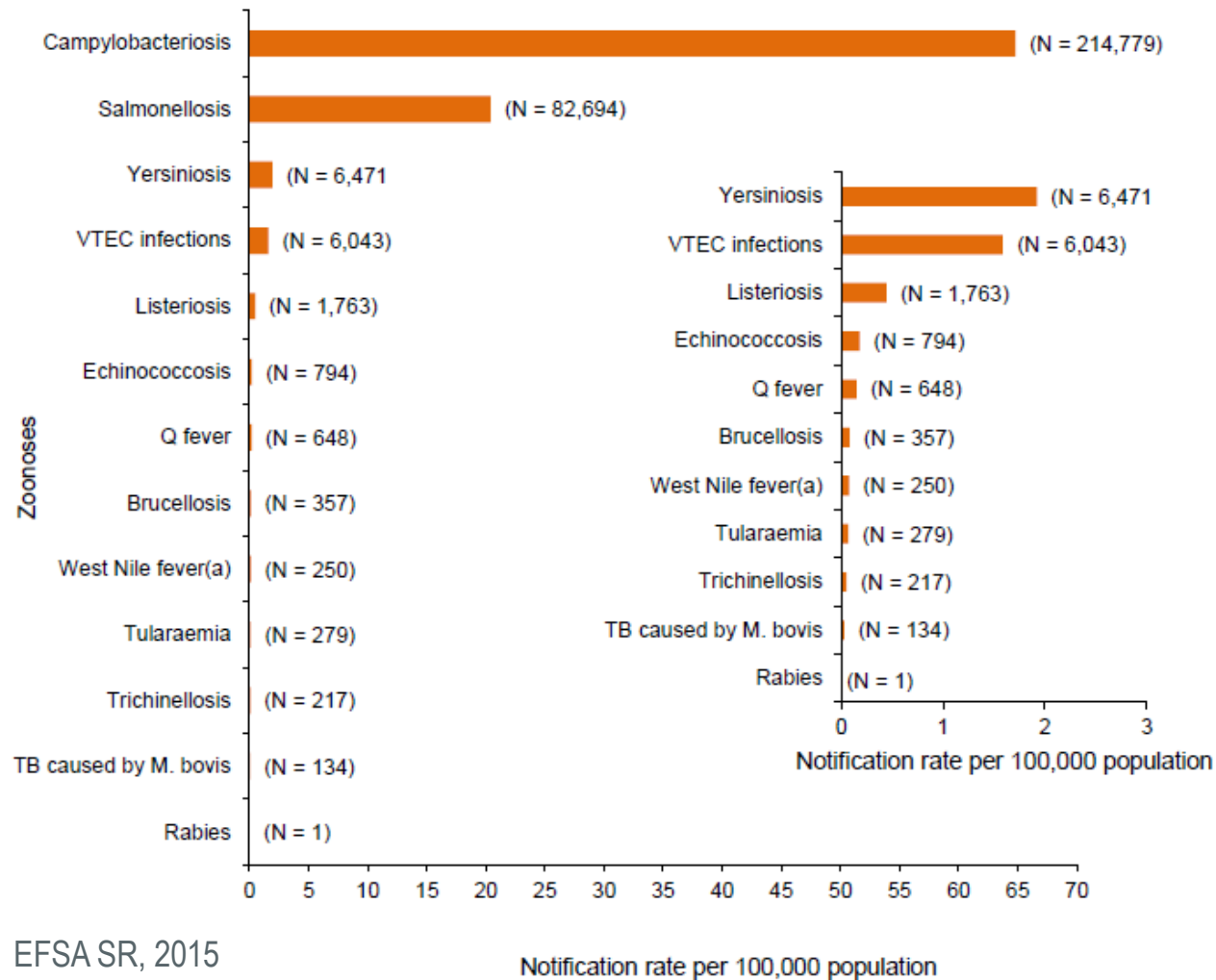


Relation between *C. jejuni* strains isolated from the clinical cases and collected from the monitoring carcass broiler plan in Belgium during 2011 to 2013

Workshop:
***Campylobacter* in slaughterhouses and cutting plants:
risks and opportunities for better control**
28 January 2016, Brussels

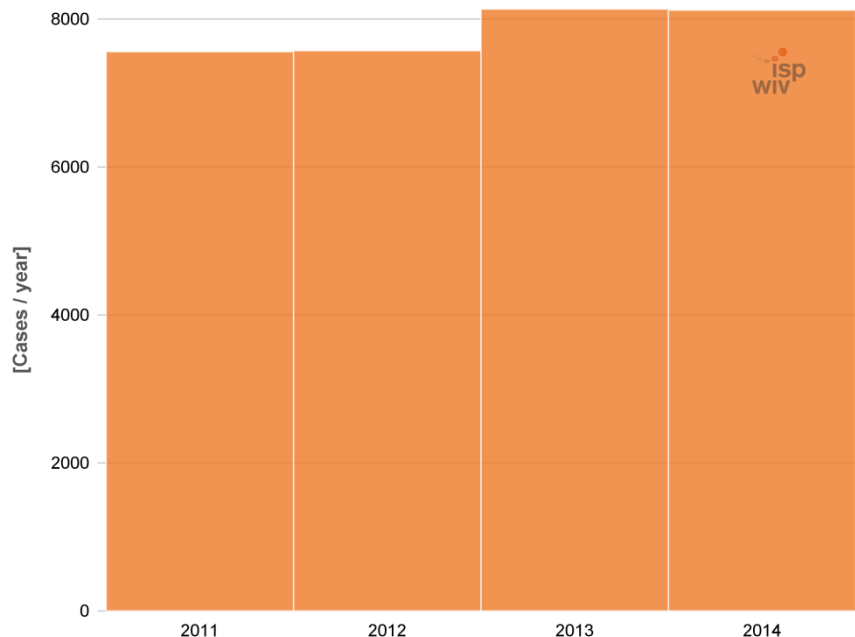
Dr. Nadine Botteldoorn
NRL *Campylobacter*

