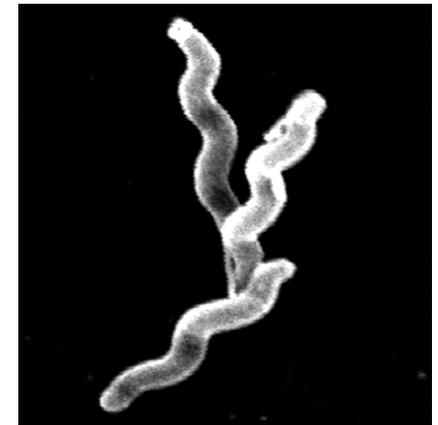


Campylobacter

- Family:** Campylobacteraceae
- Genus:** *Campylobacter*
- Species:** 17 species and 6 subspecies
- C. jejuni*, *C. coli*, *C. lari*
- = thermophilic campylobacters



- Gram-negative spirils
- 0.2-0.8 μm – 0.5-5.0 μm
- motile with corkscrew-like motion:
monotrichous or amphitrichous
- micro-aerobic
- temp: 30°C – 45°C (opt. \pm 37°C, \pm 42°C)
- pH: 4.9 – 9.0 (opt. \pm 7)



Source: www.microbes-edu.org/etudiant/campylo.html

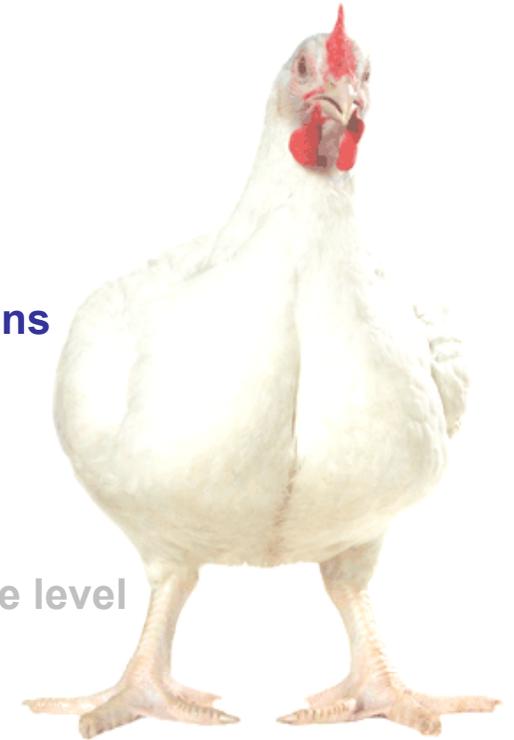
ASM, cover, Infection and Immunity 74

Virginia-Maryland Regional College of Veterinary Medicine, Virginia



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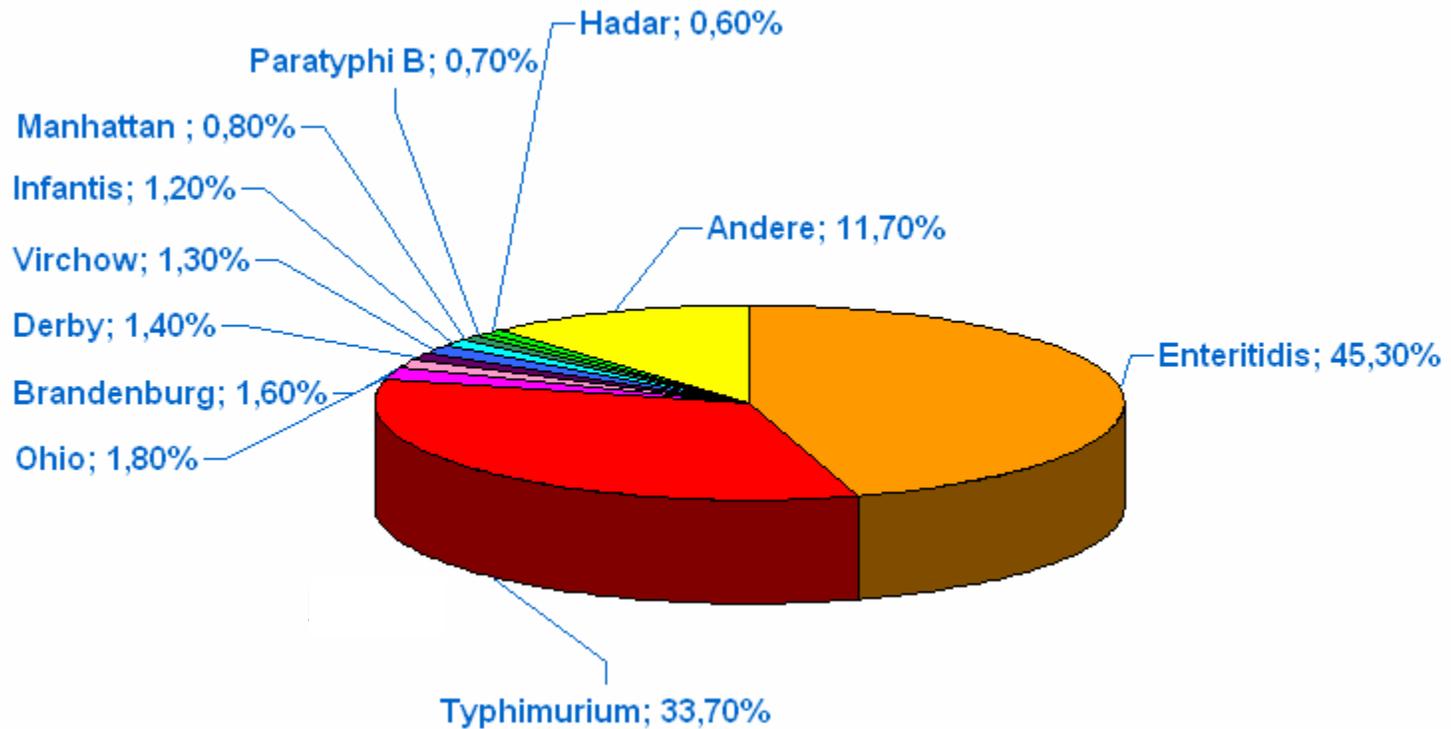
3. Clinical aspects



	<i>Salmonella</i>	<i>Campylobacter</i>
Incubation period	12 h to 5 days	18 h to 8 days
Infective dose	10 ⁵ -10 ¹⁰ bacteria (>100)	500 - 800 bacteria
Symptoms	non-bloody diarrhea abdominal pain mild fever & headache nausea & vomiting	diarrhea (may be bloody) intense abdominal pain fever & headache nausea & vomiting
Treatment	self-limiting fluoroquinolones	self-limiting erythromycin
Complications	Reactive Arthritis	Reactive Arthritis Guillain-Barré syndrome

