Campylobacter: recent knowledge using genomics and metagenomics
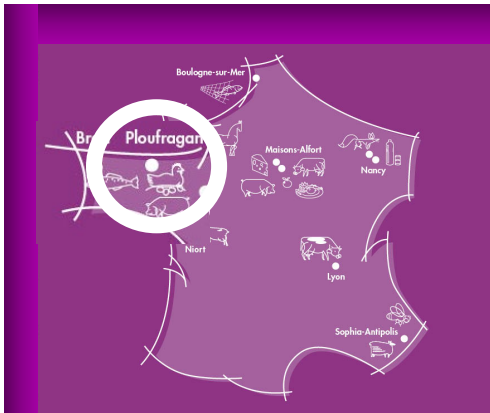
Marianne Chemaly

Anses, UHQPAP: Unit Hygiene and Quality of Poultry and Pork Products

Unit Hygiene and Quality of Poultry and Pork Products

➢ **30 members**

➢Reference activities: 4 NRLs

- *Salmonella*
- Avian salmonellosis
- *Campylobacter*
- Avian botulism

➢**Expertise**
National, EU and
international levels

➢**Research activities**
Control of zoonotic
pathogens in poultry
and pig productions
using a multifactorial
approach

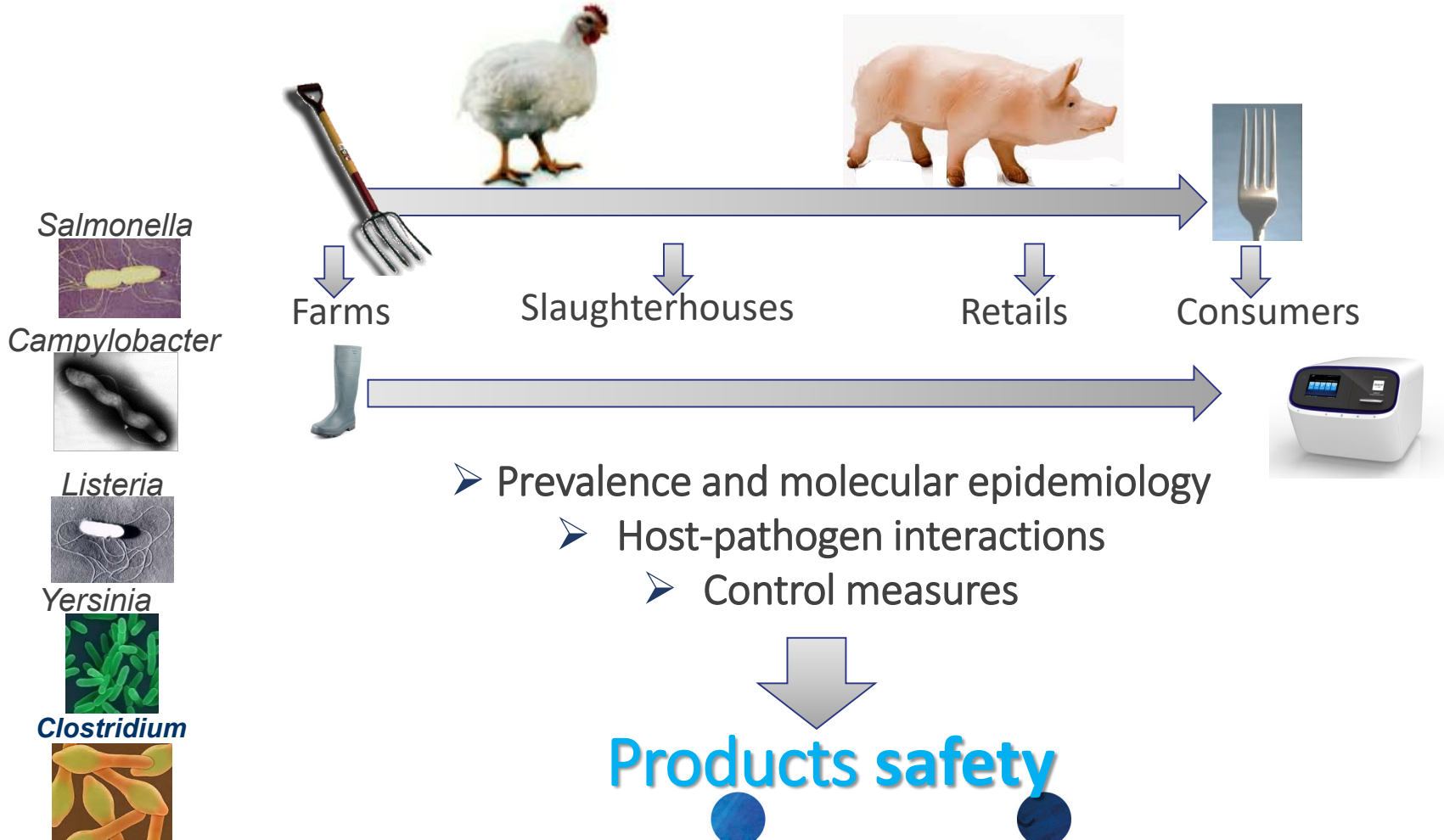# Control of zoonotic pathogens in poultry and pig productions using a multifactorial approach

Salmonella

Campylobacter

Listeria

Yersinia

Clostridium

Farms   Slaughterhouses   Retails   Consumers

➤ Prevalence and molecular epidemiology
   ➤ Host-pathogen interactions
      ➤ Control measures

Products safety

How sequencing can help to address our research questions?

➤ **Prevalence and molecular epidemiology**

- Epidemiological investigations: traceback, source attribution studies (*Salmonella*, *Campylobacter*, *Yersinia*, *Clostridium*)

- New tools when culture based methods are difficult to implement (*C. botulinum*)

➤ **Host-pathogen interactions**

- virulence determinants, colonization, gut microbiota: *Salmonella*, *Campylobacter* in poultry and pig productions

➤ **Control measures**

- Impact of gut microbiota: *Salmonella*, *Campylobacter* in poultry and pig productions

How sequencing can help to address our research questions?

What is the part of human campylobacteriosis attributable to broilers and to other animal sources?