Campylobacteriosis in EU

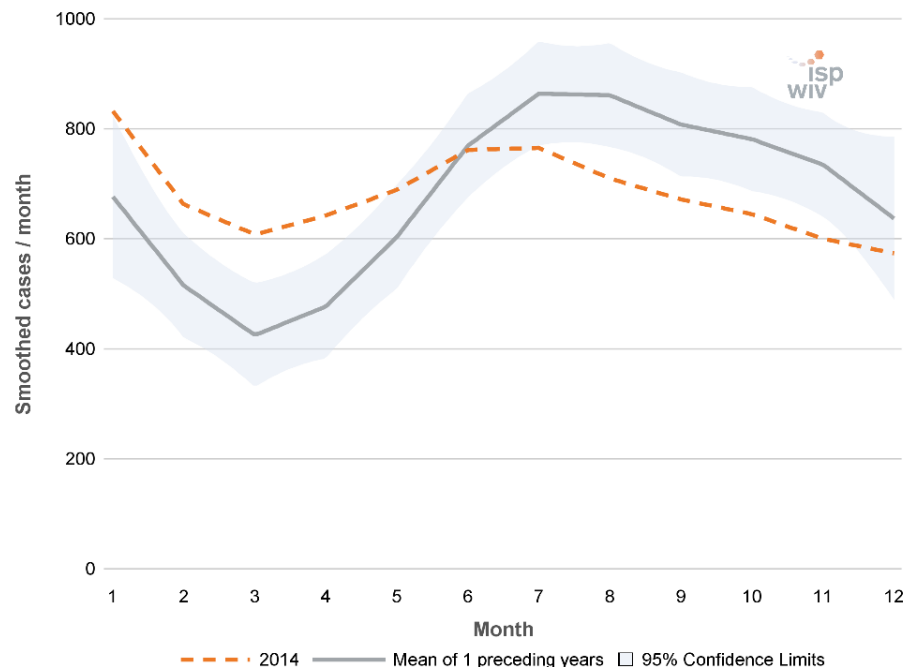


EFSA SR, 2015

Campylobacteriosis in Belgium



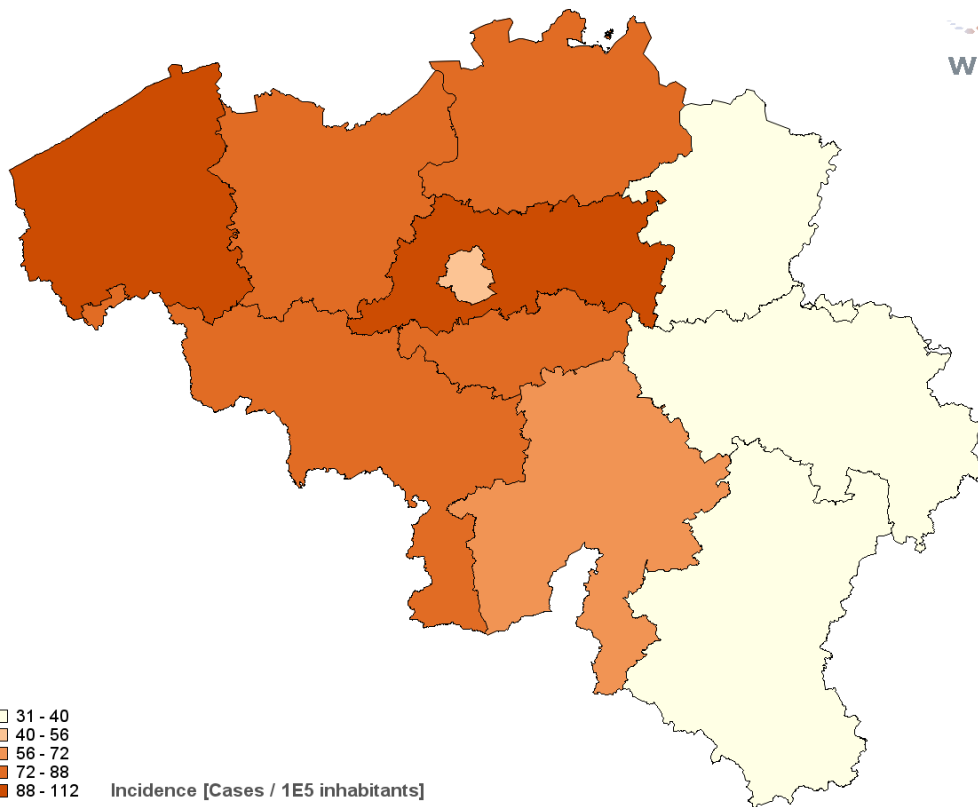
Monthly distribution of Campylobacter



> 8000 reported cases /year

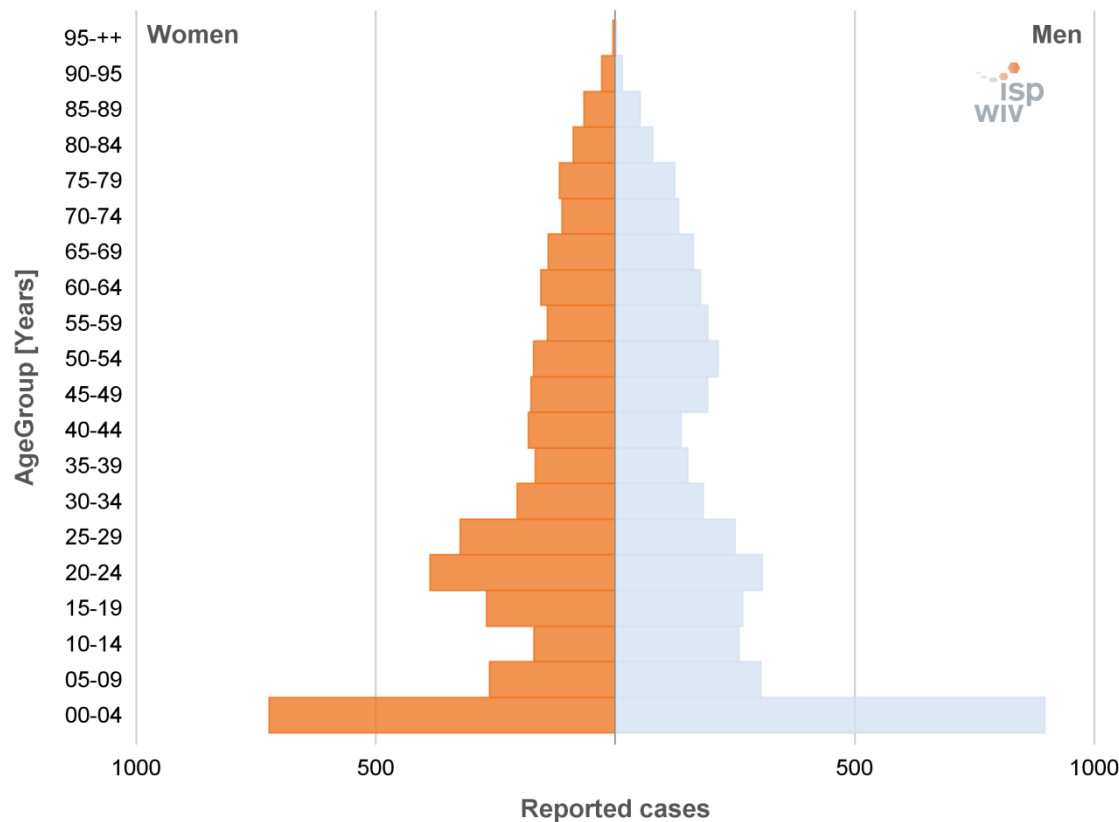
Seasonality of the reported infection (July and August most of the cases)

Incidence rate in 2014



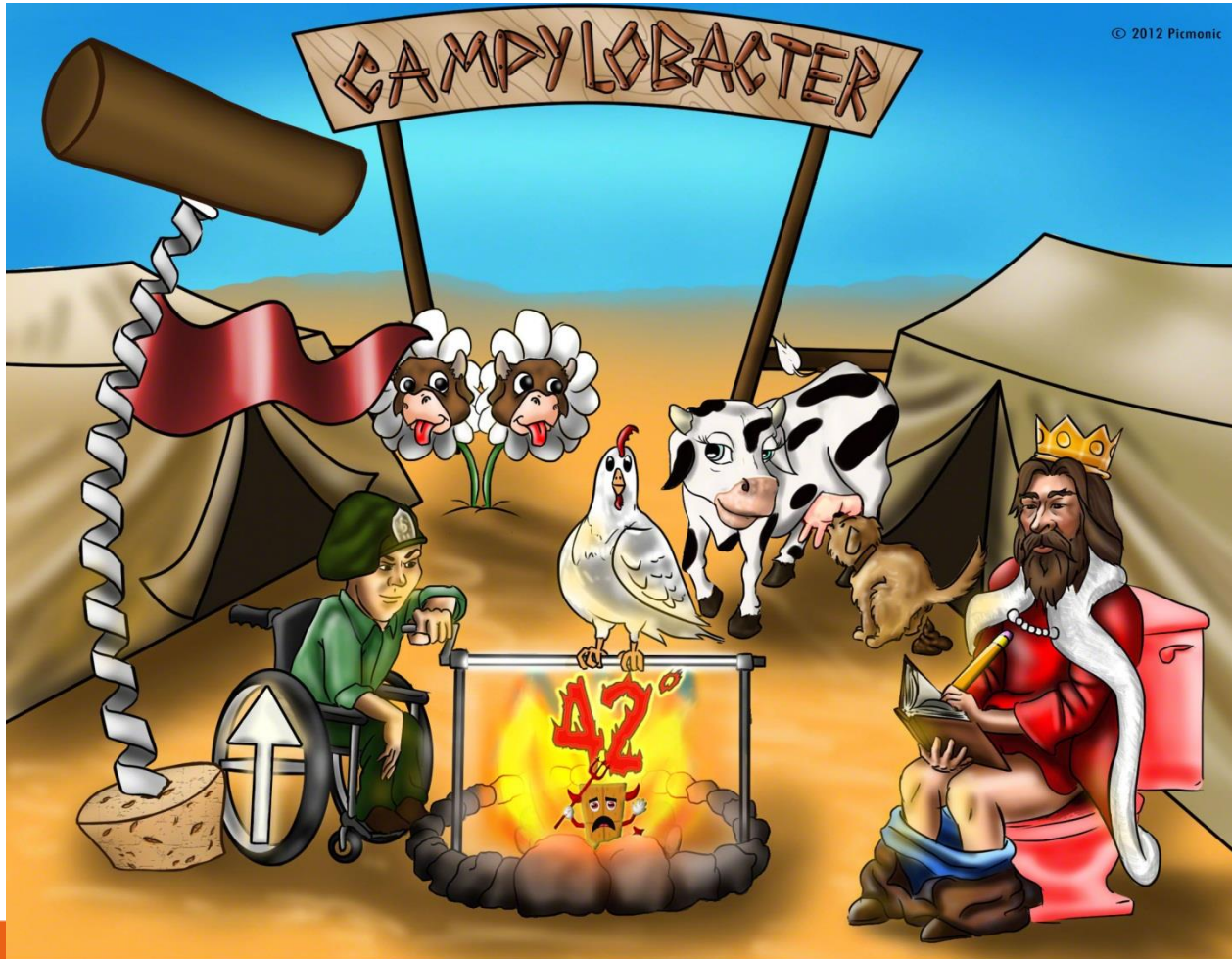
Province	NIS1_5	Cases	Population	Incidence
Vlaams-Brabant	23	1218	1086446	112.1
West-Vlaanderen	3	1063	1164967	91.2
Antwerpen	1	1546	1764773	87.6
Brabant Wallon	25	327	382866	85.4
Hainaut	5	1040	1317284	79.0
Oost-Vlaanderen	4	1112	1445831	76.9
Namur	9	289	476835	60.6
Brussels	21	590	1119088	52.7
Luxembourg	8	99	271352	36.5
Liège	6	374	1077203	34.7
Limburg	7	262	844621	31.0