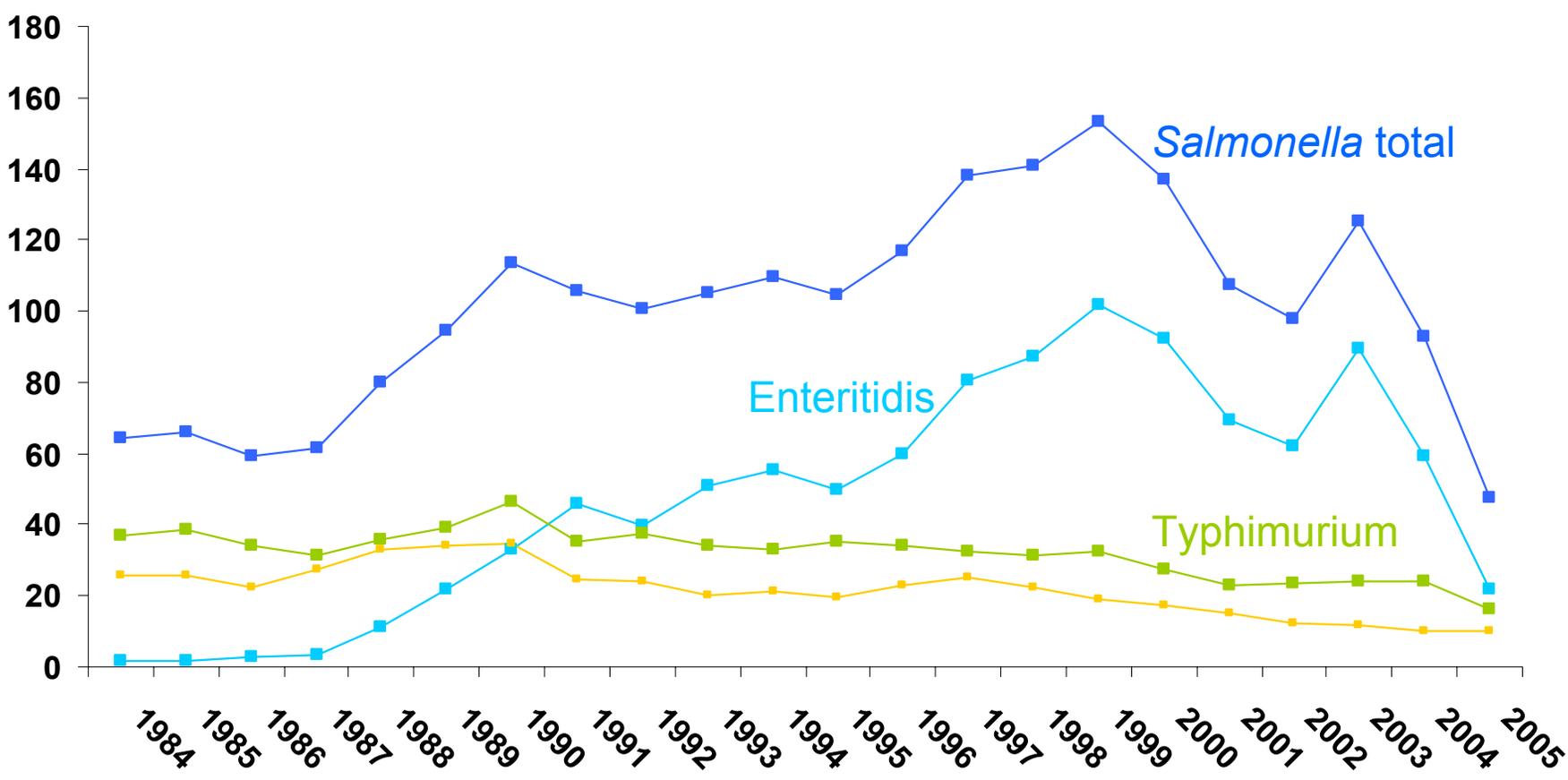
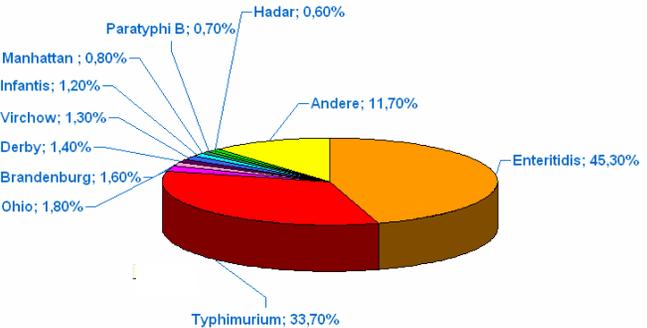


Distribution of *Salmonella* serotypes from patients in Belgium in 2005

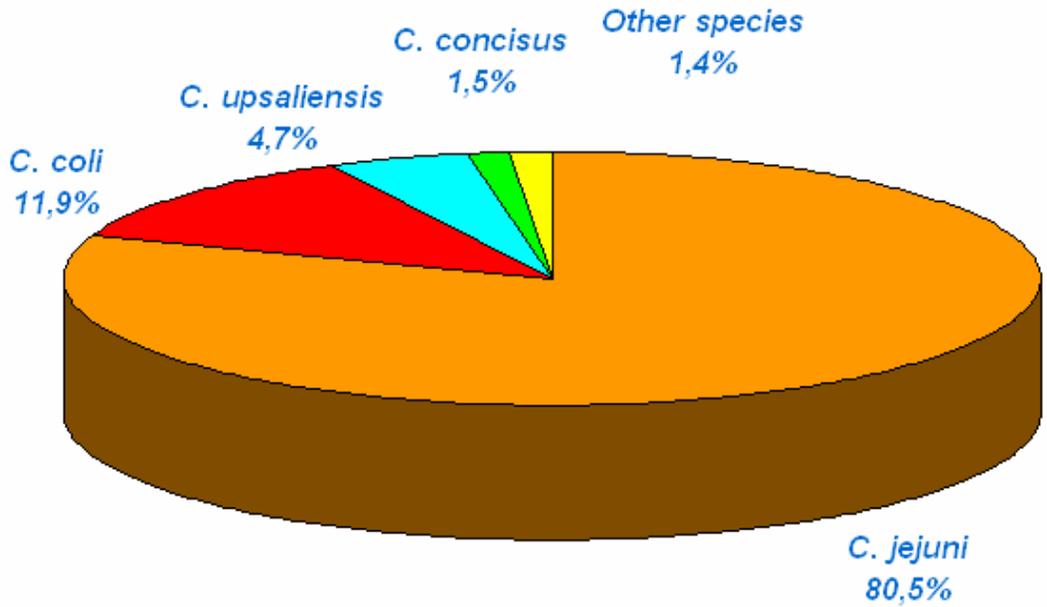


(SIPH, 2006)





