



IZSAM G. CAPORALE  
TERAMO



*Campylobacter*

Laboratorio Nazionale di Riferimento

# Bacteriology: Research, strategic guidelines, and projects

Dr. Giuliano Garofolo  
Study tour: Animal Welfare in the European Union  
Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise – January, 31 , 2017





# International and national expertise

 *Campylobacter*  
Laboratorio Nazionale di Riferimento



 Reference Laboratory  
for Brucellosis, Contagious Bovine  
Pleuropneumonia, r

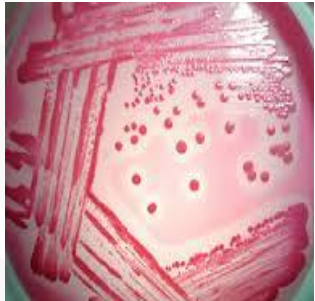


 **Brucellosi**  
Centro di Riferenza Nazionale

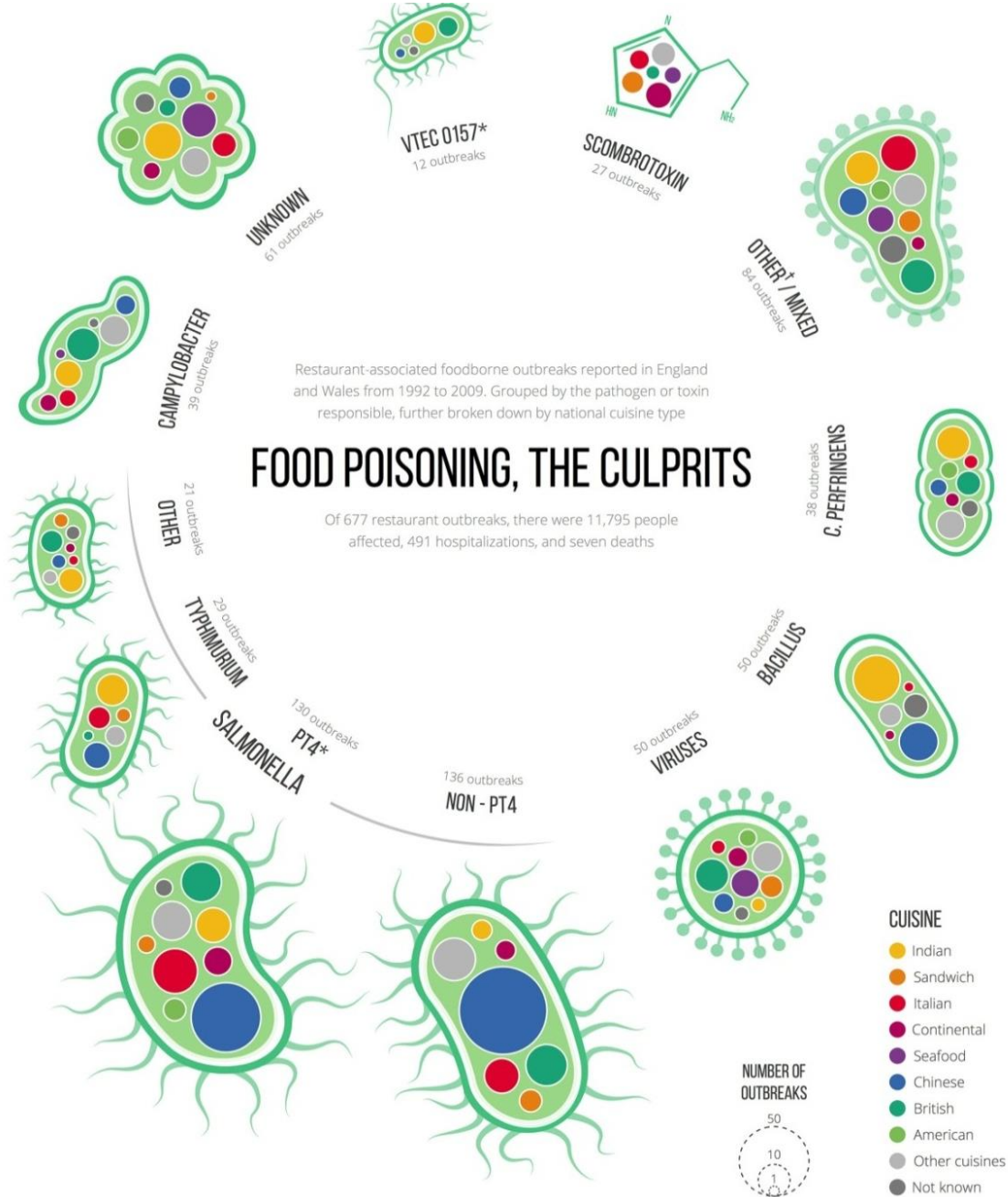


 *Campylobacter*  
Laboratorio Nazionale di Riferimento

Regional Reference Centre for *Salmonella*



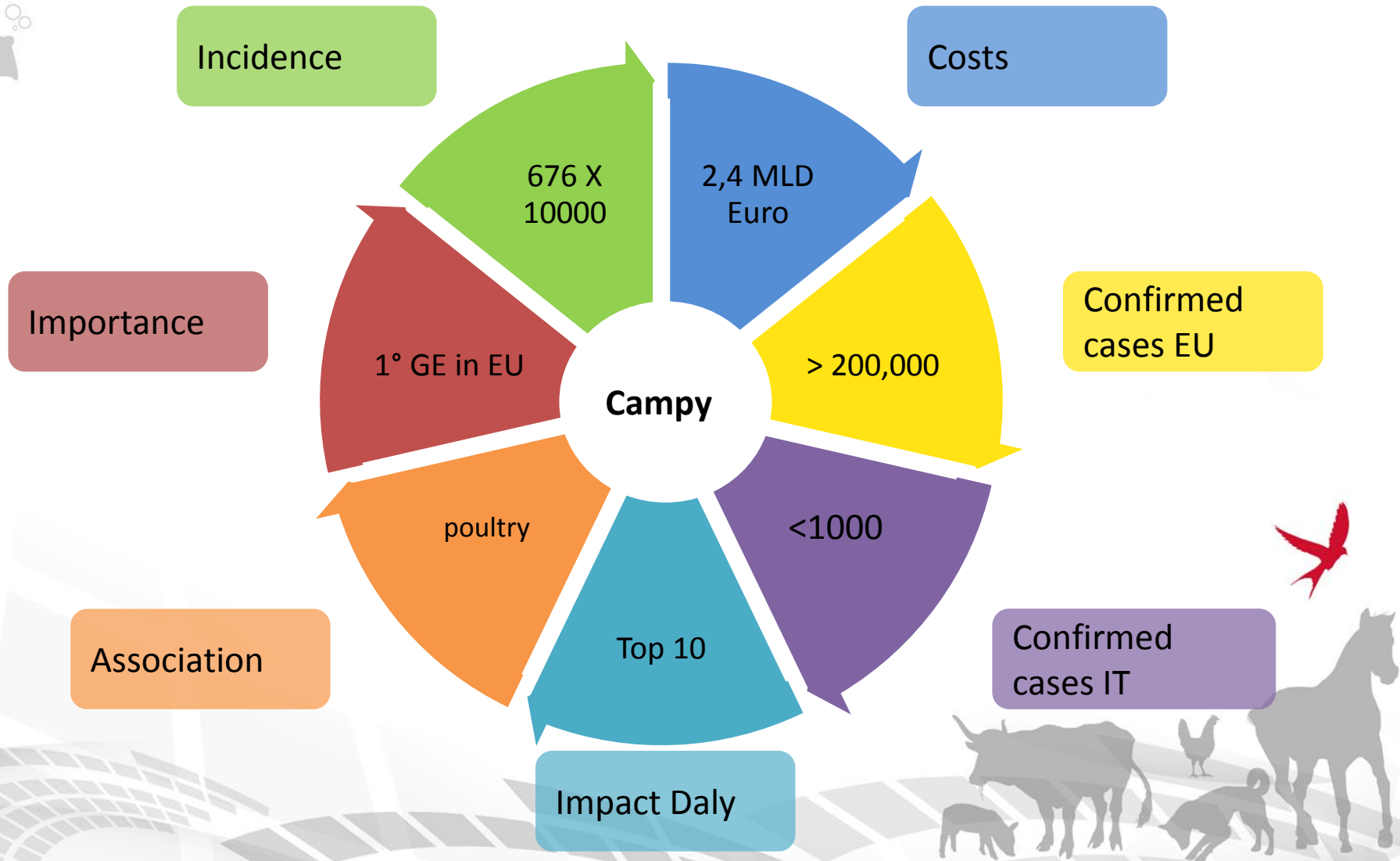
# Food safety



\* Salmonella PT4 (paratyphoid) & VTEC O157 (Verocytotoxin producing Escherichia coli (E-coli)) can cause serious illness

† Other includes Giardia lamblia, Shigella spp., Staphylococcus aureus and shellfish toxins

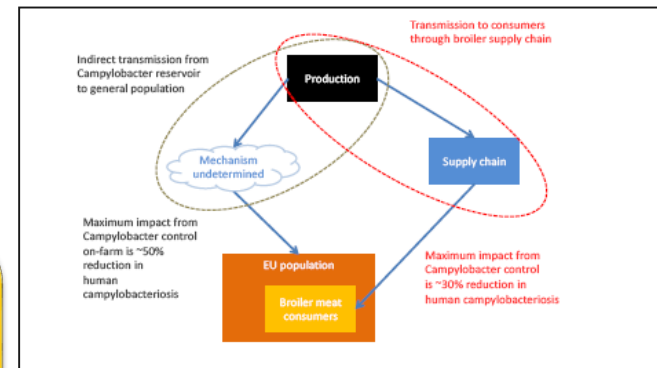
# Campylobacteriosis



# Campylobacter fact-sheet

**9 million** campylobacteriosis cases per year in the EU27  
Estimated disease burden is 0.35 million DALYs per year  
and total annual costs are **2.4 billions**

The public health benefits of controlling Campylobacter in primary production are expected to be greater than control later in the chain as bacteria may also spread from farms to humans by other pathways than broiler meat





# NRL for *Campylobacter*

 **Campylobacter**  
Laboratorio Nazionale di Riferimento

The National Reference Laboratory for campylobacter has been designated at the IZSAM by a note dated 7 March 2007 of the Ministry for Health .

The NRL for *Campylobacter* carries out the tasks referred to EC regulation no. 882/2004

Specific tasks of the NRL are:

- **Confirmation**, of the diagnosis carried out by other laboratories
- **Standardization** of methods of analysis
- **Organization** comparative tests between the official national laboratories
- **Production**, possession and distribution to other official laboratory of reference materials
- **Dissemination** of official methods of analysis
- **Organization** of training courses
- **Preparation** of field plans
- **Collaboration** with other EU Reference Centre and with Community and Third Countries
- **Support** to the Ministry of Health
- **Research**