How the microbiota can contribute to improve the control measures in broilers to prevent or reduce *Campylobacter* shedding?
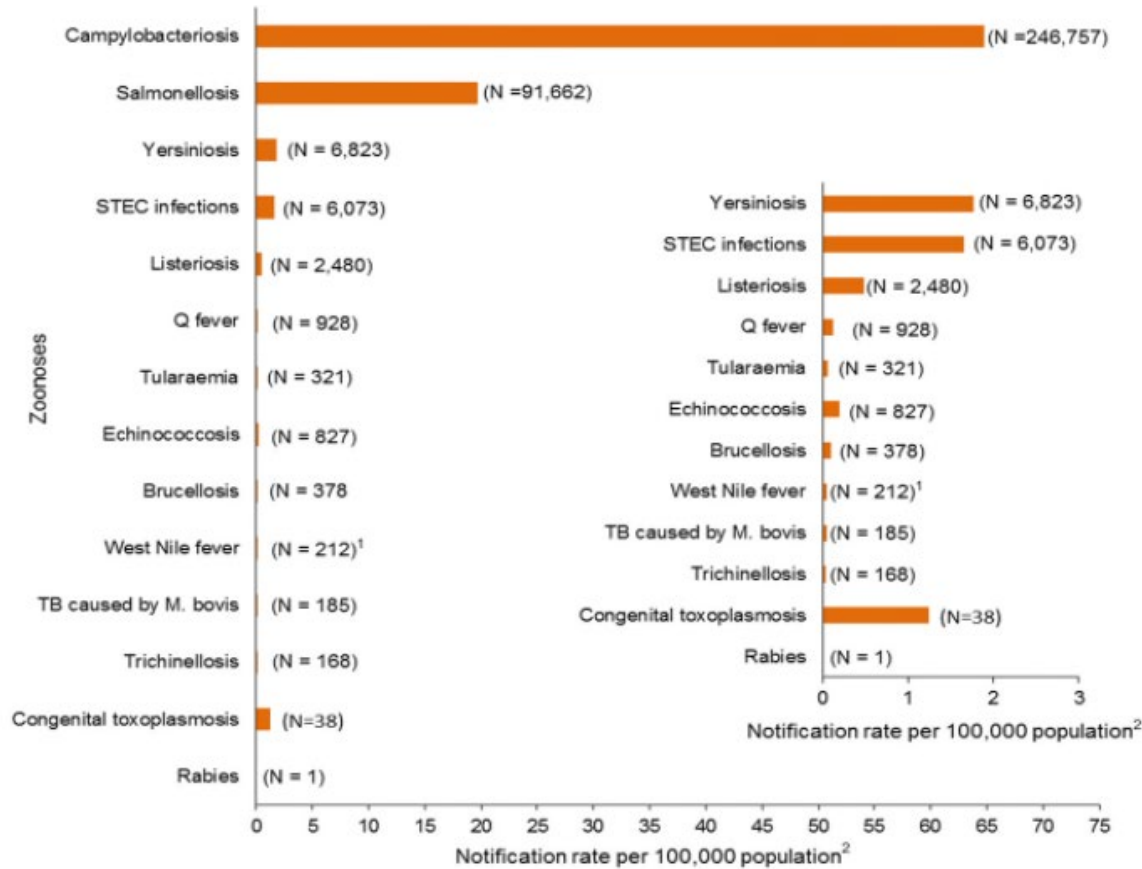
Prevalence and molecular epidemiology

source attribution: genomic approach

Control measures
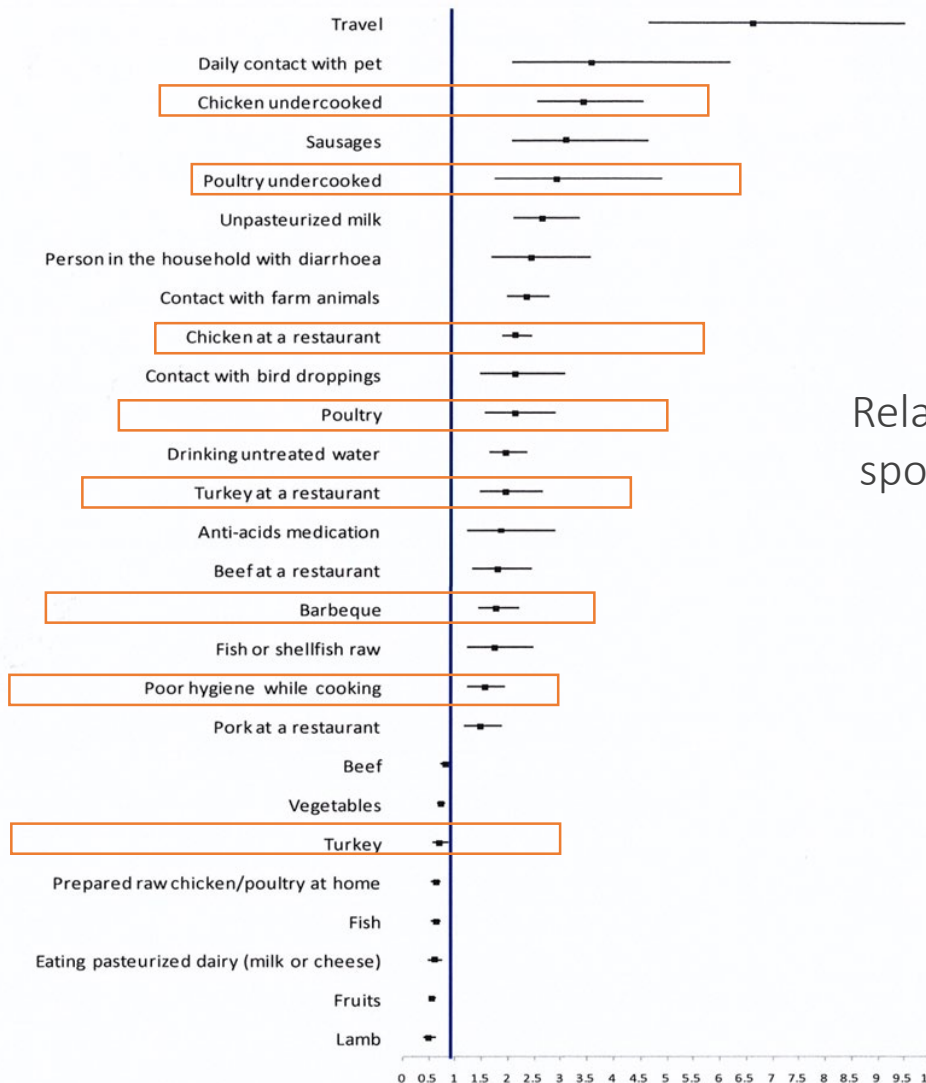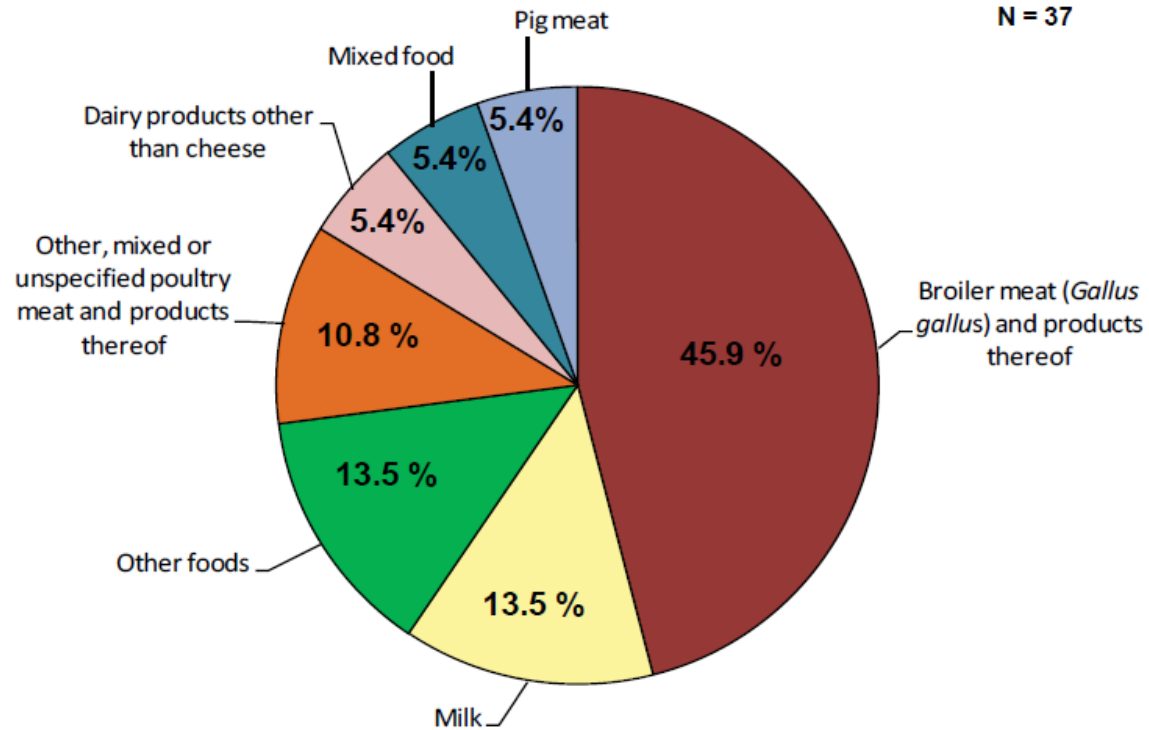
metabarcoding: metagenomic approach

Zoonoses EU, 2017

(EFSA, 2019)

Risk factors

Relative importance of RF for sporadic campylobacteriosis (EFSA, 2010)

Food sources

N = 37

- Pig meat 5.4%
- Mixed food 5.4%
- Dairy products other than cheese 5.4%
- Other, mixed or unspecified poultry meat and products thereof 10.8 %
- Other foods 13.5 %
- Milk 13.5 %
- Broiler meat (*Gallus gallus*) and products thereof 45.9 %

➢ 80% to poultry and 20-30% to poultry meat (Efsa 2013)

How sequencing can help to address our research questions?