Age distribution 2014

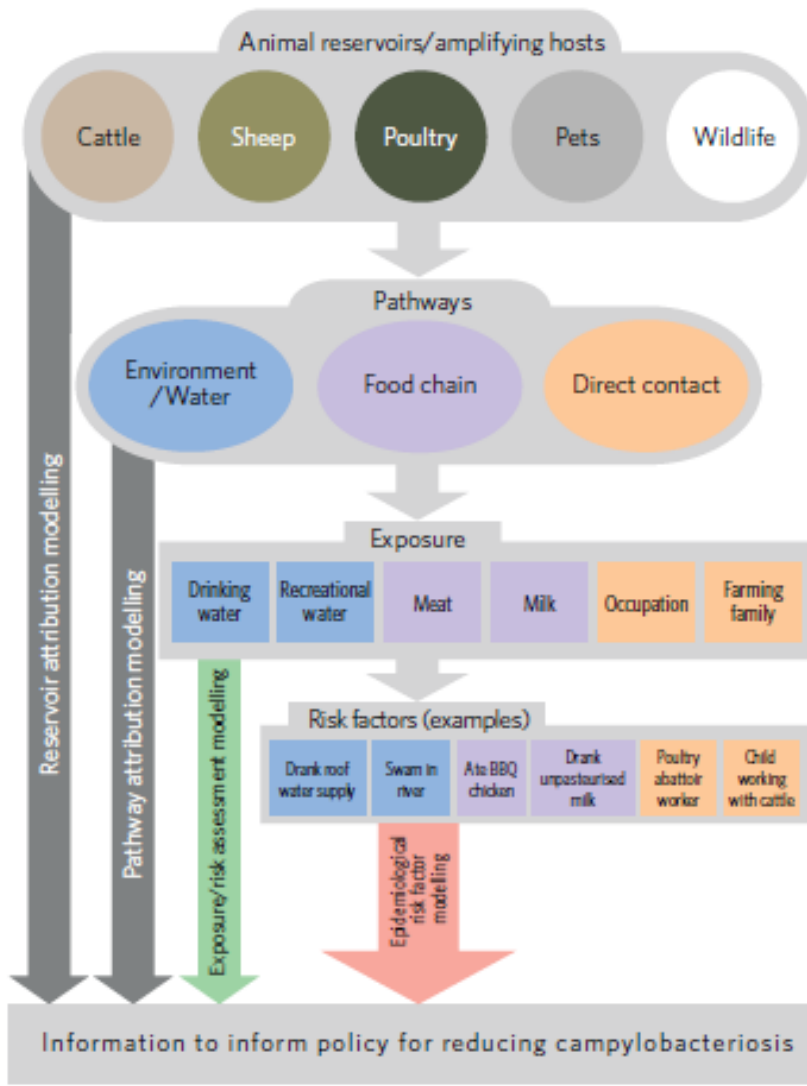


Population at risk : especially children < 5 years and young adults between 20-29 years old

Transmission routes and sources of *Campylobacter*



Source attribution and transmission



The Dioxin Crisis as Experiment To Determine Poultry-Related *Campylobacter* Enteritis

Akke Vellinga and Frank Van Loock

APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2009, p. 4277-4288
0099-2240/09/\$08.00+0 doi:10.1128/AEM.02269-08
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Vol. 75, No. 13

Correlation between Genotypic Diversity, Lipooligosaccharide Gene Locus Class Variation, and Caco-2 Cell Invasion Potential of *Campylobacter jejuni* Isolates from Chicken Meat and Humans: Contribution to Virulotyping^V

Ihab Habib,^{1,6*} Rogier Louwen,² Mieke Uyttendaele,³ Kurt Houf,¹ Olivier Vandenberg,⁴ Edward E. Nieuwenhuis,² William G. Miller,⁵ Alex van Belkum,² and Lieven De Zutter¹

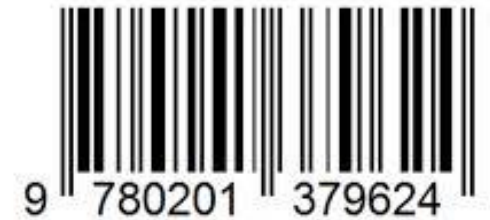
Current epidemiology !!!!!!!