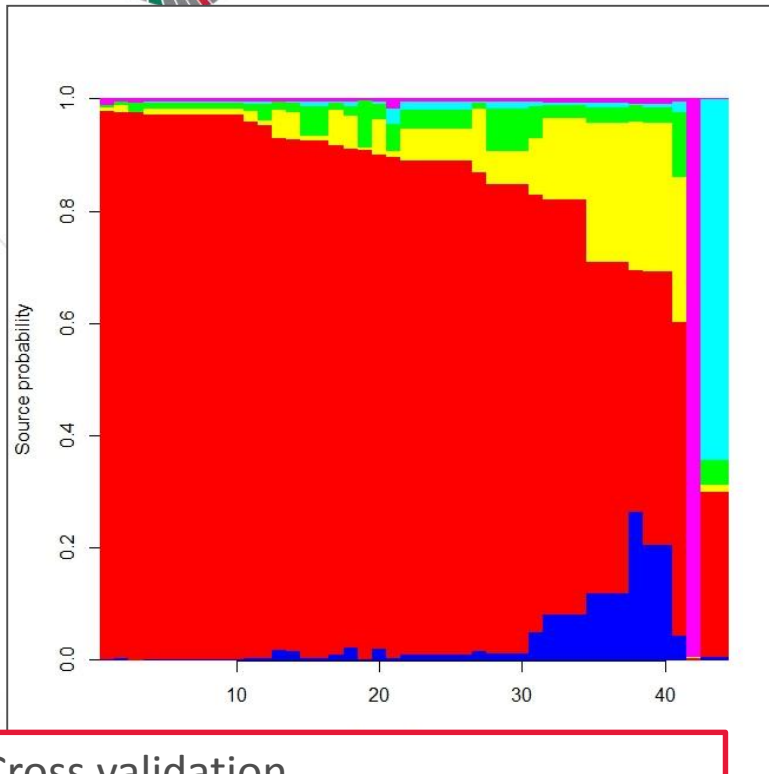


# Research projects

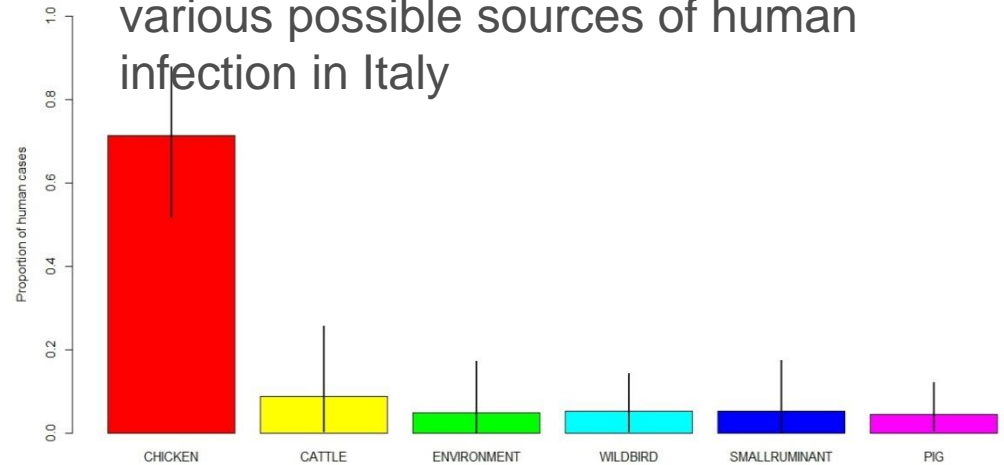
- a) I-Source tracing the sources of *Campylobacter jejuni*
- b) Camchain using symbiotic bacteria to tackle *Campylobacter*
- c) CombAT *Campylobacter*: characterization of poultry microbiota for selection of protective flora
- d) Air Sample: fast and reliable diagnosis from air samples from the farms



# I-Source project



To quantify the relative importance of various possible sources of human infection in Italy



## Tracing Back Clinical *Campylobacter jejuni* in the Northwest of Italy and Assessing Their Potential Source

Elisabetta Di Giannatale<sup>1</sup>, Giuliano Garofolo<sup>1</sup>, Alessandra Alessiani<sup>1</sup>, Guido Di Donato<sup>1</sup>, Luca Candeloro<sup>2</sup>, Walter Vencia<sup>3</sup>, Lucia Decastelli<sup>3</sup> and Francesca Marotta<sup>1\*</sup>

<sup>1</sup> National Reference Laboratory for *Campylobacter*, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy, <sup>2</sup> Department of Statistics and GIS, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale", Teramo, Italy, <sup>3</sup> Food Hygiene and Safety Department, Istituto Zooprofilattico Sperimentale del Piemonte, Liguria e Valle d'Aosta, Torino, Italy