Distribution of *Campylobacter* species from patients during 1995-2002 in Belgium



(Vandenberg *et al.*, 2004)



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4. Poultry flocks

Vertical transmission:



Horizontal transmission:

insects



rodents



feed and drinking water

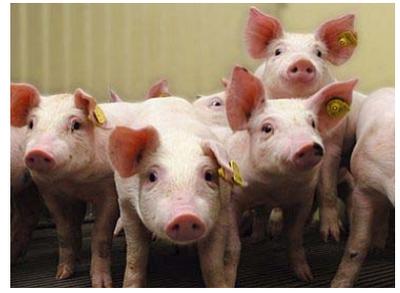
wild birds



human traffic & hygiene

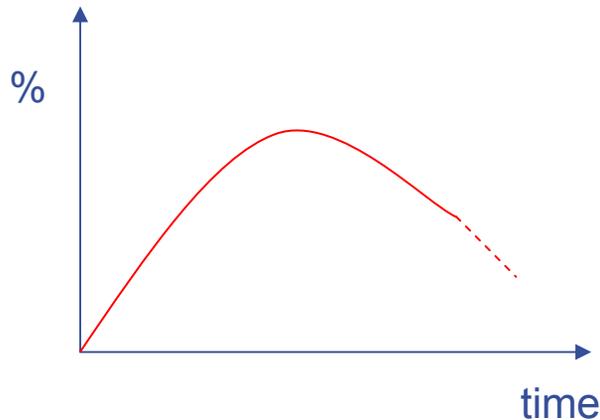


other farm animals



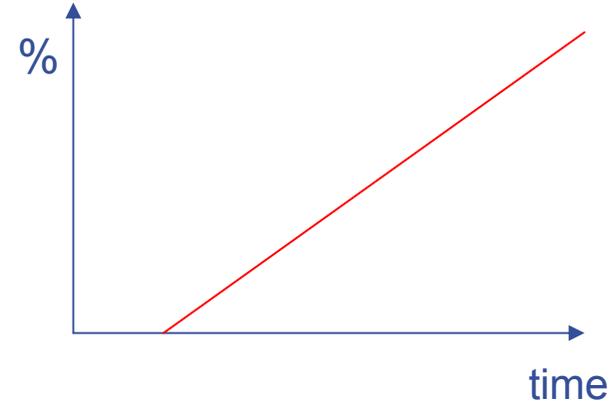
Prevalence of colonized flocks

Salmonella



- top: 2 to 4 weeks
- shedders vs. carriers
- within-flock prevalence: variable
- prevalence: 50% - 33% (Belgian study)

Campylobacter



- top: 6 weeks (lag of 2 weeks)
- shedders
- within prevalence: 100%
- prevalence: 3 - > 90%



slaughter

- loading at 5 or 6 weeks
- transport
- processing (logistic slaughter !)
 - hanging
 - stunning: electrical shock, CO₂
 - killing
 - scalding
 - defeathering
 - evisceration: removal of crop, nek, internal organs
 - chilling



Putting on transport band



hanging
stunning
killing
scalding
defeathering
evisceration



hanging
stunning
killing
scalding
defeathering
evisceration



hanging
stunning
killing
scalding
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evisceration



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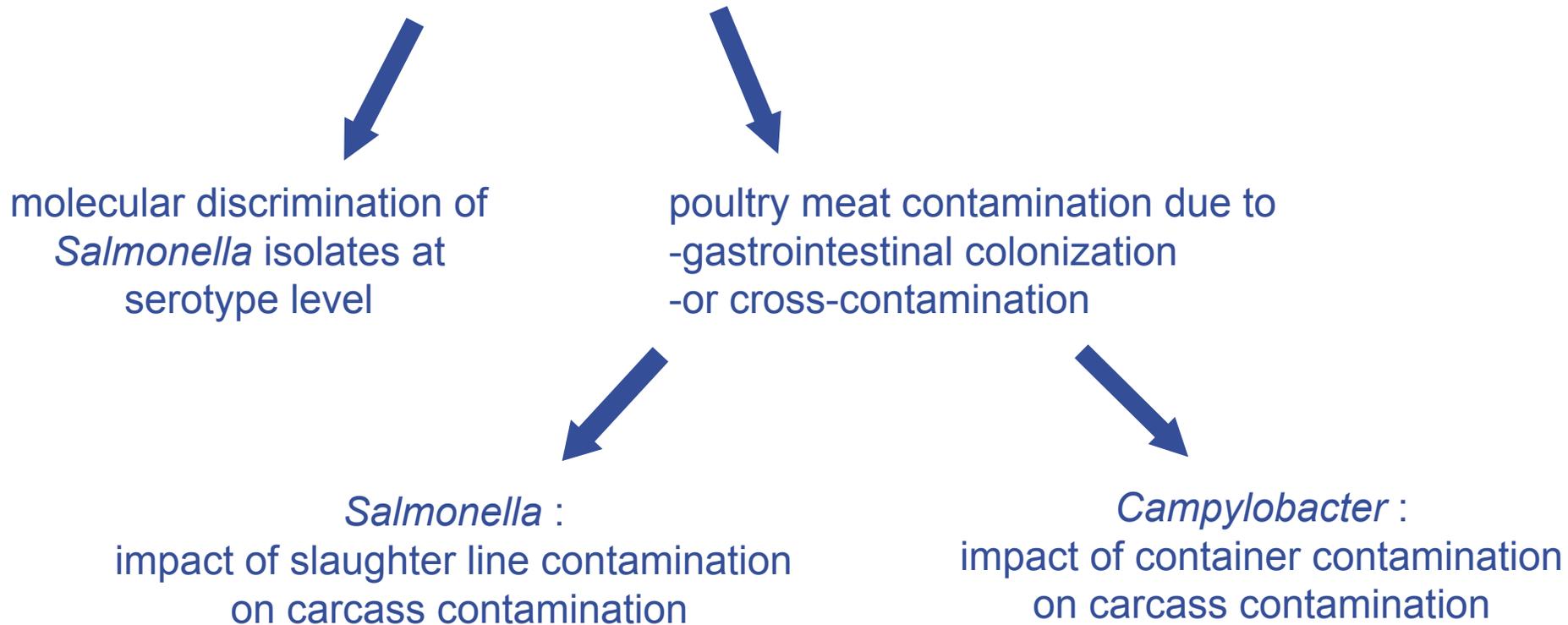
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5. Aim of the study

study of contamination of poultry with *Salmonella* and *Campylobacter* during transport and slaughter by means of molecular tools