What is the part of human campylobacteriosis attributable to broilers and to other animal sources?

How the microbiota can contribute to improve the control measures in broilers to prevent or reduce *Campylobacter* shedding?

Prevalence and molecular epidemiology

Control measures

source attribution: genomic approach

metabarcoding: metagenomic approach

Campylobacteriosis source attribution in France
(PhD 2014-2017)

➢ Large investigations: animal sources

EU baseline, NCPs

Local, on voluntary basis

Major abattoir

Collaboration: IFREMER and NRC

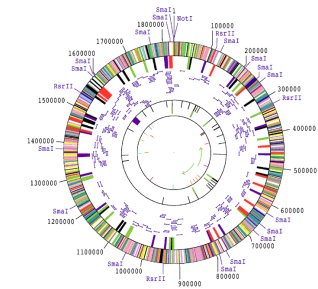# Campylobacteriosis source attribution in France
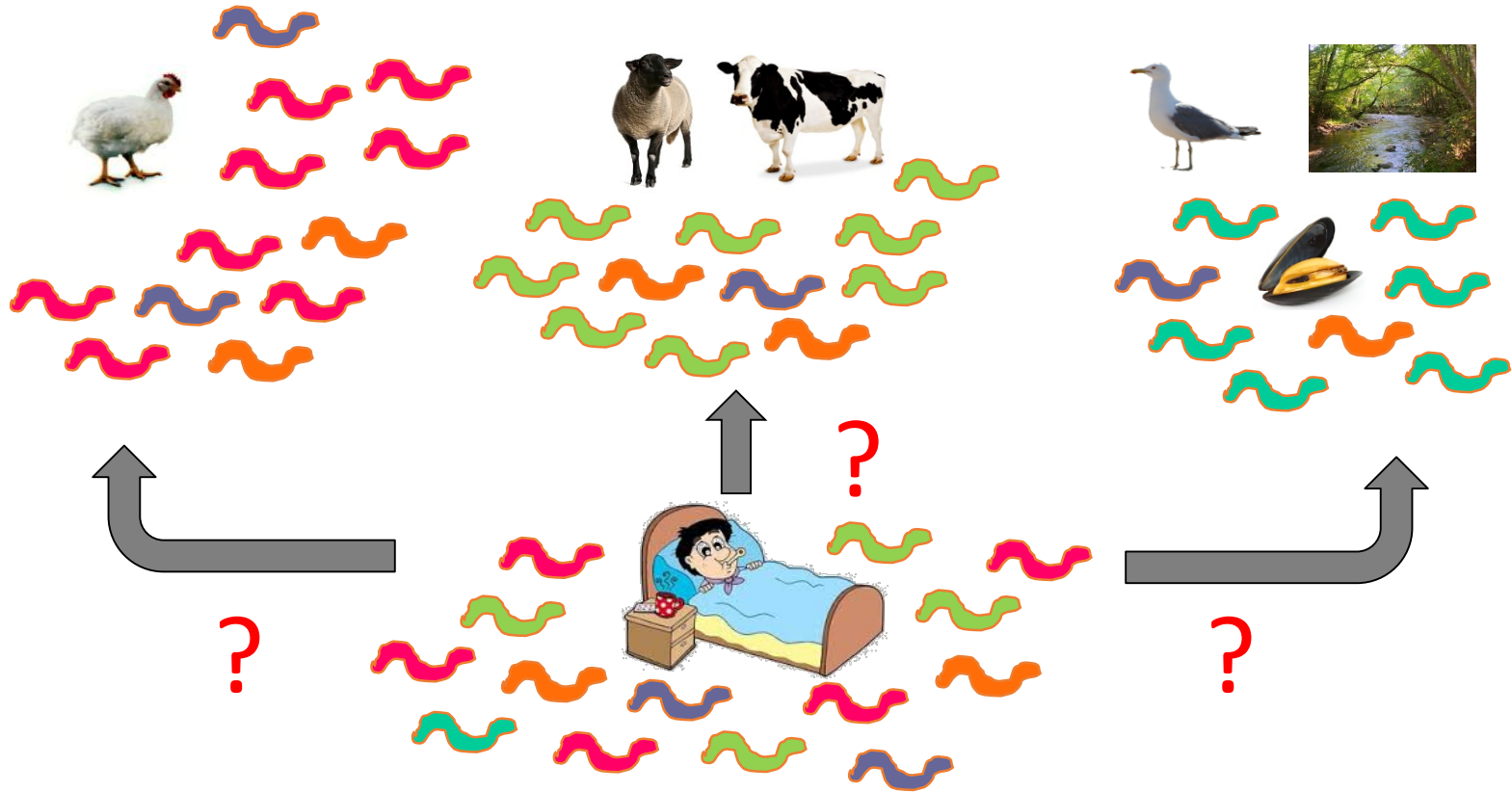
Campylobacteriosis source attribution in France
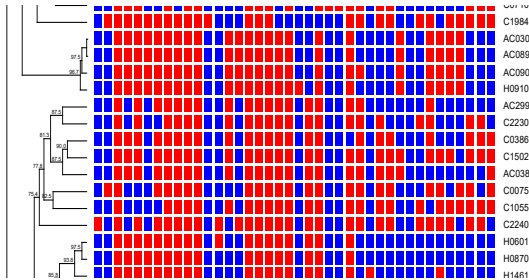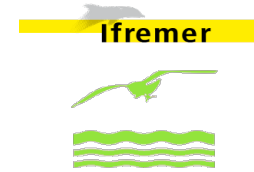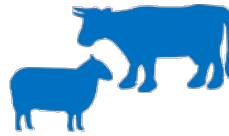

CGF40
(Taboada *et al.*, 2012)
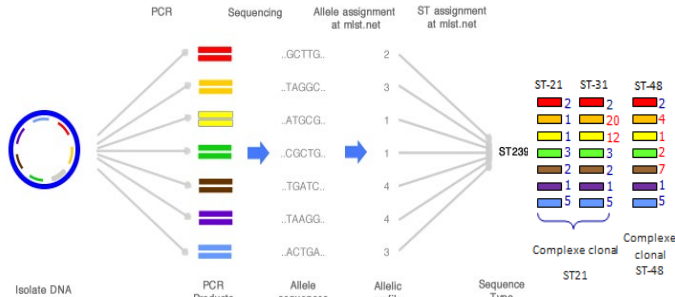
MLST
(Maiden *et al.*, 1998)

Whole Genome Sequencing
15 HS markers
(Thépault *et al.*, 2017)

➢ Bionumerics (Applied Math v6.5) & PubMLST database: MLST alleles designations

➢ BIGSdb (Jolley & Maiden, 2010): Bacterial Isolate Genome Sequence database (Genome Comparator for wg MLST)