Cross validation

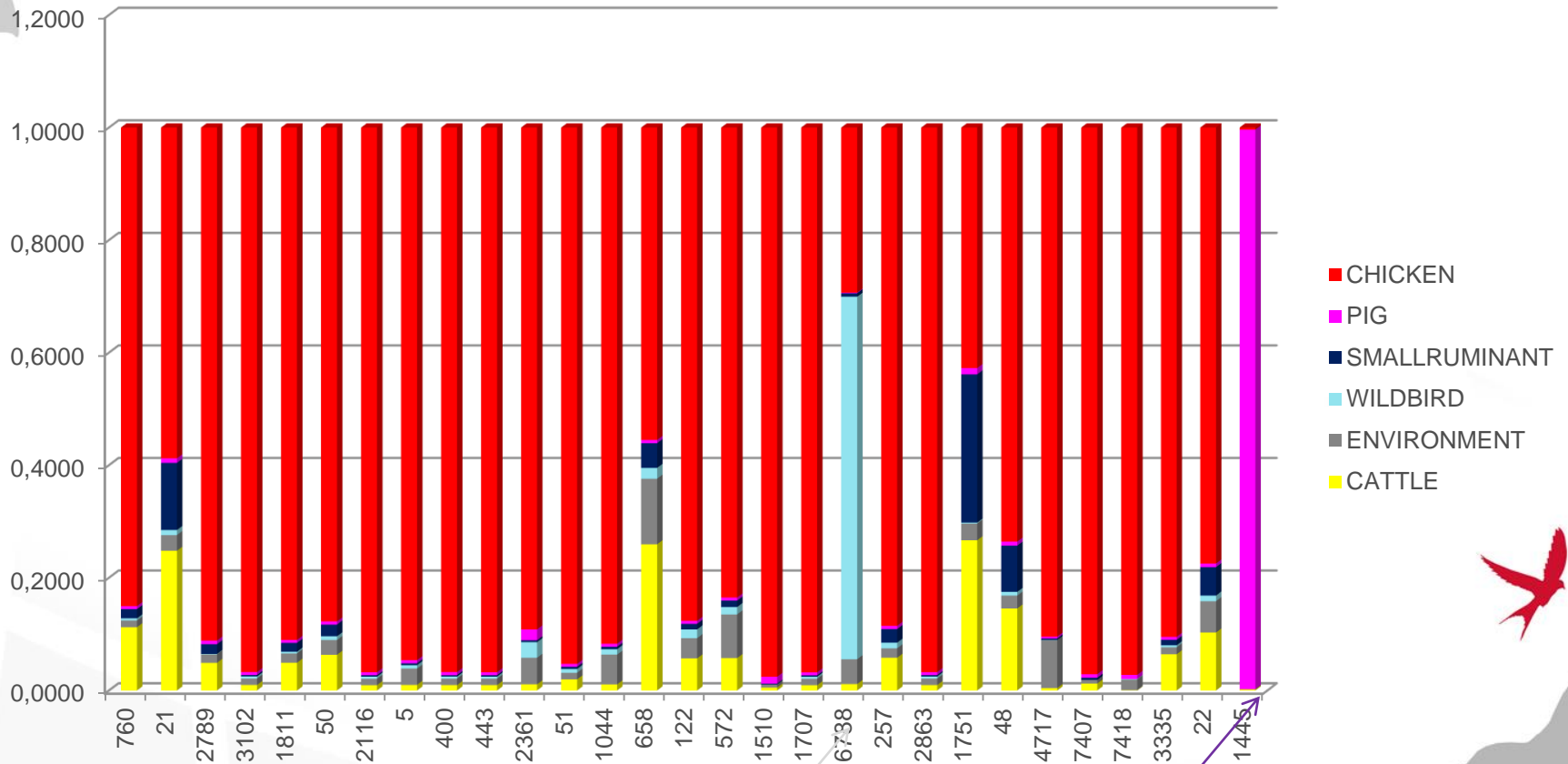
97% Poultry,

98% Ruminants

77% Wild birds, 62% environment



# Probability

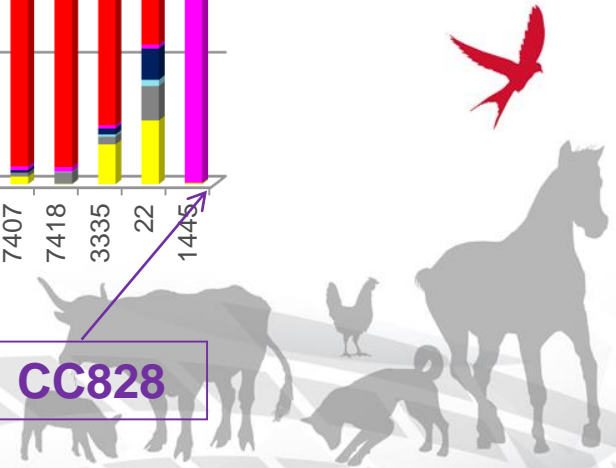


CC21

CC353

CC1332

CC828

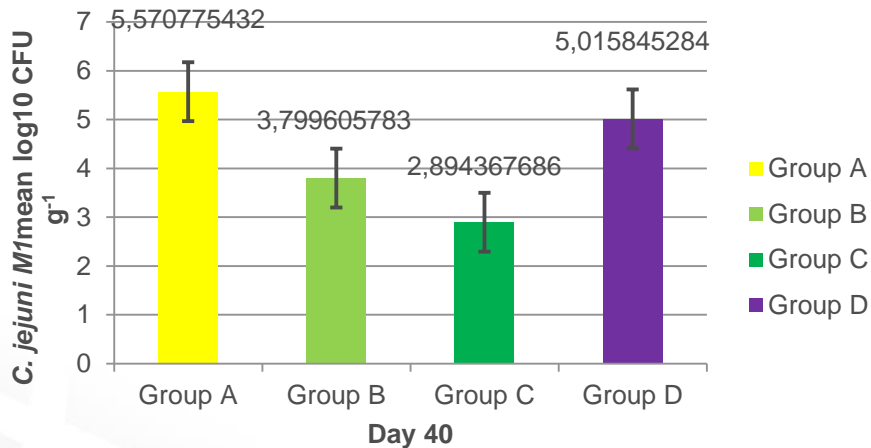


# CamChain project

## Campylobacter reduction from farm to slaughterhouse

Effect of a Synbiotic Formula for reducing *Campylobacter jejuni* in infected broilers

To verify in vivo activity of the *B. longum* subsp. *longum* PCB133 with Xylooligosaccharides (XOS) to reduce the cecal concentration of *C. jejuni*

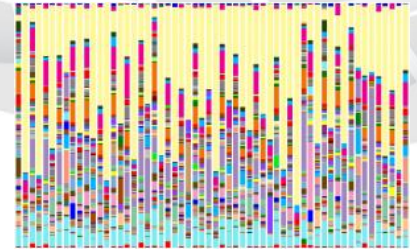


The results underlined the effectiveness of the synbiotic product in reducing *Campylobacter jejuni* in infected chickens (3 log<sub>10</sub> of reduction).

An interesting outcome concerned the high number of endogenous bifidobacteria  
5% of chickens resister

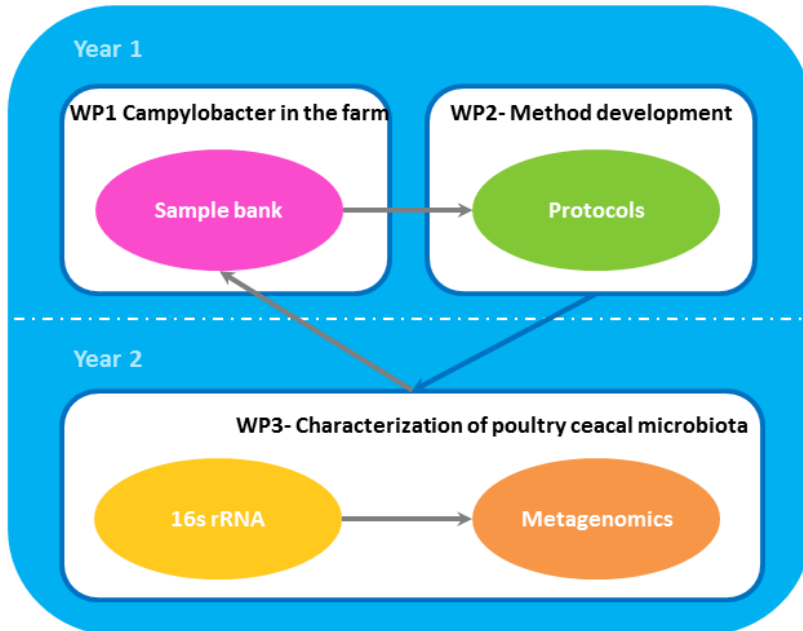


# CATCh project



**CombAT Campylobacter:** characterization of poultry microbiota for selection of protective flora

## CATCh



Compositional differences of ceecal microbiota and ceecal metagenome of poultry free and colonized by Campylobacter.

Preliminary data for the selection of transplantable flora for prophylactic or therapeutic use in broiler chickens

**Period**  
24 months





 *Campylobacter*  
Laboratorio Nazionale di Riferimento

# Air sampling:

## A Low-Cost Screening Tool in Biosecured Broiler Production

Acronym: **AIR SAMPLE**  
EU funding: **Approx. 1 mill. Euro**  
Period: **2 years (Jan 2018 - Dec. 2019).**  
Coordinator: **Jeffrey Hoorfar, DTU Food.**

### Partners:

Julia Christensen & Jeffrey Hoorfar, DTU Food.  
Gro Johannessen, Mona Torp & Camilla Sekse, NVI.  
Renáta Karpíšková & Ivana Koláčková, VRI.  
Kinga Wiczorek & Jacek Osek, NVRI.  
Elisabetta Di Giannatale & Giuliano Garofolo, IZSAM.

Copenhagen, Denmark.  
Oslo, Norway.  
Brno, Czech Republic.  
Pulawy, Poland.  
Teramo, Italy.





Future method, air sampling



- *Campylobacter* not detected in boot swabs.