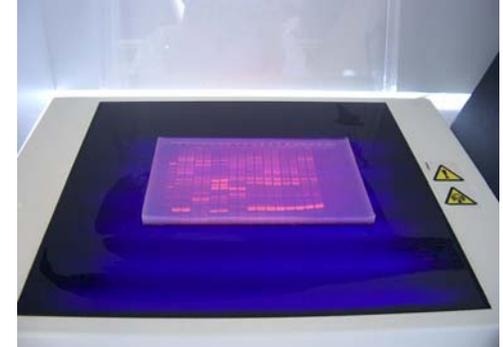
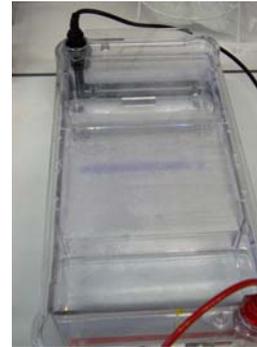
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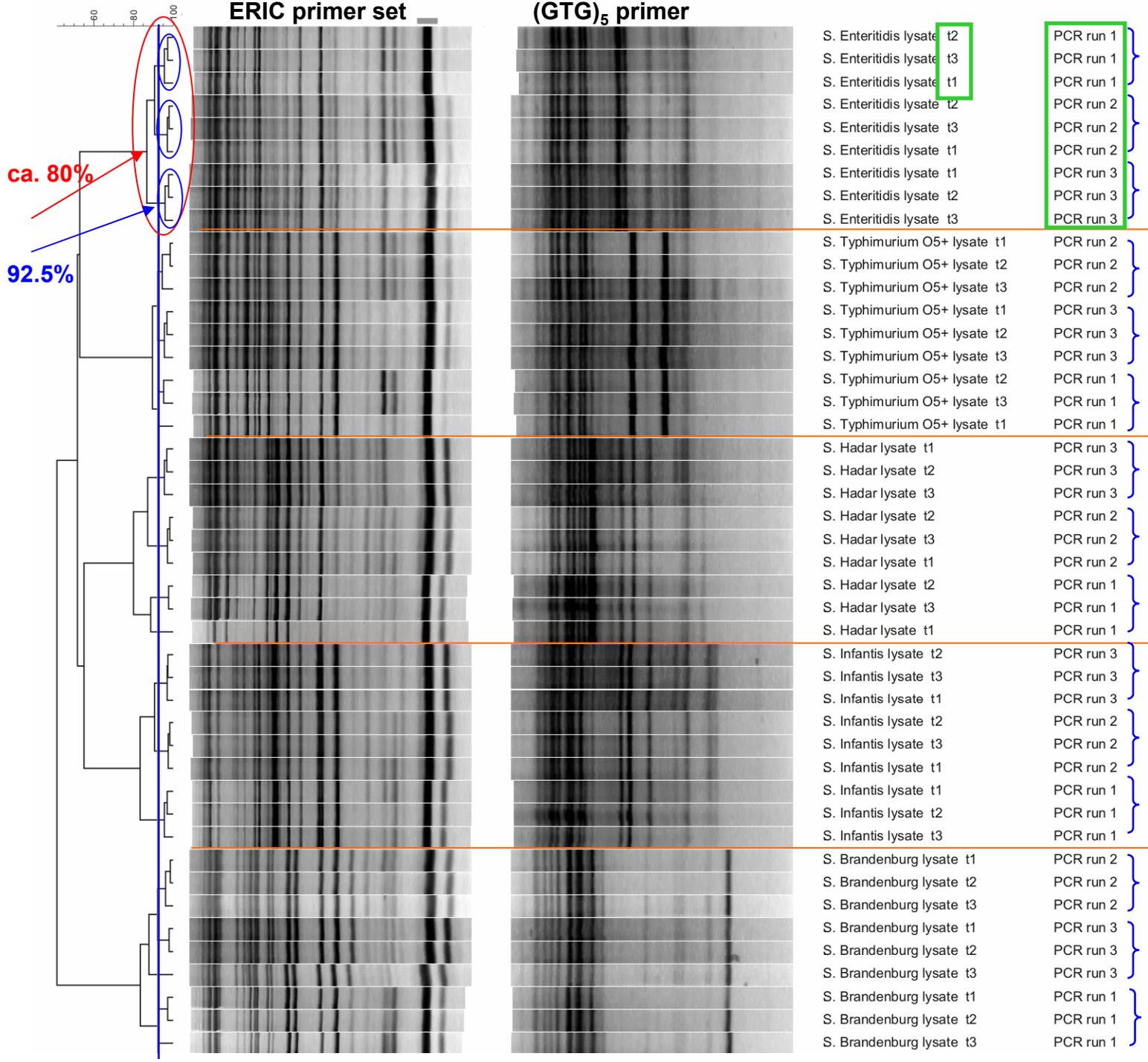
6. Molecular discrimination of *Salmonella* isolates at serotype level

Serotyping ↔ PCR-based techniques
(e.g., Repetitive-sequence-based PCR)