Campylobacter characterization

Guidelines for the validation and application of
typing methods for use in bacterial epidemiology
(Van Belkum A. et al. 2007)

Typing

- analysis of bacterial isolates below the species/subspecies level
- phenotypic and/or genotypic
- Generate strain/clone specific data



- long term surveillance
- elucidate bacterial transmission patterns
- find reservoirs or sources of infection in humans
- give outbreak support (short term surveillance)

Selection of the *Campylobacter* isolates

Monitoring broiler carcass (MBC) isolates

Collected from the official control of *Campylobacter* in broiler meat by the Belgium National Reference Laboratory

Human (H) isolates

Collected from human cases in Brussels by the Belgium Human National Reference Center

Period = during 2011, 2012 and 2013

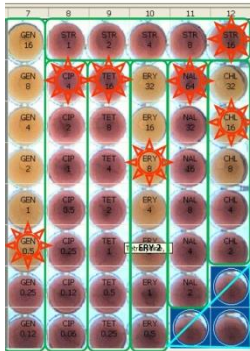
Isolates randomly selected

Year	<i>C. jejuni</i>	
	H	MBC
2011	24	32
2012	24	31
2013	24	34
Total	72	97

Typing methods used

Antibiotic microbiological resistance profile (AMRp)

Sensititre EUCAMP plate

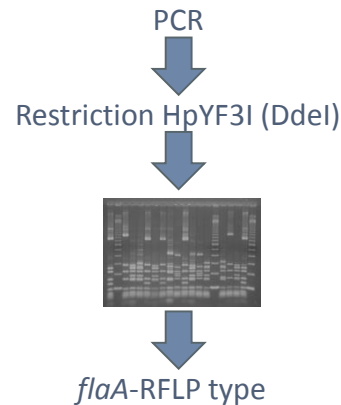


GEN Gentamicin
STR Streptomycin
CIP Ciprofloxacin
TET Tetracycline
ERY Erythromycin
NAL Nalidixic Acid
POS Positive Control
CHL Chloramphenicol

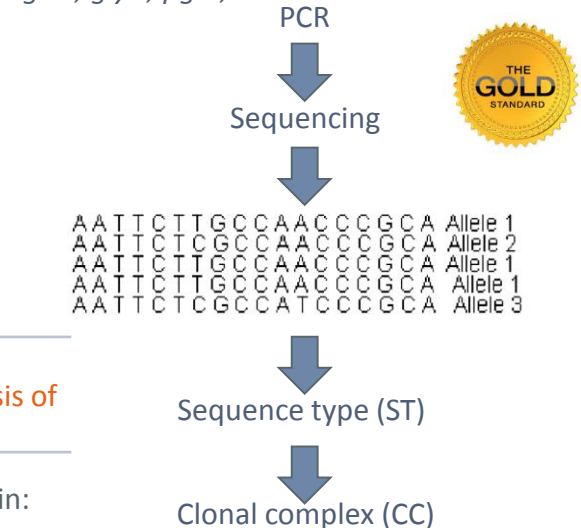
Cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, <http://mic.eucast.org/Eucast2>).

flaA-RFLP typing

PCR and restriction fragment length polymorphism of flagellin gene A (PCR/*flaA*-RFLP)



Multi Locus Sequence typing (MLST) of 7 housekeeping genes: *aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt* and *uncA*



Lipooligosaccharide (LOS) class determination

Determination by PCR and electrophoresis

Target	Virulent factor involved in:
LOS	- Binding, adhesion and invasion
	- Mimicry of LOS to human gangliosides, in particular sialylated ones present a higher invasion potential and may be in the origin of Guillain-Barré syndrome.

Virulence profile (Vp)

Determination by PCR and electrophoresis of the presence or absence of the gene

Gene	Virulent factor involved in:
<i>cadF</i>	Binding, adhesion and invasion
<i>cdtA</i> , <i>cdtB</i> and <i>cdtC</i>	Toxin production
<i>ceuE</i>	Invasion

How to compare methods?

Measure the discriminatory ability of typing systems

C. jejuni: Different typing methods



Simpson's Index of Diversity - indicates the probability of 2 strains sampled randomly from a population belonging to 2 different types.

Adjust Wallace coefficient - indicates directional information concerning the agreement between partitions indicating the probability of two isolates with the same category in the 1st typing method fall in the same category when the 2nd typing method is applied.

Comparison of the different typing methods

Simpson's index of Diversity (ID)

Method	# partitions	Simpson's ID	CI (95%)
ST	74	0.975	(0.968-0.983)
<i>flaA</i> -RFLP	48	0.930	(0.906-0.955)
CC	22	0.885	(0.859-0.908)
LOS	6	0.801	(0.778-0.823)
AMRp	14	0.758	(0.720-0.795)
Vp	9	0.148	(0.073-0.222)

Which methods to combine ?

Adjust Wallace coefficient (AW)