➢ Structure (Pritchard *et al.,* 2010): probabilistic assignment of human isolates to a source

Campylobacter jejuni isolates

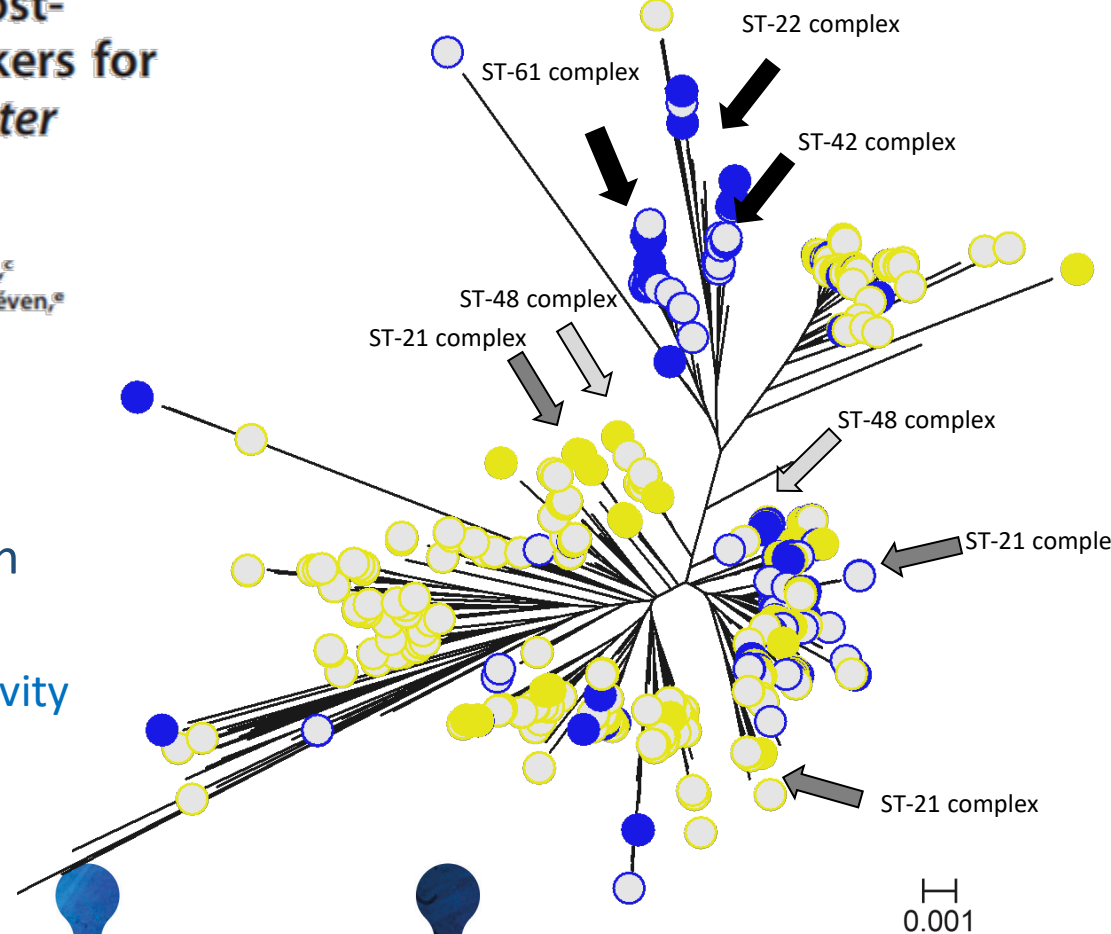|	🐔	🐄🐑	(squirrel)	Ifremer	2009	2015
CGF40	**644**	**691**	**161**	**122**	**143**	**371**
MLST	**644**	**119**	**55**	**39**	**143**	**79**
WGS	**392**	**195**	**55**	**98**	**42**	**79**

Genome-Wide Identification of Host-Segregating Epidemiological Markers for Source Attribution in *Campylobacter jejuni*

Amandine Thépault,[a,b] Guillaume Méric,[c] Katell Rivoal,[a] Ben Pascoe,[c] Leonardos Mageiros,[d] Fabrice Touzain,[e] Valérie Rose,[a] Véronique Béven,[e] Marianne Chemaly,[a] Samuel K. Sheppard[c,f]

15 HS markers for source attribution

→ Potential improvement of sensitivity in the probabilistic assignment
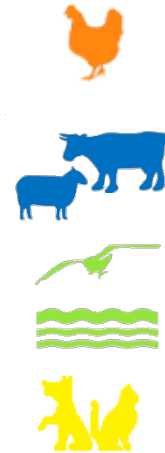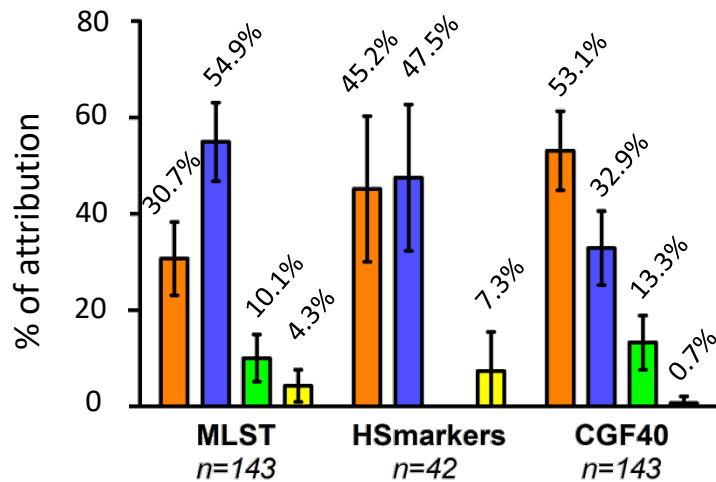
FR chicken   FR ruminants
UK chicken   UK ruminants

ST-22 complex
ST-61 complex
ST-42 complex
ST-48 complex
ST-21 complex
ST-48 complex
ST-21 complex
ST-21 complex