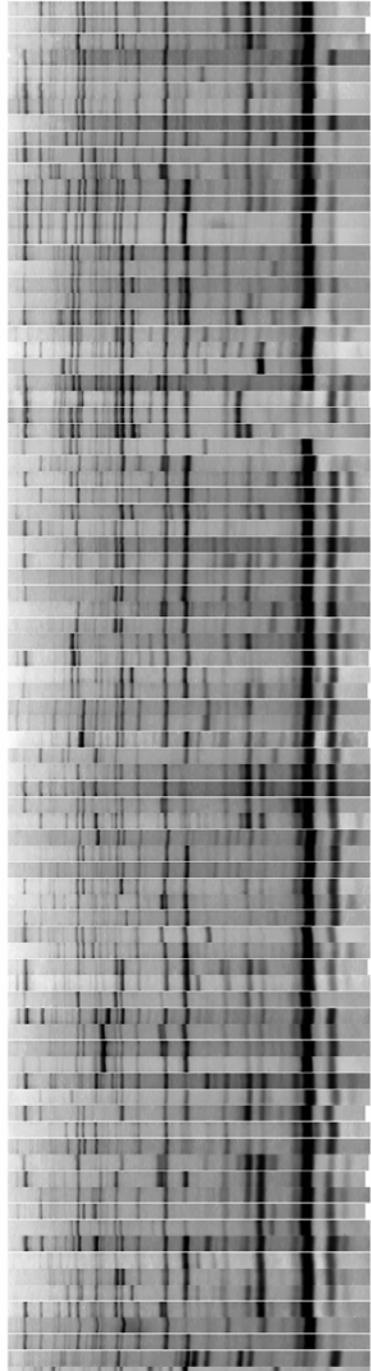
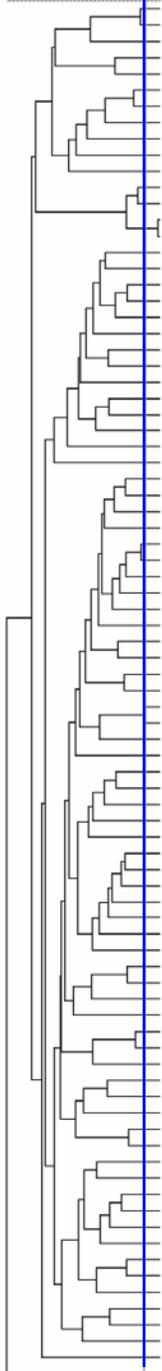
- Experiment 1 : 5 different primers tested
- Experiment 2 : reproducibility
- Experiment 3 : typeability & discriminatory power
- Experiment 4 : stability