1st Typing method




2nd Typing method

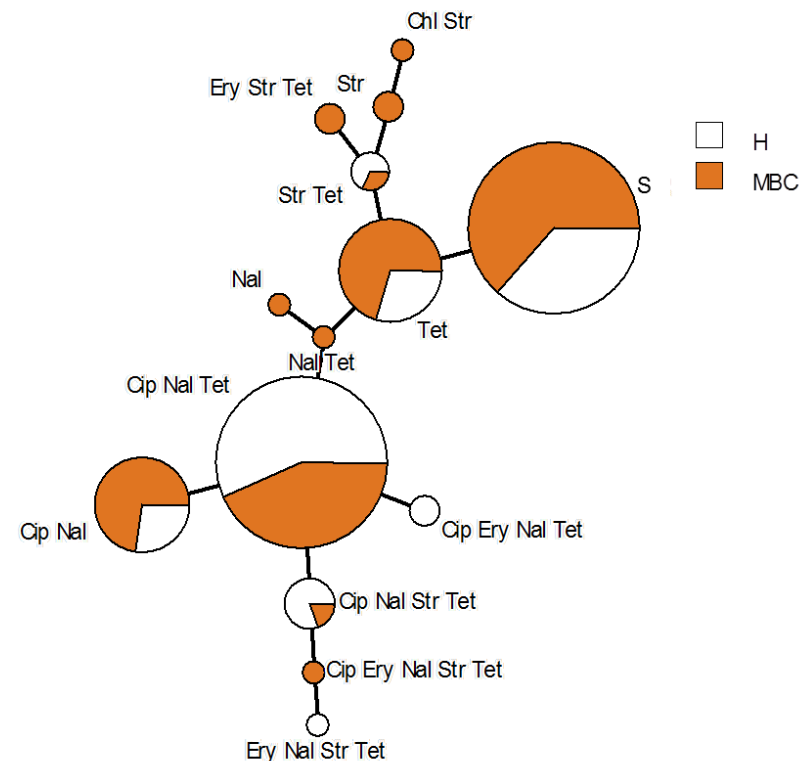
	AMRp	flaA	LOS	Vp	ST	CC
AMRp		0.005 (0.000-0.033)	0.045 (0.000-0.091)	0.000 (0.000-0.588)	0.016 (0.000-0.032)	0.033 (0.000-0.071)
flaA	0.022 (0.000-0.090)		0.256 (0.161-0.351)	0.144 (0.000-0.793)	0.091 (0.053-0.129)	0.271 (0.152-0.390)
LOS	0.058 (0.000-0.128)	0.077 (0.025-0.129)		0.274 (0.000-0.632)	0.092 (0.057-0.127)	0.335 (0.248-0.423)
Vp	0.000 (0.000-0.052)	0.002 (0.000-0.031)	0.012 (0.000-0.048)		0.003 (0.000-0.012)	0.005 (0.000-0.037)
ST	0.199 (0.080-0.318)	0.268 (0.189-0.348)	0.900 (0.827-0.974)	0.672 (0.362-0.982)		1.000 (1.000-1.000)
CC	0.080 (0.008-0.152)	0.156 (0.090-0.221)	0.640 (0.530-0.750)	0.230 (0.000-0.624)	0.195 (0.147-0.242)	

AMRp = *flaA*-RFLP
(p-value = 0,501)

AWc → Additional information given by the 2nd method

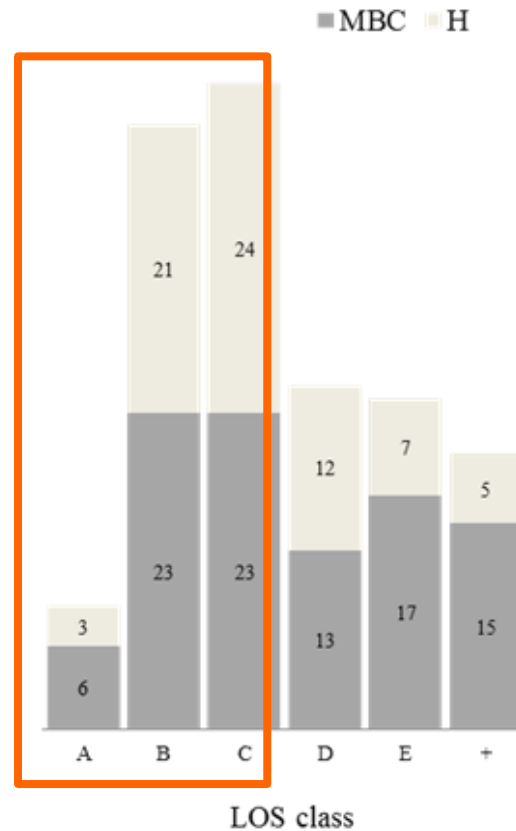
Antimicrobial resistance AMR(%)

Antibiotic	H (n=72)	MBC (n=97)
Chloramphenicol (Chl)	0,0	1,0
Ciprofloxacin (Cip)	58,3	40,2 
Erythromycin (Ery)	4,2	3,1
Gentamicin (Gen)	0,0	0,0
Nalidixic acid (Nal)	59,7	42,3 
Streptomycin (Str)	9,7	8,2
Tetracycline (Tet)	63,9	45,4 