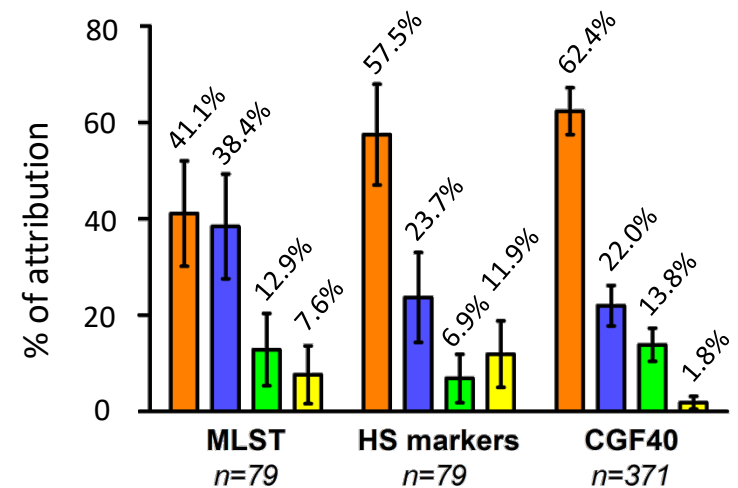0.001

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Campylobacteriosis source attribution in France
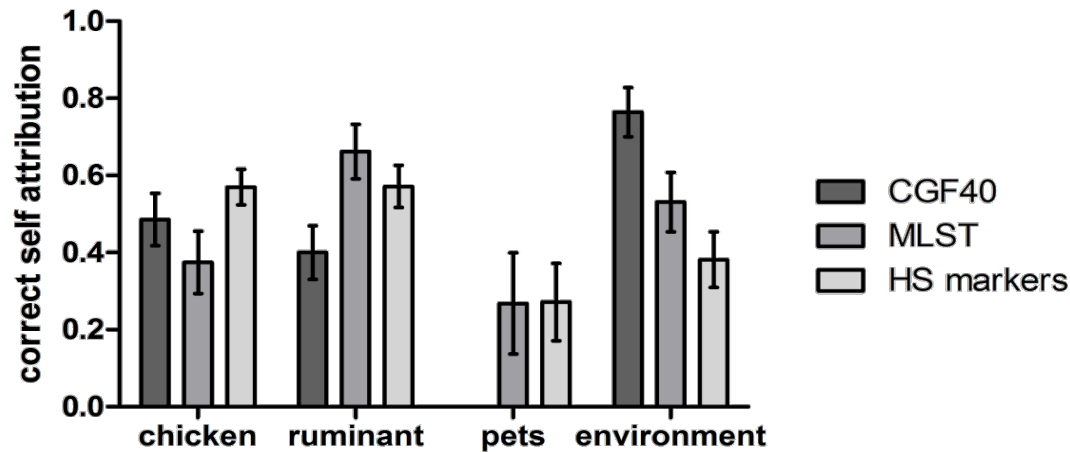
- 15 HS markers : the most accurate
- MLST: 2nd
- CGF40: the less accurate

Campylobacteriosis source attribution in France

2009

2015

Amandine Thépault[1,2], Valérie Rose[1], Ségolène Quesne[1], Typhaine Poezevara[1],
Véronique Béven[3], Edouard Hirchaud[3], Fabrice Touzain[3], Pierrick Lucas[3], Guillaume Méric[4],
Leonardos Mageiros[5], Samuel K. Sheppard[4,6], Marianne Chemaly[1] & Katell Rivoal[1]

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How sequencing can help to address our research questions?

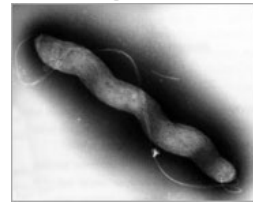**What is the part of human campylobacteriosis attributable to broilers and to other animal sources?**

Prevalence and molecular epidemiology

source attribution: genomic approach

- 15 markers : better sensitivity (MLST) and CGF40 : not suitable

➢ Chicken reservoir: highly implicated in human contamination

➢ Ruminant reservoir: significant role in French campylobacteriosis

➢ Source attribution: yearly basis

➢ Risk management tool: adapt control measures

How sequencing can help to address our research questions?

What is the part of human campylobacteriosis attributable to broilers and to other animal sources?

How the microbiota can contribute to improve the control measures in broilers to prevent or reduce *Campylobacter* shedding?
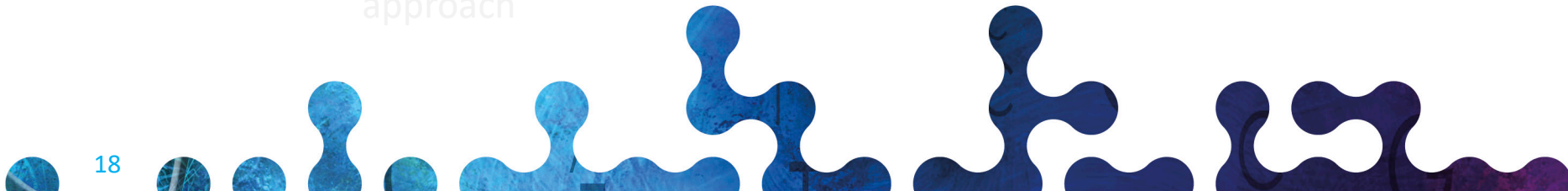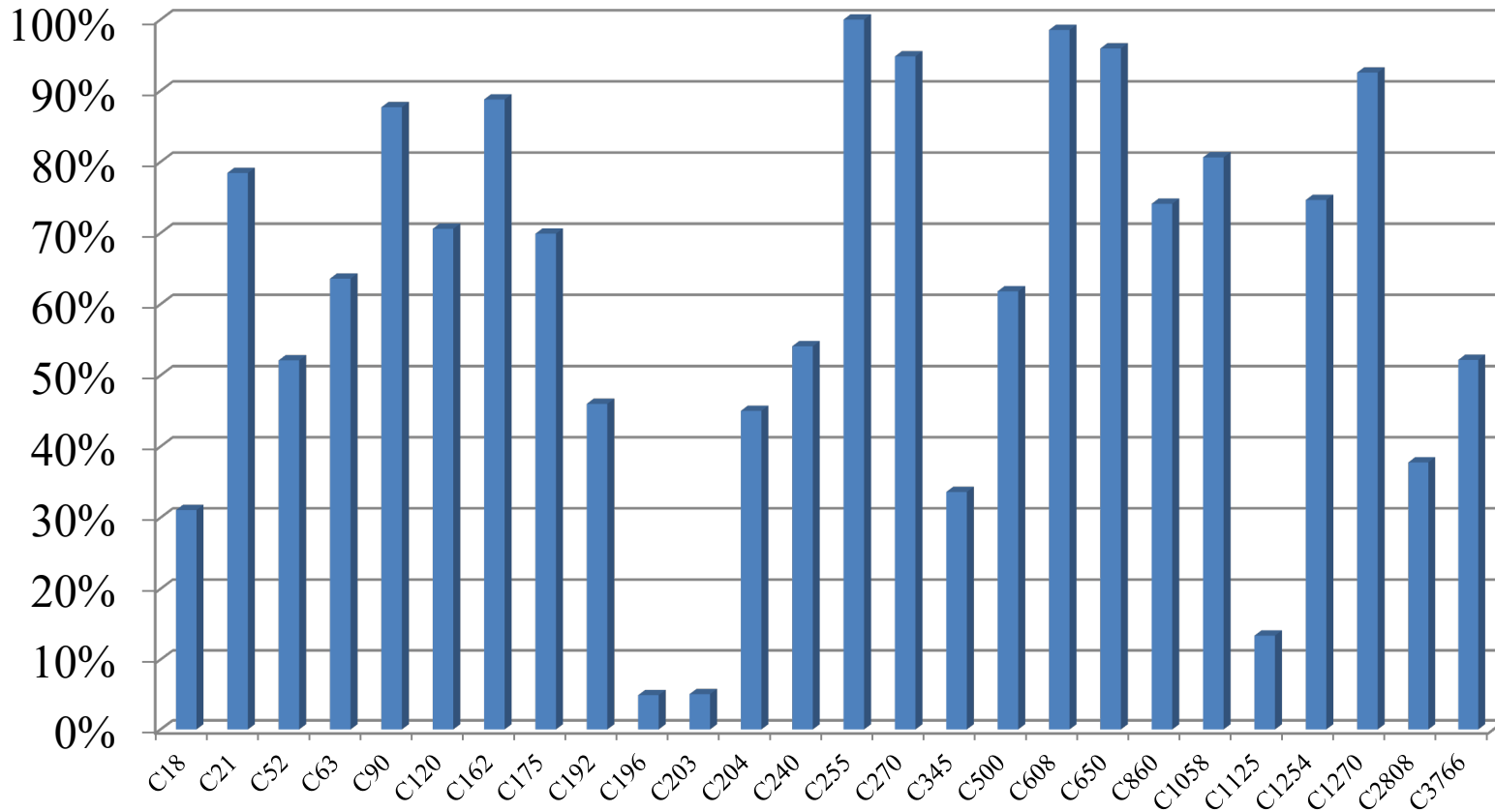
Prevalence and molecular epidemiology