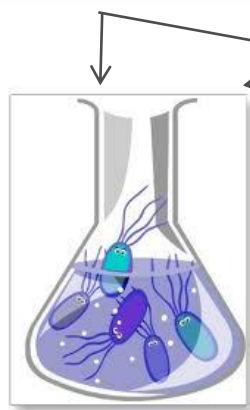


# Experimental setup

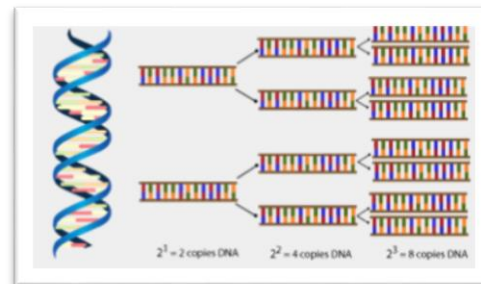


Air samples

Boot swabs



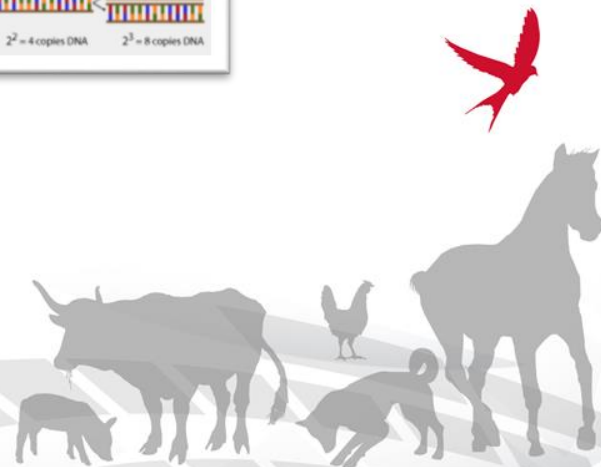
Enrichment qPCR  
Diagnostic  
Metagenomics



Enrichment broth


Selective plates

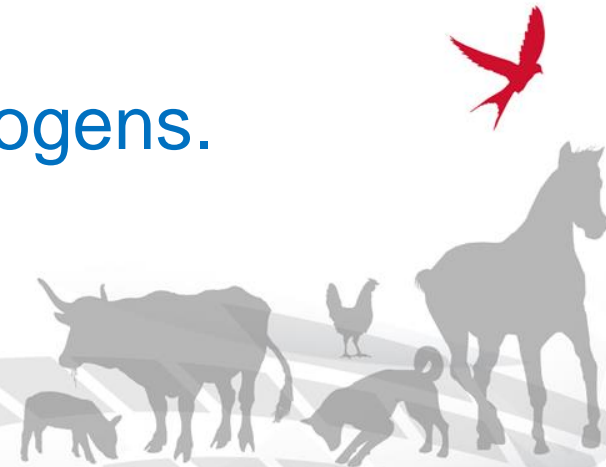
Colony PCR



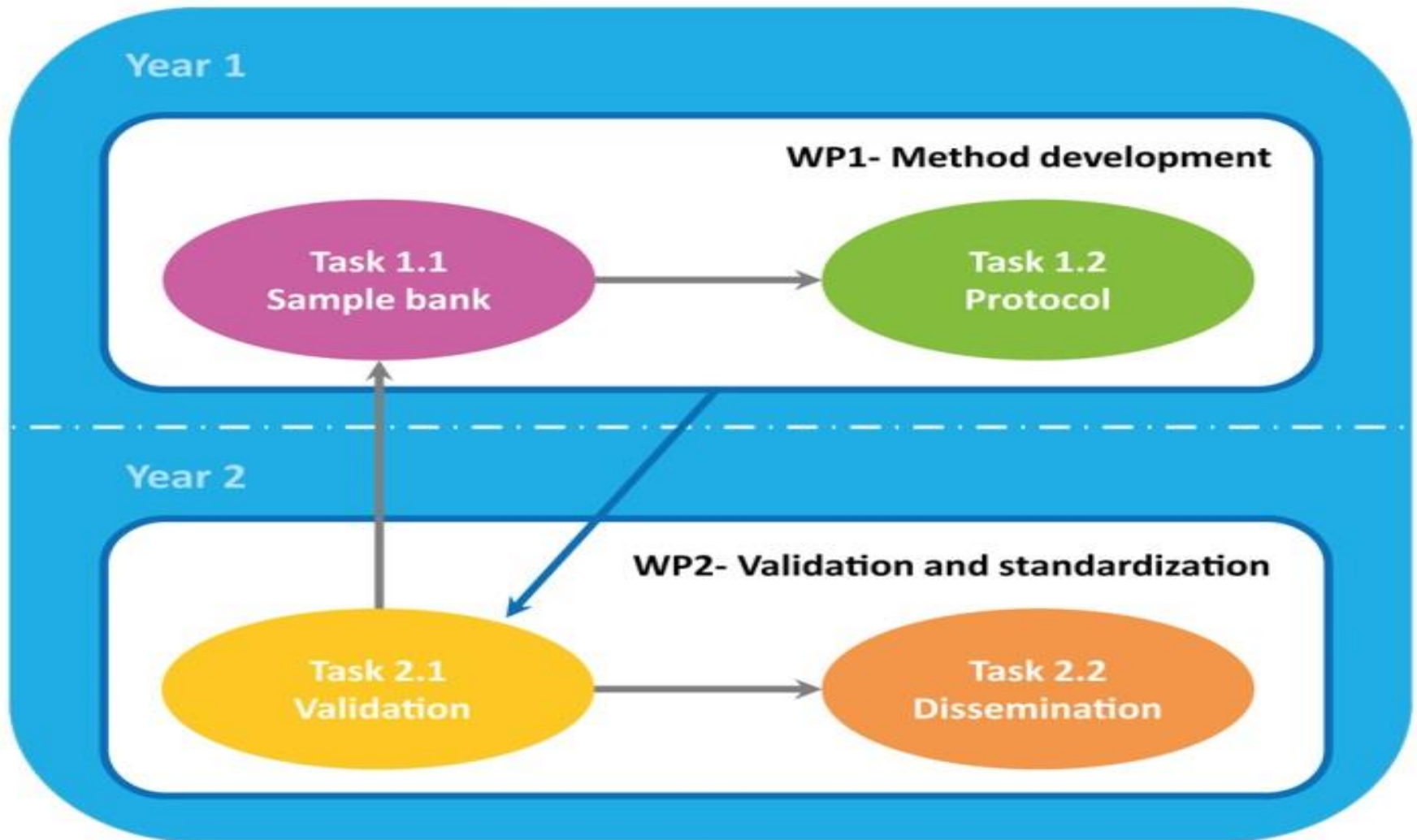


# Advantages could be:

- 
- ✓ Earlier test results.
  - ✓ Simpler sampling and shipping.
  - ✓ Lower overall costs.
  - ✓ Cleaner sample or less PCR inhibitors.
  - ✓ Suitable for culturing, as well as PCR and sequencing
  - ✓ Generic sample for multiple pathogens.