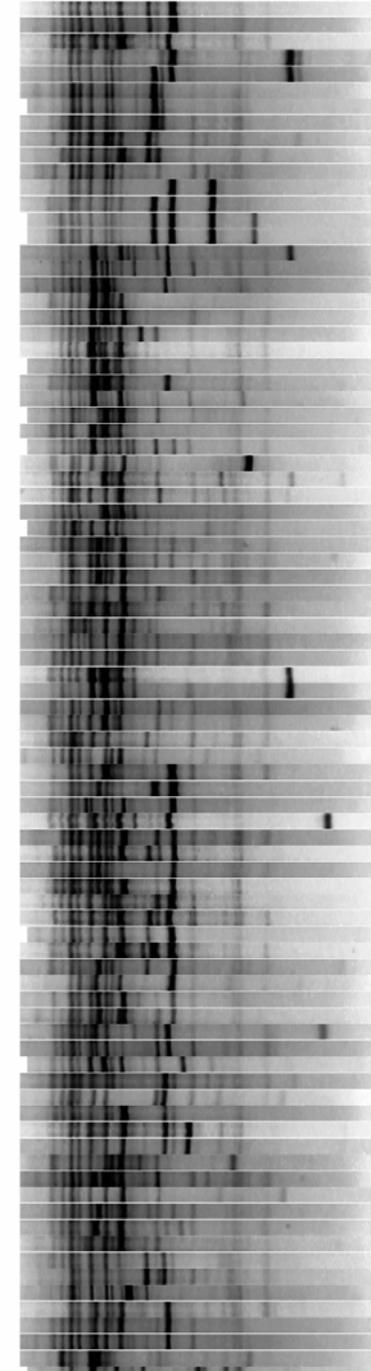


0 10 20 30 40 50 60 70 80 90 100

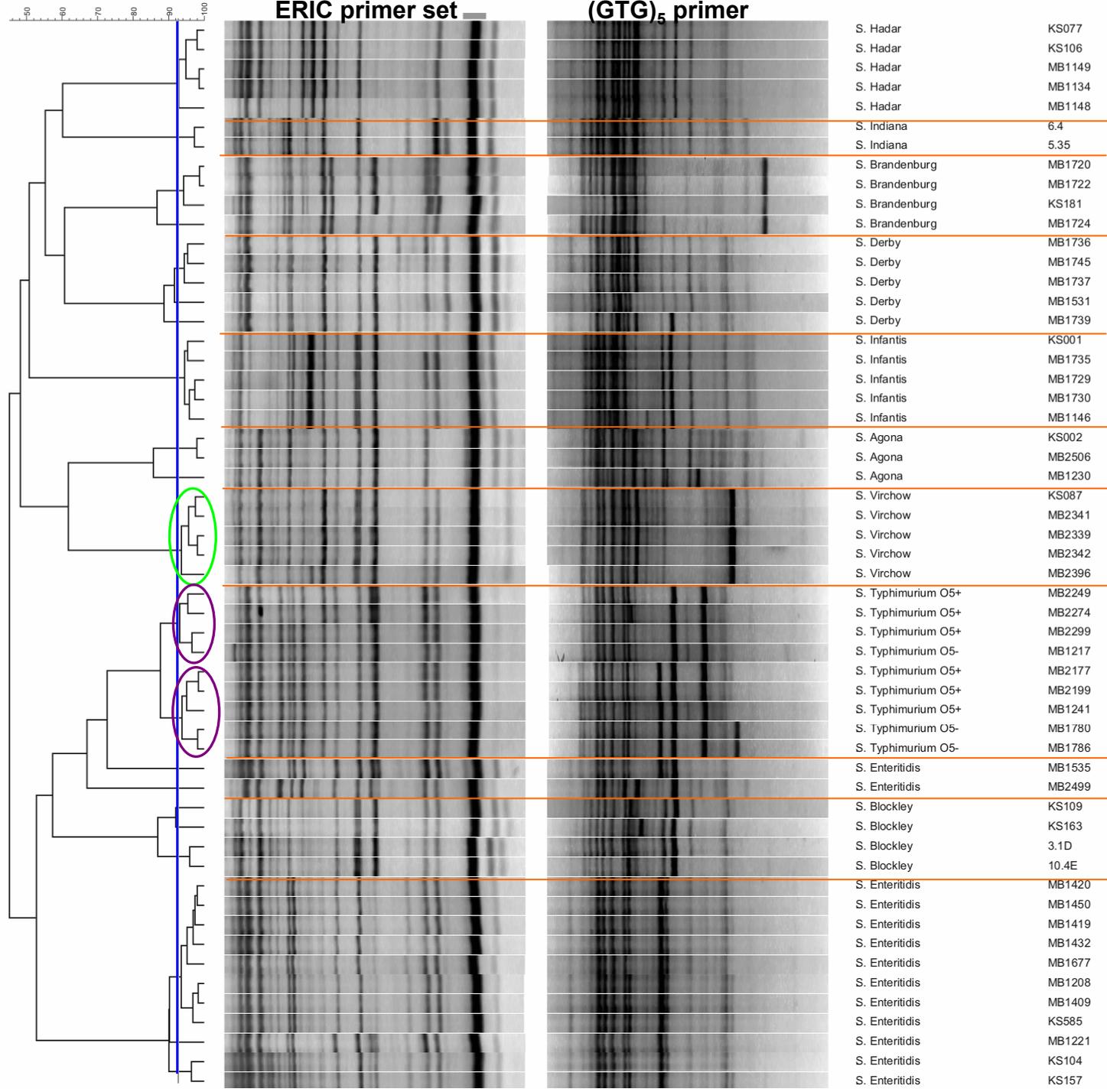
ERIC primer set



(GTG)₅ primer

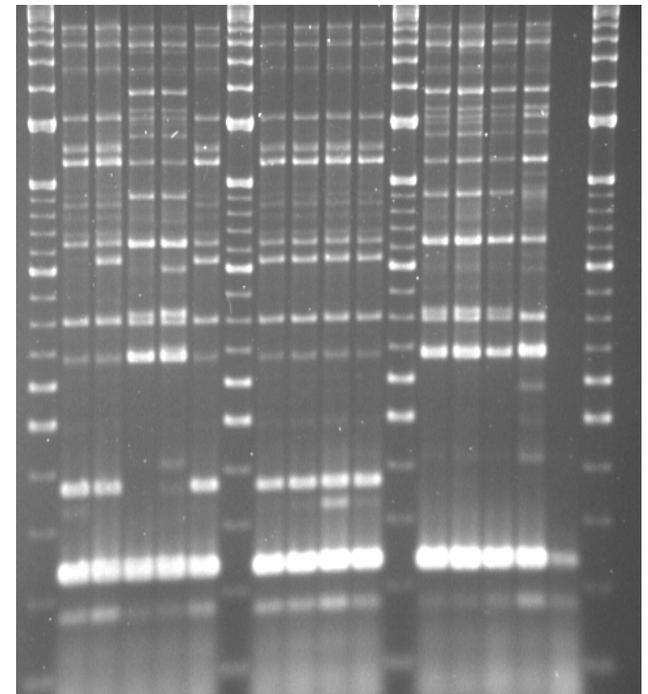


- S. 6.8 : - : 1.2
- S. Muenchan
- S. Adelaide
- S. Gallinarum
- S. Pullorum
- S. 9 : - : -
- Not typable
- S. Enteritidis
- S. London
- S. Manhattan
- S. Stanleyville
- S. Typhimurium
- S. Typhimurium var. Cc
- Not typable
- Not typable
- S. Panama
- S. Poona
- S. Oranienburg
- S. Urbana
- S. Montevideo
- S. Wien
- S. Bredeney
- S. Minnesota
- S. Give
- S. Sandiego
- S. Waycross
- S. Sundsvall
- S. Teleskebr
- S. Virchow
- S. Aba
- S. Agona
- S. Munster
- S. Apapa
- S. Derby
- Not typable
- S. Kentucky
- S. Kedougou
- S. Concord
- S. Mtandaka
- S. 4 : - : -
- S. Stourtridge
- S. Brandenburg
- S. Chester
- S. Paratyphi B
- Not typable
- S. Havana
- S. Isang
- S. Coeln
- S. Dublin
- S. Newport
- S. 47 : 2423 : -
- S. Kingston
- S. Bareilly
- S. Livingstone
- S. Braenderup
- S. Rivkyey
- S. Anatum
- S. Aberdeen
- S. Bovismorbificans
- S. Alona
- S. Plymouth
- S. Milan
- S. Hadar
- S. Infantis
- S. 6.7 : r : -
- S. Rubislaw
- S. Rissen
- S. Meleagridis
- S. Larochele
- S. 6.7 : - : 5
- S. Thompson
- S. Paratyphi A
- S. Kottbus
- S. Tennessee
- S. Uitchfield
- S. Indiana
- S. Goldcoast
- S. Senftenberg
- S. Swartzengrund
- S. Putten
- S. Cerro
- S. Kiambu
- S. Ohio
- S. Kintambo
- S. IV 48 : g251 : -



Conclusions

- clusters of isolates belonging to same serotype
- profiles of non-serotypeable isolates (molecular 'serotyping')
- profiles obtained by one PCR can be compared (or representative of each cluster)
- ERIC primer set and $(GTG)_5$ are equally useful for discrimination



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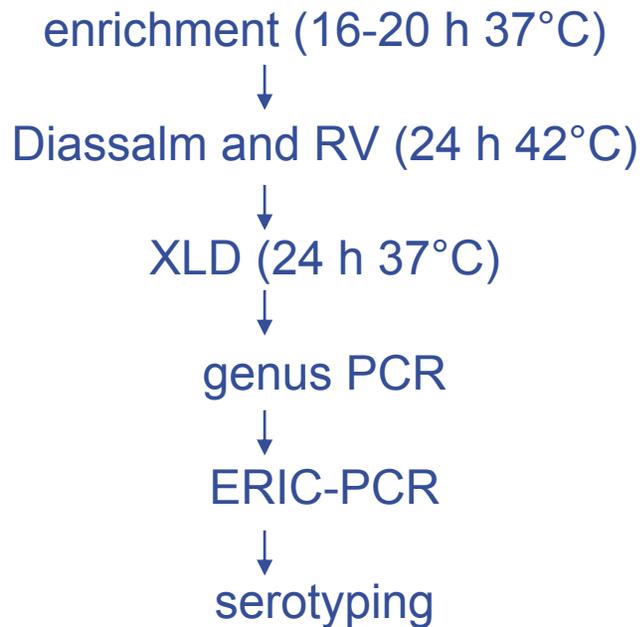


7. Materials and Methods

Bacteriological culture & Identification

crop swabs, neck skins, gastrointestinal tracts (duodenum & ceca), ...
diluted in 1:10 BPW

Salmonella



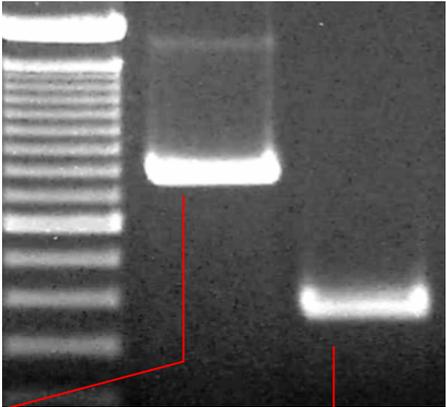
Bacteriological culture & Identification

crop swabs, neck skins, gastrointestinal tracts (duodenum & ceca), ...
diluted in 1:10 BPW

Campylobacter



1:10 dilution in preston
↓
enrichment (24 – 48 h at microaerobic conditions at 42°C)
↓
CCDA (24 h at 42°C)
↓
species-mPCR