Control measures

source attribution: genomic approach

metabarcoding: metagenomic approach

Prevalence primary production flocks EU

Control measures primary production

efsa — European Food Safety Authority

EFSA Journal 2010; 8(1):1437

SCIENTIFIC OPINION

Scientific Opinion on Quantification of the risk posed by broiler meat to human campylobacteriosis in the EU[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

efsa — European Food Safety Authority

EFSA Journal 2012;10(6):2741

SCIENTIFIC OPINION

Scientific Opinion on the public health hazards to be covered by inspection of meat (poultry)[1]

EFSA Panel on Biological Hazards (BIOHAZ), EFSA Panel on Contaminants in the Food Chain (CONTAM) and EFSA Panel on Animal Health and Welfare (AHAW)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

This Scientific Opinion, published on 10 July 2012, replaces the earlier version published on 29 June 2012.[4]

efsa — European Food Safety Authority

EFSA Journal 2011; 9(4):2105

SCIENTIFIC OPINION

Scientific Opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

Control measures primary production

Farms　　**Slaughterhouse**　　**Retail**　　**Consumers**

Reduction in live animals

Reduction on carcasses

Reduction on products

Reduction of campylobacteriosis

Interventions	Estimated risk reduction
In primary production	
Improved hygiene/biosecurity in indoor flocks	16%[a]
Application of fly screens in indoor flocks	60%[a]
Discontinued thinning of indoor flocks	1.8–25%[b]
Reduction of slaughter age of indoor flocks	
- to 42 days	0–5%[b]
- to 35 days	0.6–18%[b]
- to 28 days	21–43%[b]
Reduced colonization in caecal contents of indoor and outdoor flocks by	
- 1 $\log_{10}$ units	48–83%[b,c]
- 2 $\log_{10}$ units	76–98%[b,c]
- 3 $\log_{10}$ units	90–100%[b,c]
- 6 $\log_{10}$ units	100%[b,c]

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journal homepage: www.elsevier.com/locate/foodcont

ELSEVIER

Quantitative microbiological risk assessment (QMRA) of food-borne zoonoses at the European level

P. Romero-Barrios, M. Hempen, W. Messens, P. Stella, M. Hugas[*]

Unit on Biological Hazards (BIOHAZ), European Food Safety Authority (EFSA), Via Carlo Magno 1A, 43126 Parma, Italy
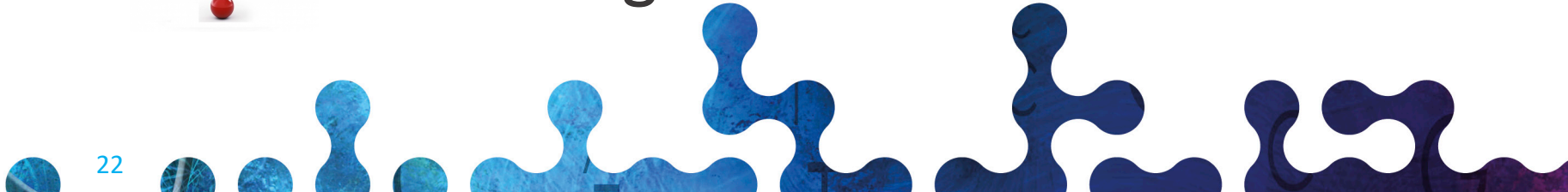
21
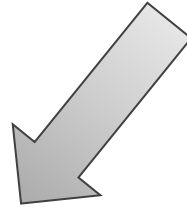
Biosecurity
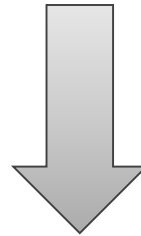
Additives
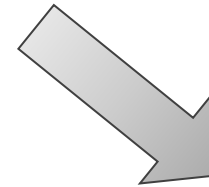
Vaccination

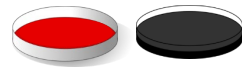Reproducibilty, variability

Role of gut microbiota

21 or 35-day old chicks from control or treated groups (litter and drinking water)
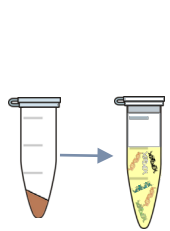
Caeca

Microbiological Approach
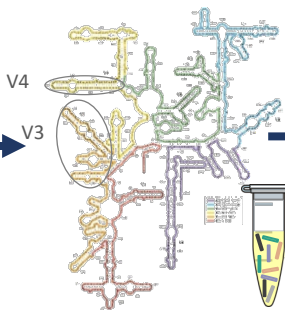*Campylobacter* detection and enumeration NF-ISO 10272

No significant difference between control and treated groups in *Campylobacter* loads

Metabarcoding Approach
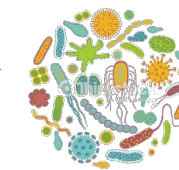
DNA extraction

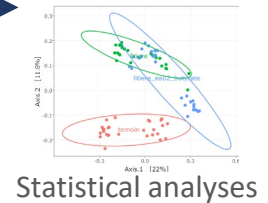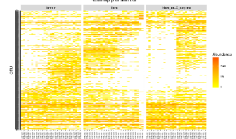V4
V3

16S region amplification

Next Generation Sequencing (NGS)