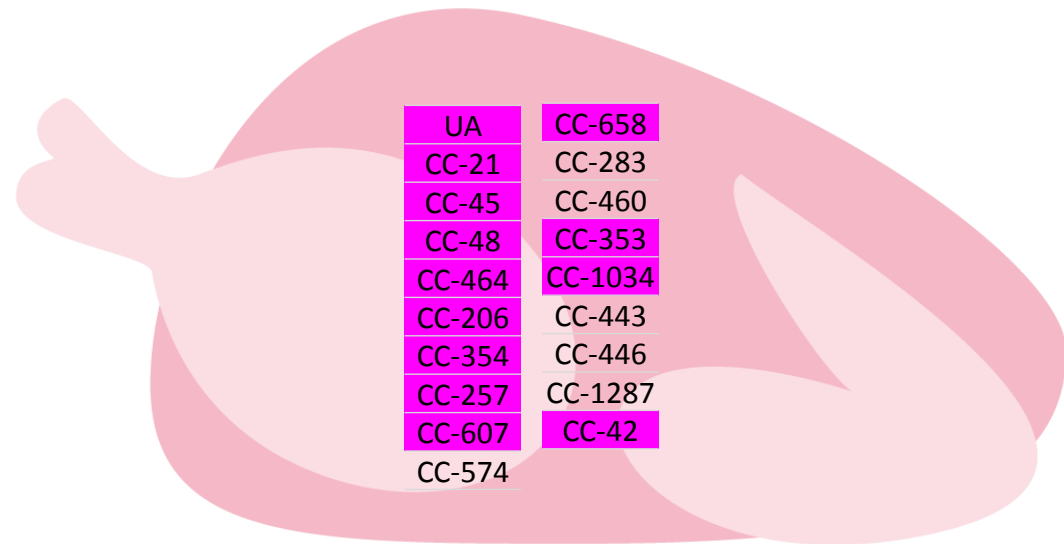
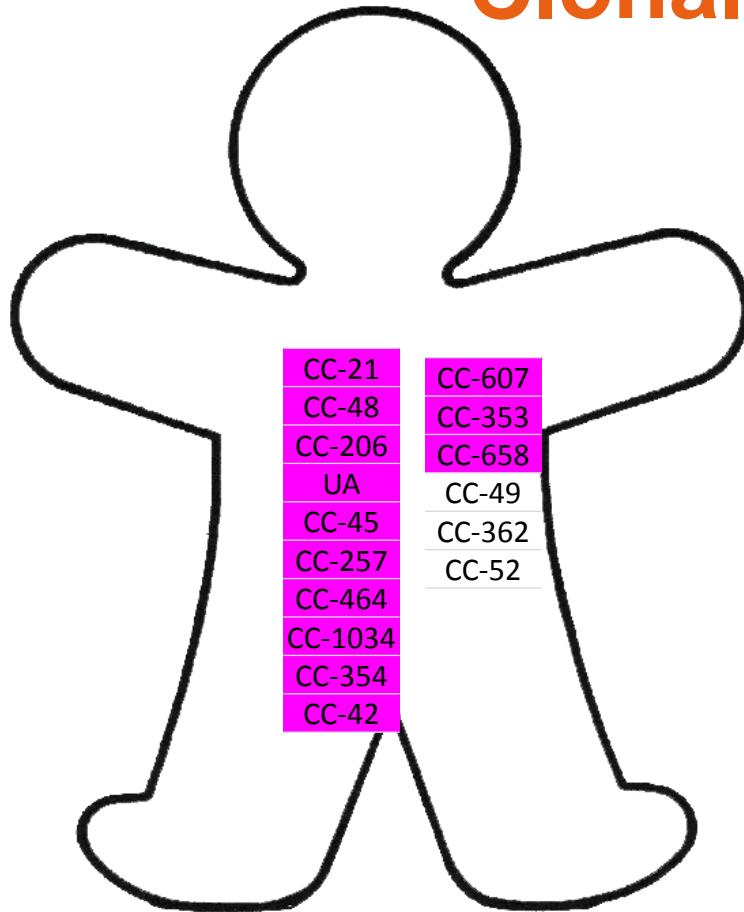
- The human *C. jejuni* isolates showed a significant higher resistance than the monitoring isolates for Cip, Nal and Tet

LOS class classification



- LOS class B and C were the most commonly found for H isolates
- All but the LOS class A were equally found MBC isolates
- LOS class A+B+C H = 66.7 %; MBC= 53,6%

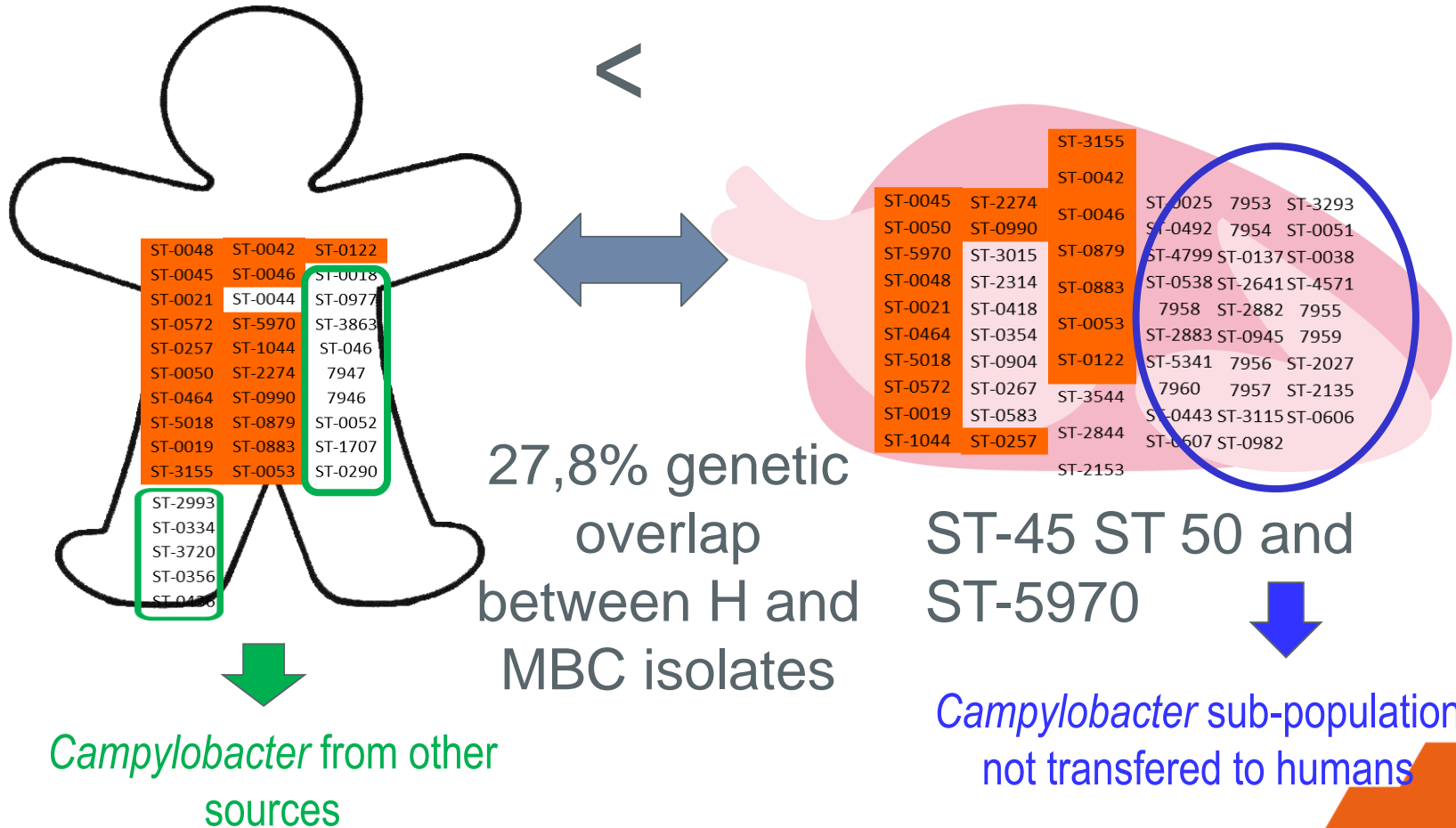
Clonal complex (CC)