# AIR SAMPLE



**Task 1.1** Creates a sample bank (air boot-swab samples) from different regions

**Task 1.2** Develops of a non-complex DNA extraction protocol for qPCR or diagnostic metagenomics of gelatin-filter samples

**Task 2.1** Focuses on validation of air sampling and DNA extraction methods

**Task 2.2** Will promote the outcomes of the project to relevant stakeholders

# Animal health



- Aerobic and anaerobic culture for the presence of bacterial pathogens
- Mycoplasma
- Culture for fungal pathogens
- Campylobacter
- Brucella
- Listeria
- Salmonella



# Brucellosi, 2016 l'anno peggiore: a Messina 136 casi Problemi al mercato di Uil: «Commissariare la Sicilia»

SALVO CATALANO 5 GENNAIO 2017

**CRONACA** – R  
mercato clar  
articolazio

TRAVERSETOLO  
Allevamento di capre colpito dalla  
brucellosi

no quadruplicati, tornando ai livelli di fine anni 90. A contribuire il  
sudorazione si aggiunge il rischio di complicazioni alle  
a formaggi e verdure»



Bruc  
sequ

mette:  
"complice"

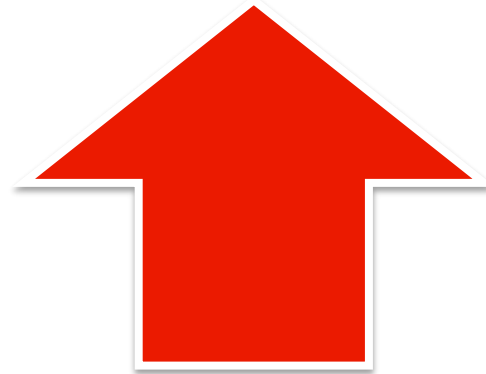
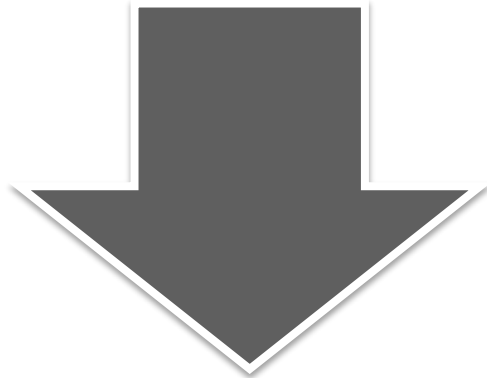




 *Campylobacter*  
Laboratorio Nazionale di Riferimento



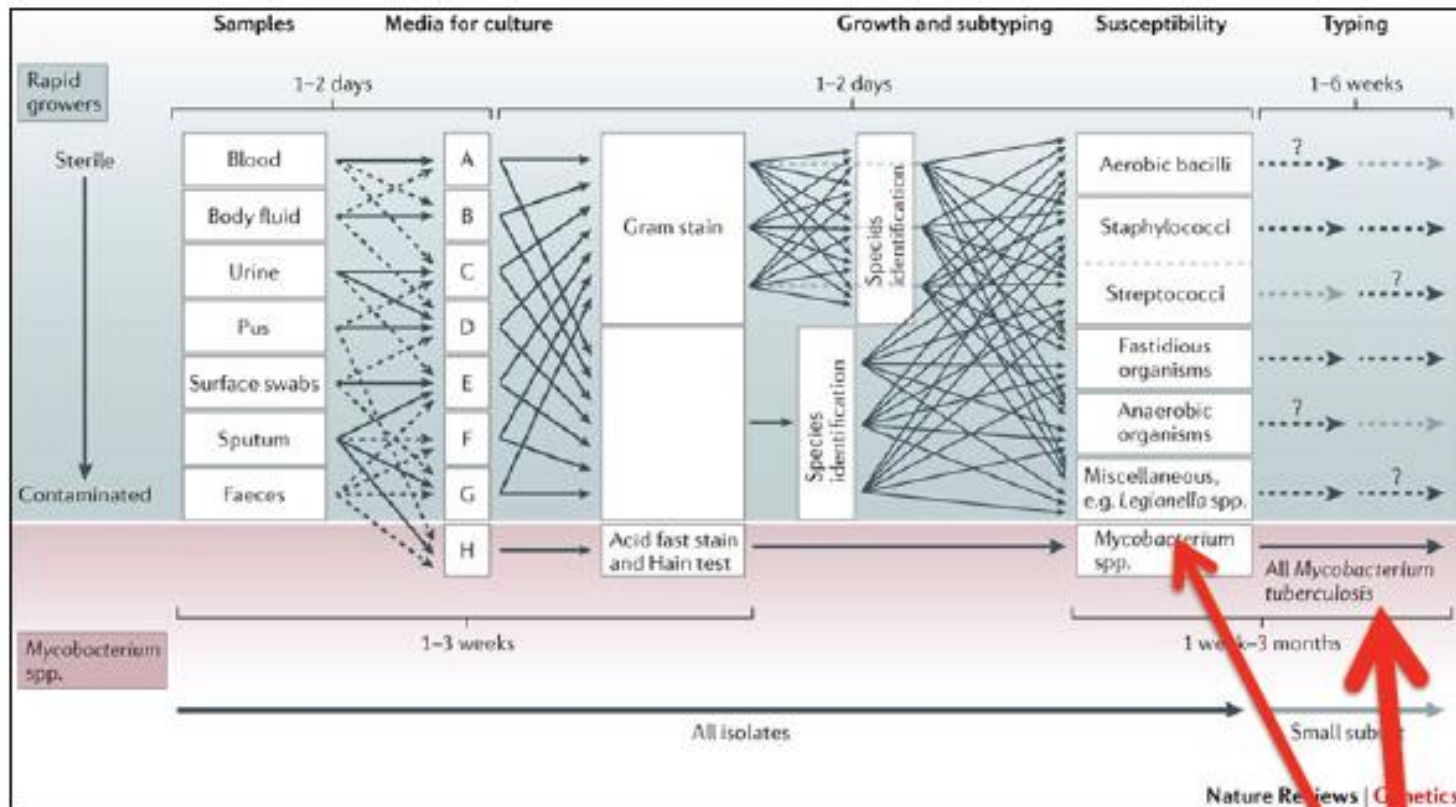
# GLOBAL EPIDEMIOLOGY



# Diagnostic Workflow in Clinical Bacteriology

Meta-genomics | Genomics

Cultivation Identification Susceptibility Genotyping



Didelot et al. (2012). *Nature Rev. Genet.* doi: 10.1038/nrg3226 [PubMed].

\*Claydon et al. (1996). *Nat. Biotechnol.* 14:1584 [PubMed].  
 Mellmann et al. (2008). *J. Clin. Microbiol.* 46:1946 [PubMed].

NGS\* WGS\*\*

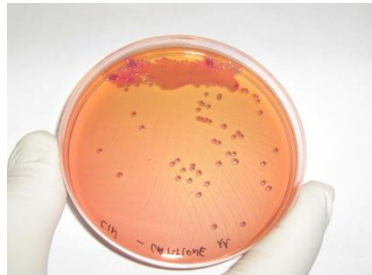
\*NGS, next generation sequencing; WGS, whole genome (shotgun) sequencing.