C. jejuni

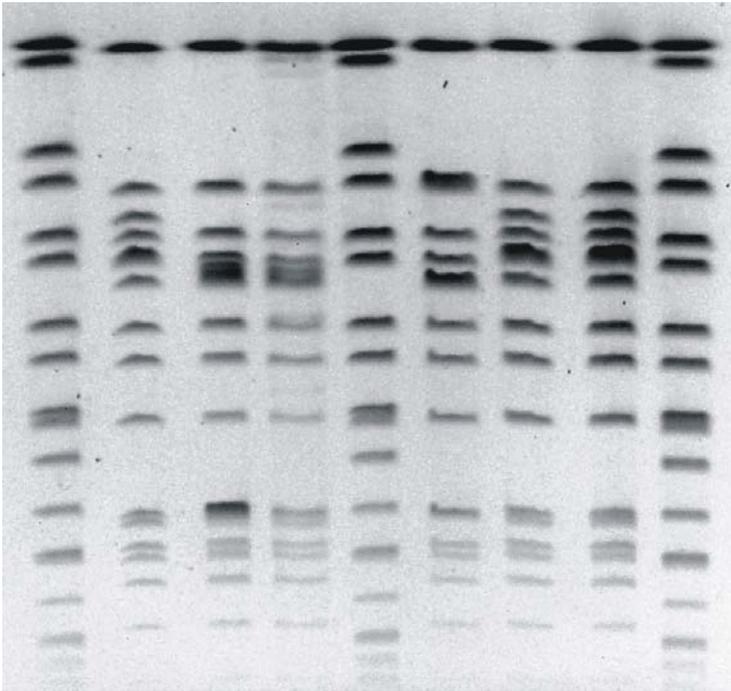
C. coli



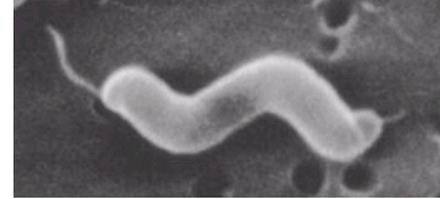
Characterization of the isolates

Salmonella:

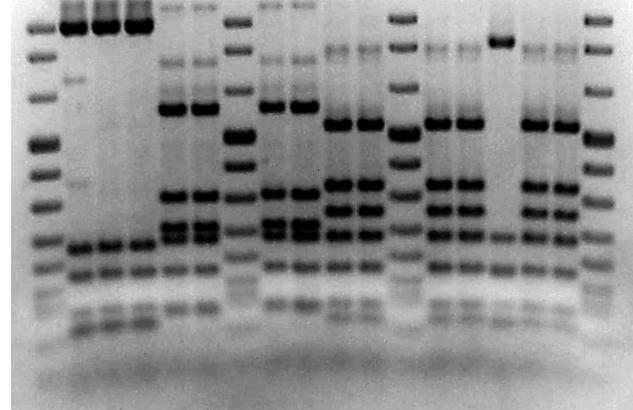
PFGE (*Sma*I, *Not*I, *Spe*I)



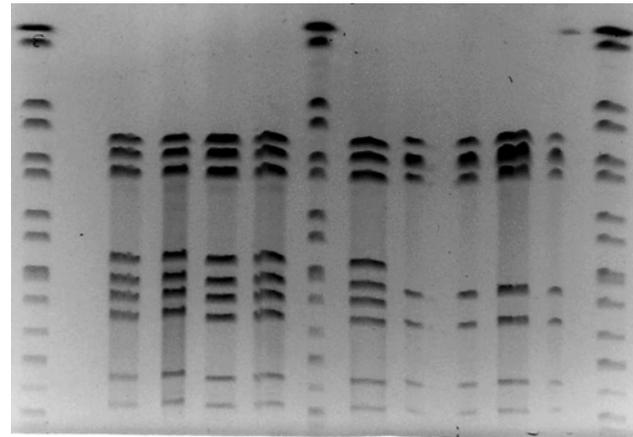
Campylobacter:



FlaA PCR/RFLP



PFGE (*Sma*I, *Kpn*I)



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8. Contribution of gastrointestinal colonization & cross-contamination to carcass contamination during slaughter

Salmonella

M&M:

- 30 crop swabs
- 30 gastrointestinal tracts
- 20 neck skins

} 4 broiler
slaughterhouses (x 3)



Results:

- 13% of flocks were colonized with *Salmonella* ↔ status (5%)
- 55% of flocks were contaminated with *Salmonella* after slaughter
→ Agona, Hadar, Infantis, Typhimurium, Virchow, Indiana, ...



gastrointestinal contribution

external contamination

gastrointestinal tract

Flock	NECK SKINS		CROP		DUODENUM		CECA	
	Numb. /20	Serotype & genotype	Numb. /3	Serotype genotype	Numb. /3	Serotype genotype	Numb. /3	Serotype genotype
1	1	Indiana I-1			3	Typhimurium T-1	1	Typhimurium T-1
2	1	Newport N-1			1	London		
3	1	NT-1						
4					1	NT-1		
5								
1								
2	1	Agona A-1						
3	19	Hadar H-1	1	Hadar H-1				



cross-contamination

Flock	NECK SKINS		CROP		DUODENUM		CECA	
	Numb. /20	Serotype & genotype	Numb. /3	Serotype genotype	Numb. /3	Serotype genotype	Numb. /3	Serotype genotype
1	3	Hadar H-1						
2	2	Hadar H-1						
3								
4	2	Hadar H-1						
1	12	Agona A-2 Virchow V-1 Typhimurium T-2						
2	9	Agona A-2 Typhimurium T-2 Ealing Ea-1	1	Typhimurium T-2				
3	8	Agona A-2 Typhimurium T-2 NT-2						
4	6	Agona A-2, A-3 Typhimurium T-2 O4:d:-						
5	5	Typhimurium T-2						
6	3	Typhimurium T-2						
7	3	Typhimurium T-2 O4:d:-						



Conclusions:

- gastrointestinal contribution is limited
- cross-contamination is high
- origin of majority of strains is unknown
- logistic slaughter is difficult



8. Contribution of gastrointestinal colonization & cross-contamination to carcass contamination during slaughter

Campylobacter

M&M:

- 30 crop swabs
 - 30 gastrointestinal tracts
 - 20 neck skins
- } 3 broiler slaughterhouses (x 3)



Results:

- 72% of flocks were colonized with *Campylobacter*
- 79% of flocks were contaminated with *Campylobacter* after slaughter
→ *C. jejuni* (89%), *C. coli* (8.7%) and *C. lari* (2.3%)