Bioinformatic processing

DNA database

Bacterial composition identification

Statistical analyses

Community diversity
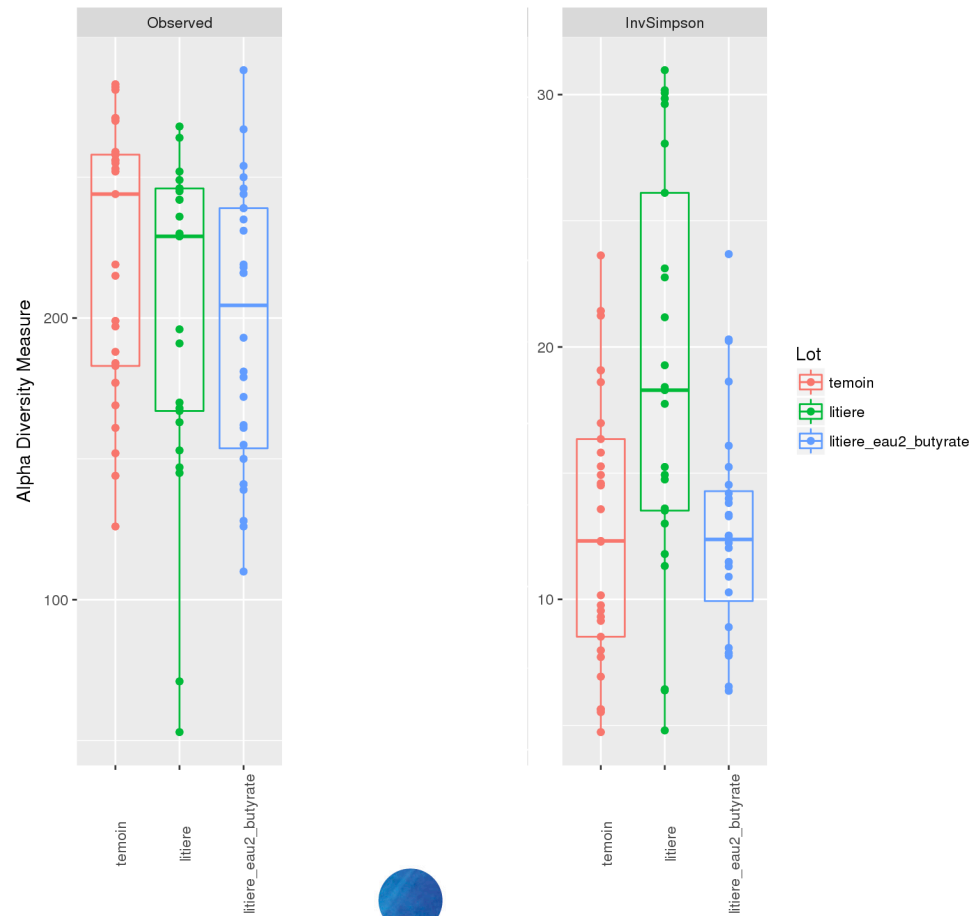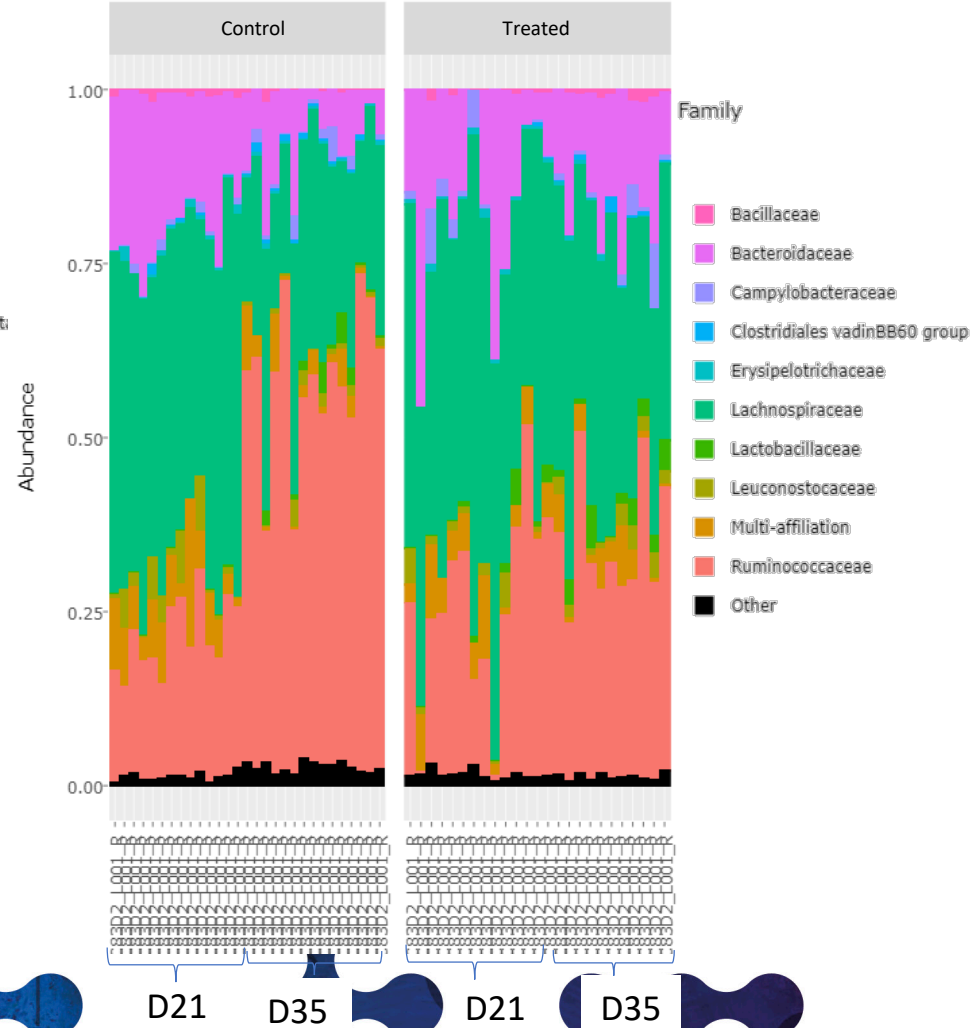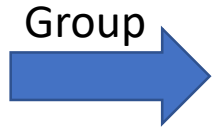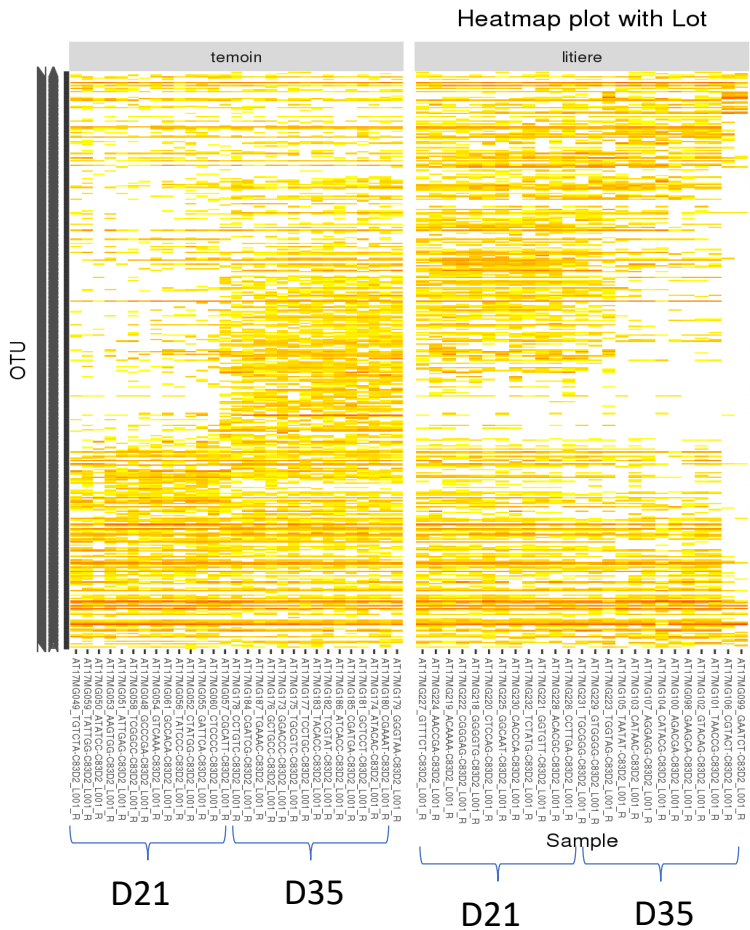
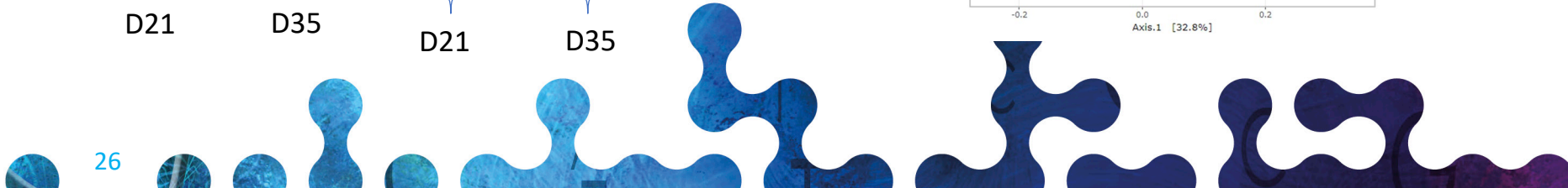# Community diversity
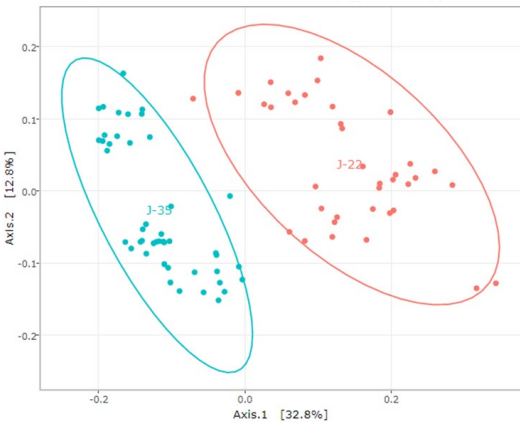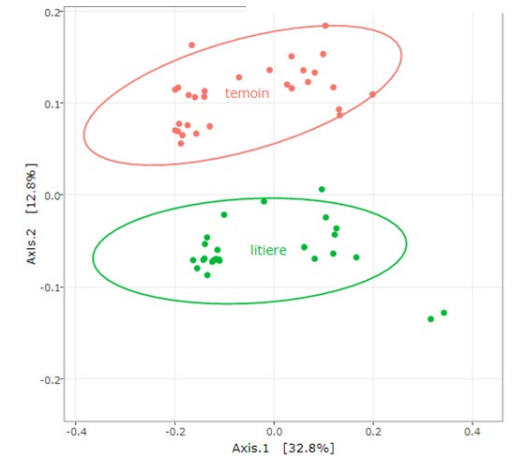