# Workflow Bacteriology


 Campylobacter  
 Laboratorio Nazionale di Riferimento

Colony morphology is a method that scientists use to describe the characteristics of an individual colony of bacteria growing on agar in a Petri dish.



## Phenotyping and Antimicrobial Susceptibility Testing



Con gli esempi su riportati non si intende sostituire la tavola di lettura della scheda tecnica.

## Molecular identification



- **source**
- **diffusion of diseases.**



# Epidemiology

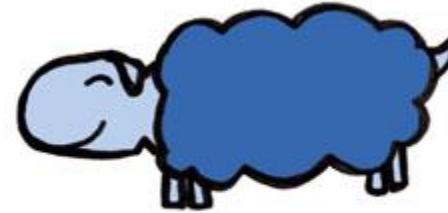
**Traditional epidemiology** discovers relationship among people, animals, places and items that might have the opportunity to spread the disease



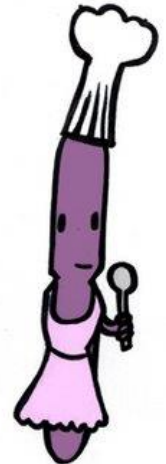
*Broad Street del 1854*



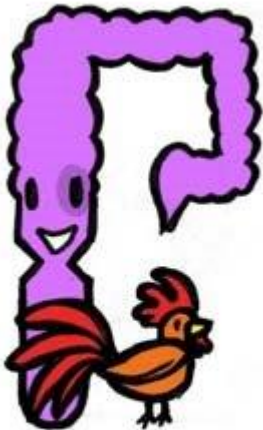
# we want to interview PATHOGENS!!!!!!



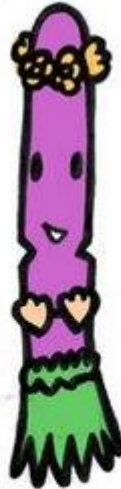
BRUCELLA MELITENSIS



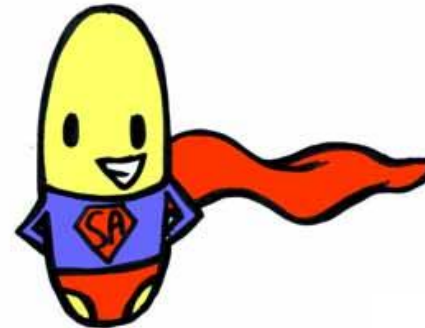
SALMONELLA  
ENTERITIDIS



CAMPYLOBACTER  
JEJUNI



BURKHOLDERIA  
PSEUDOMALLEI



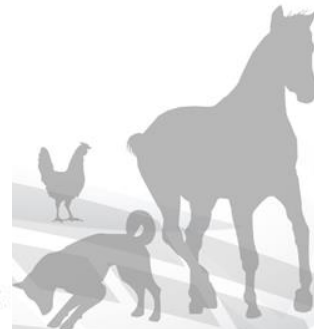
STAPHYLOCOCCUS  
AUREUS



CLOSTRIDIUM  
BOTULINUM



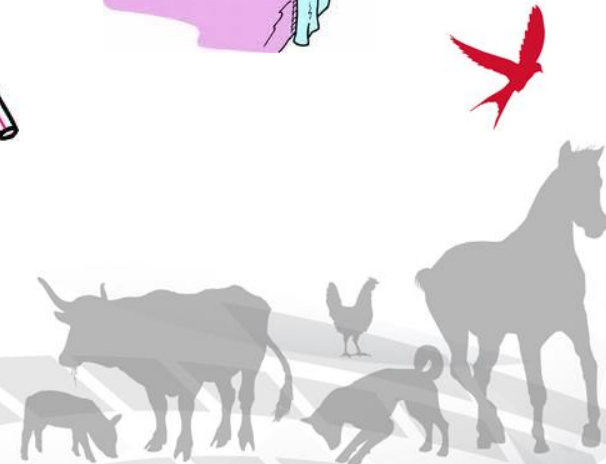
BACILLUS  
ANTHRACIS





# Molecular Epidemiology

- **Molecular epidemiology** finds out phenotypical and genetic relationship among infectious agents to suggest the source of infection, the transmission path and the biological relation



# Molecular epidemiology tools



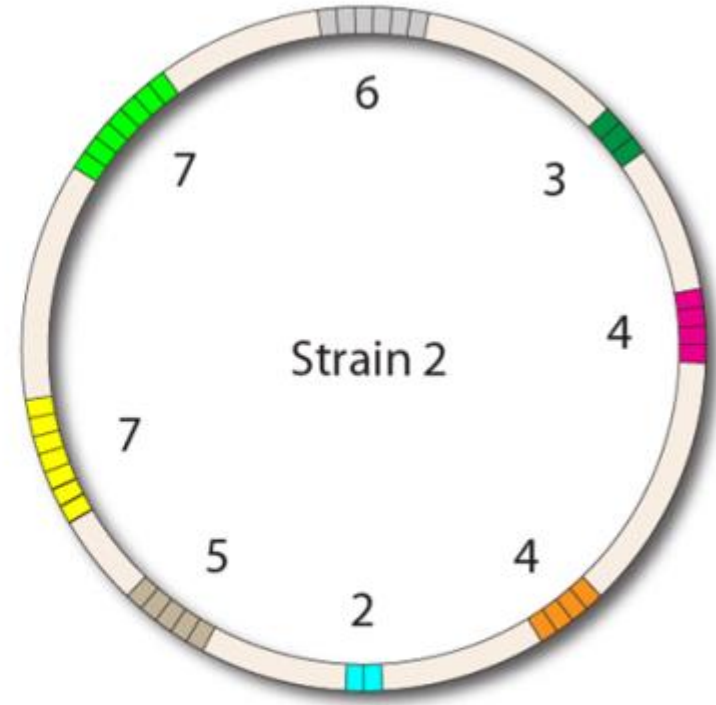
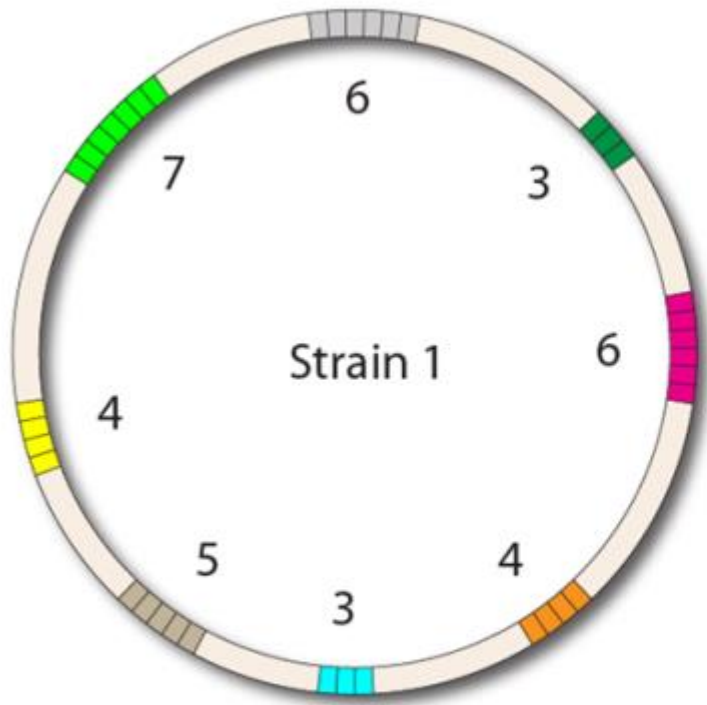
MLVA