	NECK SKINS		CROP		DUODENUM		CECA	
Flock	Numb /20	Species & genotype	Numb /3	Species genotype	Numb /3	Species genotype	Numb /3	Species genotype
1	18	<i>C. jejuni</i> G-1	1	<i>C. jejuni</i> G-1	3	<i>C. jejuni</i> G-1	3	<i>C. jejuni</i> G-1
2								
3	20	<i>C. jejuni</i> G-2	3	<i>C. jejuni</i> G-2	3	<i>C. jejuni</i> G-2 <i>C. coli</i> G-3	3	<i>C. coli</i> G-3
4	18	<i>C. jejuni</i> G-2						
5	7	<i>C. jejuni</i> G-2 <i>C. coli</i> G-3						
1	16	<i>C. jejuni</i> G-4	2	<i>C. jejuni</i> G-5	3	<i>C. jejuni</i> G-6	1	<i>C. jejuni</i> G-6
2	20	<i>C. jejuni</i> G-5 <i>C. jejuni</i> G-7	3	<i>C. jejuni</i> G-5	3	<i>C. jejuni</i> G-7	3	<i>C. jejuni</i> G-7
3	20	<i>C. jejuni</i> G-8 <i>C. jejuni</i> G-5	3	<i>C. jejuni</i> G-8	3	<i>C. jejuni</i> G-8	3	<i>C. jejuni</i> G-8
4	20	<i>C. jejuni</i> G-8	3	<i>C. jejuni</i> G-8	3	<i>C. jejuni</i> G-8	2	<i>C. jejuni</i> G-8



Conclusions for *Salmonella*:

- gastrointestinal contribution is limited
- cross-contamination is high
- origin of majority of strains is unknown
- logistic slaughter is difficult

Conclusions for *Campylobacter*:

- gastrointestinal contribution is high
- cross-contamination is limited
- origin of only a few strains is unknown
- flocks slaughtered on same day had same strains in intestines ???



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9. *Salmonella*: impact of the slaughter line contamination on carcass contamination

Observation: origin of majority of strains is unknown

Hypothesis: slaughter line contamination

M&M:

- 6 flocks (3 slaughterhouses, x2)
- samples of slaughter line
- samples of first flock
 - gastrointestinal tracts
 - neck skins
 - feathers
 - containers



slaughterline

first flock

Samples	Number	Serotype & genotype
Scalding tank (+ water)	6/19	Typhimurium T-1 Blockley B-1 Paratyphi B P-1 Indiana I-1
Plucking machine	8/15	Typhimurium T-1 Blockley B-1 Paratyphi B P-1 Indiana I-1 Montevideo M-1 M-2, M-3 Agona A-1, A-2
Evisceration	2/14	Typhimurium T-1 Paratyphi B P-1
Crates	1/6	Typhimurium T-1
Feathers before scalding	3/3	Typhimurium T-1
Feathers after scalding	2/3	Agona A-2
Feathers during plucking	3/3	Montevideo M-1 Rissen
Neck skins after plucking	13/30	Typhimurium T-1, T-2 Blockley B-1 Paratyphi B P-1
Neck skins after evisceration	15/30	Minnesota Mi-1

from flock
slaughtered 4
day before



Results:

- 2/3 slaughterhouses contaminated on slaughter line before start slaughter activities:
 - shackles & wheels, plucking machine, scalding
 - containers

strains from *Salmonella* colonized flocks in the week before



slaughter line



contamination of flocks



OVERVIEW

1. Situation of the problem
2. Characteristics of *Salmonella* and *Campylobacter*
3. Clinical aspects of *Salmonella* and *Campylobacter* infections
4. Poultry flocks
5. Aim of the study
6. Molecular discrimination of *Salmonella* isolates at serotype level
7. Materials and Methods
8. Contribution of gastrointestinal colonization and cross-contamination to carcass contamination during slaughter
9. *Salmonella*: impact of the slaughter line contamination on carcass contamination
- 10. *Campylobacter*: contamination or colonization of poultry flocks after transport**
11. Conclusions



10. *Campylobacter*: contamination or colonization of poultry flocks after transport

Observation: flocks slaughtered on same day had same strains in intestines

Hypothesis: flocks become colonized during transport

M&M:

- 7 *Campylobacter*-free flocks
- at the farm: just before depopulation
 - samples from cecal droppings & breast
 - samples from 5 containers
- at the slaughter house:
 - samples from breast, feet, head
 - samples from crop & gastrointestinal tract



Results:

-25/35 container *Campylobacter* positive (54% *C. jejuni*, 46% *C. coli*)

-15 by direct plating

-10 by enrichment

cfu/cm²

	Cont. 1	Cont. 2	Cont. 3	Cont. 4	Cont. 5
Flock 1	2	90	5	3	+
Flock 2	+		+	1	+
Flock 3	82	12	2	14	27
Flock 4	5	NC	+	1	
Flock 5	+	NC	1	+	+
Flock 6					
Flock 7			+		+



Results:

- 25/35 container *Campylobacter* contaminated (54% *C. jejuni*, 46% *C. coli*)
- 3/7 flocks became colonized in last days before slaughter
 - no colonization, no co-colonization
 - limited external contamination

Birds at the farm	5 Containers	Birds at slaughterhouse (external contamination)	Birds at slaughterhouse (intestines)
	<i>C. jejuni</i> G-1 <i>C. jejuni</i> G-2 <i>C. coli</i> G-3 <i>C. jejuni</i> G-4 <i>C. jejuni</i> G-5 <i>C. jejuni</i> G-6 <i>C. jejuni</i> G-7 <i>C. jejuni</i> G-8	<i>C. jejuni</i> G-2 <i>C. jejuni</i> G-9	



OVERVIEW

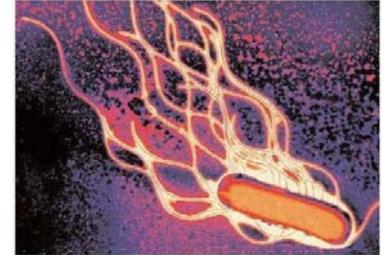
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11. Conclusions

Salmonella

- 13% of flocks colonized; 55% of flocks contaminated after slaughter
- cross-contamination was important
- caused by contamination of the slaughter equipment



adaption of cleaning & disinfection process

Campylobacter

- 72% of flocks colonized; 79% of flocks contaminated after slaughter
- carcass contamination caused by gastrointestinal colonization
- transport containers often contaminated → limited external contamination



reduction of number of colonized flocks at the farm
adaption of cleaning & disinfection process of containers





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