- CC-21 most frequent CC for H (18/72) and MBC (21/97) isolates
- The first H and MBC CCs were the same but in a different order.

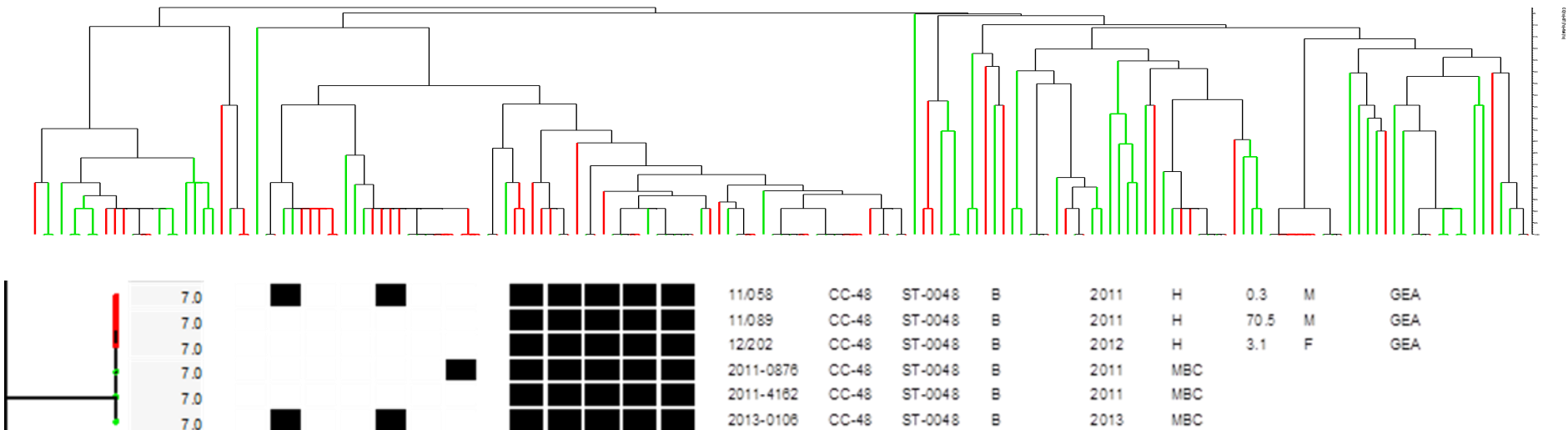
Sequence Type (ST)

Diversity very big



Clusters found between human and broiler strains at 100%

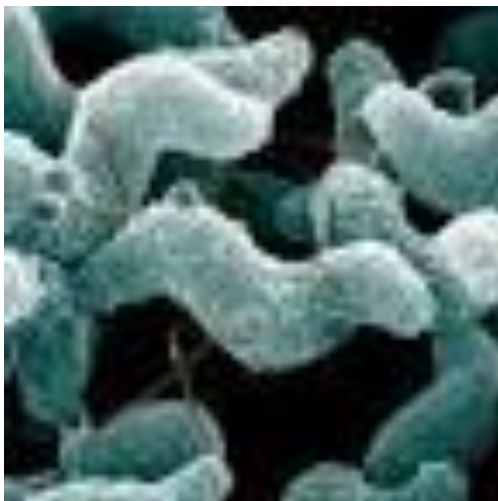
ST (7x) + *flaA*-RFLP(1x)



Methods	Obtained clusters at 100%	Good temporal relation
ST (7x) + <i>flaA</i> -RFLP	20	16/20
ST + AMRp	15	11/15

Conclusions

- The resistance of *C. jejuni* strains to Cip, Nal and Tet was higher for human isolates compared to the broiler isolates.
- Cip+Nal+Tet the most prevalent AMRp.
- By using MLST typing together with the *flaA* typing a very good discriminative power could be obtain
- A bigger variability was found in broiler isolates compared to the human ones by all typing techniques, specially by using the ST
- By using *flaA*-RFLP, Sequence typing (ST) and Clonal complex (CC) it is clear that poultry meat is a source of human infections.(28,7%)
- Not all types of *Campylobacter* present on the poultry carcass causes human illness
- In humans also other strains from another origin are causing human infections.



Acknowledgments:

A. Duarte, Dr. K. Dierick, Prof. M. Uyttendaele, Prof. L. De Zutter, Dr. T. Seliwiorstow

The NRC of *Campylobacter* : St Pierre Hospital, Dr. O. Vandenberghe

To WIV-ISP Foodborne pathogens section

To Wim Coucke and Wesley Mattheus

Thank you for listening!