Composition within Bacteria (9 top Phylum)
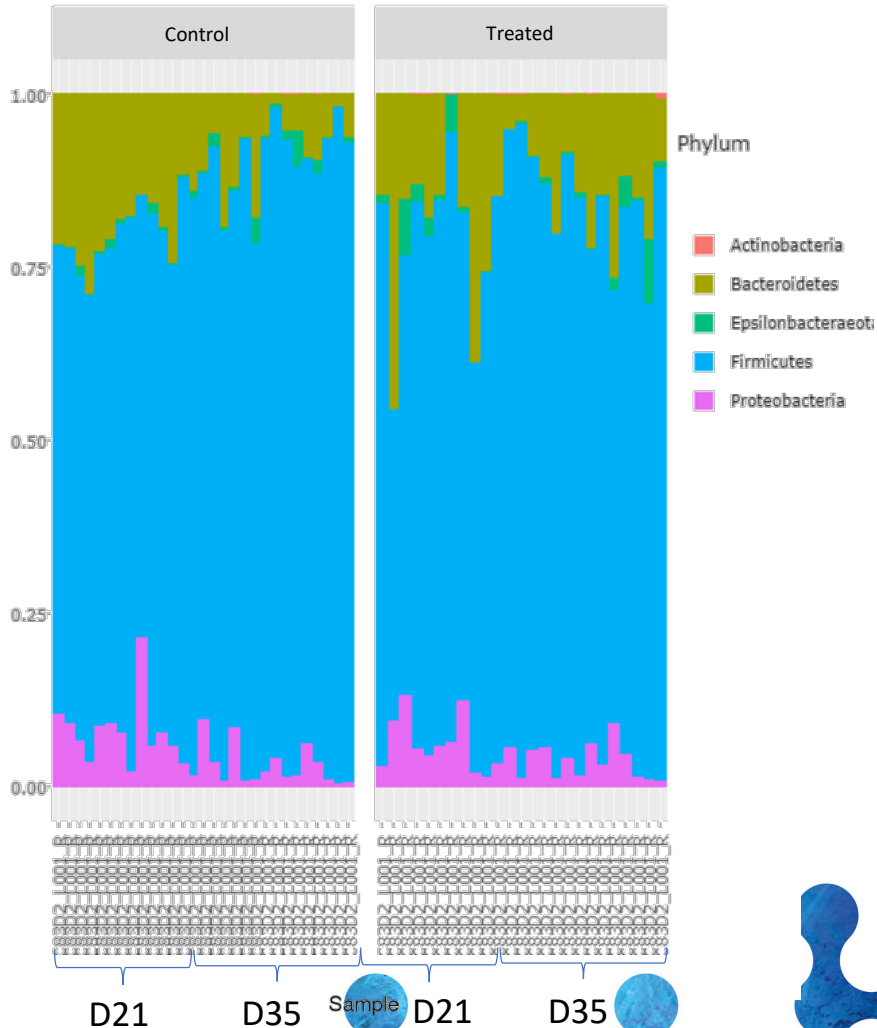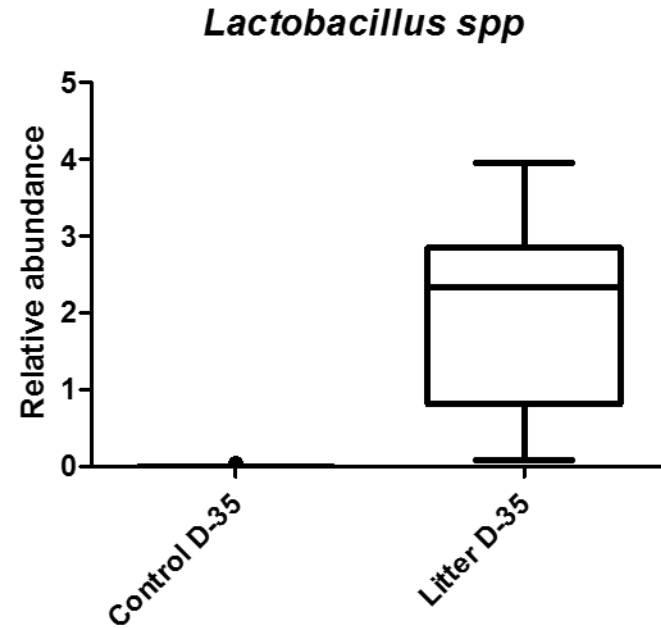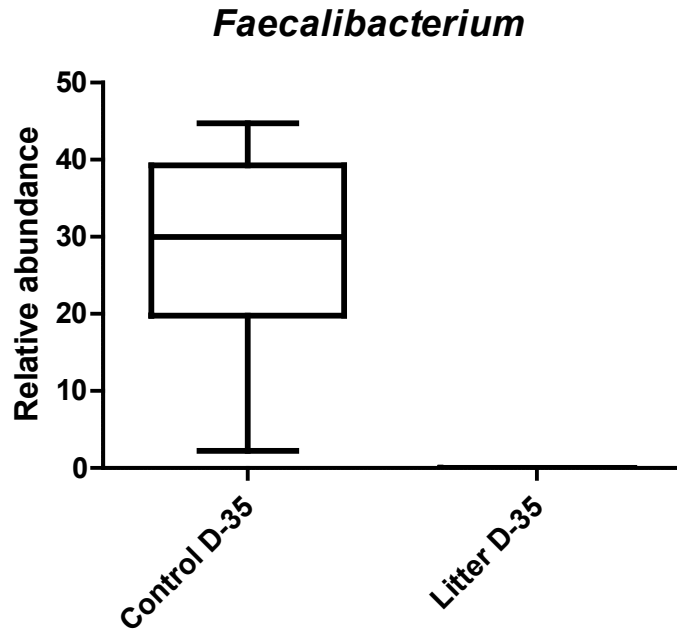
Composition within Bacteria (10 top Family)

Community structure

Community structure

Faecalibacterium

Lactobacillus spp

How sequencing can help to address our research questions?

➤ No impact on *Campylobacter*

➤ Gut microbiota affected by the treatment

➤ Impact of age as often described

➤ Identification of specific communities

➤ Association between *Campylobacter* and a specific community?

➤ Work in progress...

How the microbiota can contribute to improve the control measures in broilers to prevent or reduce *Campylobacter* shedding?

Control measures

metabarcoding:
metagenomic approach

Acknowledgments

HQPAP Unit

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Ifremer

M. Gourmelon, J. Cozien

M. Guyard **A. Thépault K. Rivoal**

F. Mégraud, P. Lehours

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S. K. Sheppard, G. Méric, B. Pascoe, L. Mageiros, E. Mourkas

RECAP Consortium

Dietaxion, Terrena

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