WGS



# Multi locus VNTR analysis



MLVA profile of strain 1: 6-3-6-4-3-5-4-7

MLVA profile of strain 2: 6-3-4-4-2-5-7-7







ELSEVIER



 *Campylobacter*  
Laboratorio Nazionale di Riferimento

## Investigating genetic diversity of *Brucella abortus* and *Brucella melitensis* in Italy with MLVA-16



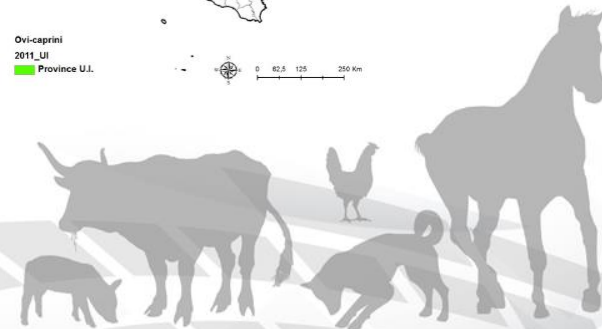
Giuliano Garofolo<sup>a,\*</sup>, Elisabetta Di Giannatale<sup>a</sup>, Fabrizio De Massis<sup>a</sup>, Katiuscia Zilli<sup>a</sup>, Massimo Ancora<sup>a</sup>, Cesare Cammà<sup>a</sup>, Paolo Calistri<sup>a</sup>, Jeffrey T. Foster<sup>b</sup>

<sup>a</sup>Istituto Zooprofilattico Sperimentale dell'Abruzzo e Molise "G. Caporale", National and OIE Reference Laboratory for Brucellosis, Via Campo Boario, 64100 Teramo, Italy

<sup>b</sup>Center for Microbial Genetics & Genomics, Northern Arizona University, Flagstaff, AZ 86011-4073, USA

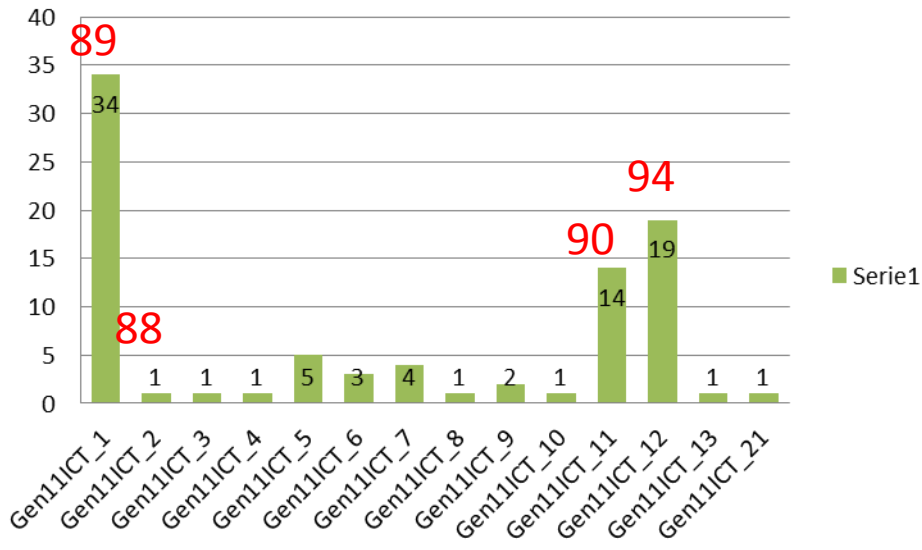


Fig. 2. Geographical representation (GIS data) of A) *B. abortus* isolates and B) *B. melitensis* isolates, with its outbreaks in Southern Italy. Further geographical information with colors at collection sites correspond to the clades from Figs. 1 and 4 are provided in the supplementary data with .kml files usable with Google Earth/Maps.



# MLVA 11 *B. melitensis* phylogeny

 *Campylobacter*  
Laboratorio Nazionale di Riferimento



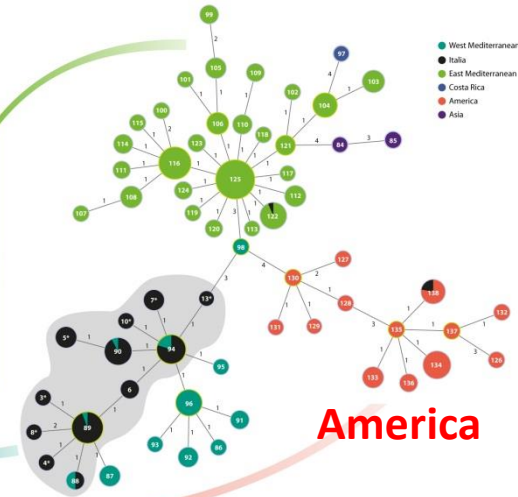
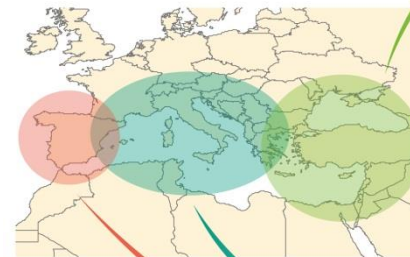
200 strains from 2011, 2012, 2013

12 MLVA11 genotypes 96,2 % strains  
Belong to West Mediterranean

1 MLVA11 genotype East Mediterranean

1 MLVA11 genotype America

East Mediterranean



West Mediterranean

America





 *Campylobacter*  
Laboratorio Nazionale di Riferimento

# Molecular Epidemiology

Salerno southern Italy

*B. melitensis biovar 3*

MLVA :

MLVA 11 – lineage West Mediterranean

MLVA 16 – 100% of identity with isolates from livestock of a farm in the same region



Relationship between human and animal cases.







QUESTIONS

Campy & Brucella girls

Thank you