



 *Campylobacter*
Laboratorio Nazionale di Riferimento

Studio di genomica comparativa del *Campylobacter jejuni* nella filiera avicola italiana

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Giornata di studio: il laboratorio nazionale di riferimento per il *Campylobacter*.
Teramo 13 dicembre 2017 – Centro Internazionale per la Formazione e l'Informazione Veterinaria Francesco Gramenzi



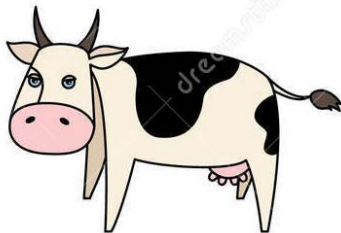
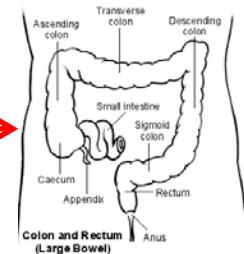
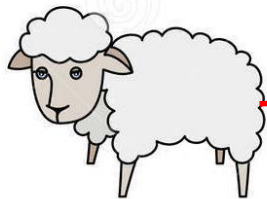
C. jejuni



CAMPYLOBACTER
JEJUNI

Sono C. jejuni. Sono un Gram negativo. Mi puoi trovare negli animali domestici. Infetto l'uomo tramite l'acqua e carne poco cotta.

Principalmente causo tossinfezioni alimentari, con diarrea autolimitante, crampi addominali e febbre



The Anthropocene: Human Impact on the Environment

An epoch is one of the smaller divisions of geologic time. Our current epoch, the Holocene, began about 11,600 years ago. But there is growing evidence that we are entering a new epoch that could be named the Anthropocene because it is marked by extensive human impacts on the environment. This poster explores evidence that future geologists might use to define the Anthropocene.

Atmosphere

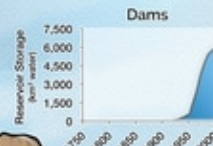
Air pollution has many components, like the emission of greenhouse gases that lead to climate change. Carbon dioxide from burning fossil fuels and changes in land use; nitrous oxide from the increased use of fertilizers; and methane from irrigated rice agriculture, cattle, and landfills are changing climate at a rate faster than most changes seen in the geologic record.

Atmospheric Concentrations of Greenhouse Gases



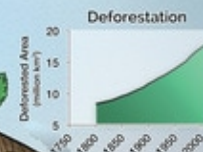
Mining

Humans literally reshape the Earth through mining and construction, causing erosion and polluting waterways. These activities also disrupt natural geochemical cycles of metallic elements, carbon, nitrogen, and phosphorus.



Water Use

Humans have drastically altered rivers and watersheds all over the world. More than half of all available fresh water is being used for agricultural, industrial, and municipal uses, drastically changing seasonal runoff patterns and downstream ecosystems.



Invasive Species

One unintended consequence of extensive global trade and travel is the rapid spread of non-native species across many localities. Invasive species change the species makeup of the environment, which will be detectable as fossils in the future.

Human Population

Cities: More than 7 billion people inhabit the planet. Their demands for land, water, food, and energy are reshaping the planet.

Farms

Natural ecosystems are converted to managed agricultural land in order to feed the world. This results in release of carbon to the atmosphere and a loss of biodiversity.

Agricultural Land Use

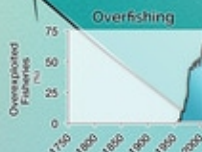


Forests

Clearing native forests for agriculture or other human development reduces biodiversity and fragments habitats, impeding the ability of species to change their geographic ranges to adapt to global warming.

Ocean

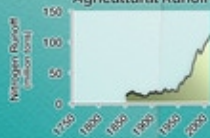
Overfishing depletes fish populations. Many traditional fisheries such as the Atlantic Cod have collapsed, causing immediate economic hardship, and also reverberating throughout the marine ecosystem, harming numerous other species.



Coastal Habitats

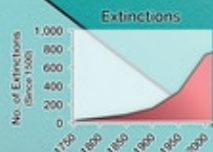
Coastal waters and nearshore ecosystems are vulnerable to pollution such as agricultural runoff, which carries nitrogen and phosphorus into coastal waters that feed plankton blooms that end up suffocating fish and shellfish. Industrial pollutants such as toxic heavy metals and organic compounds can also be deadly to coastal life.

Agricultural Runoff



Biodiversity

Almost 900 species have gone extinct in the past 500 years and the pace of extinctions has greatly accelerated in the past few decades, perhaps trending toward a mass extinction.



Begins with: Start of Ice Ages affecting both hemispheres of Earth
 Characterized by: Ice Age animals, e.g. saber-toothed tiger, mammoth

Begins with: End of the Last Ice Age
 Characterized by: Modern animal species, modern landscape

Begins with: Atomic Age, industrialization, globalization
 Characterized by: Changes in landscape, ocean and

Defining the Anthropocene

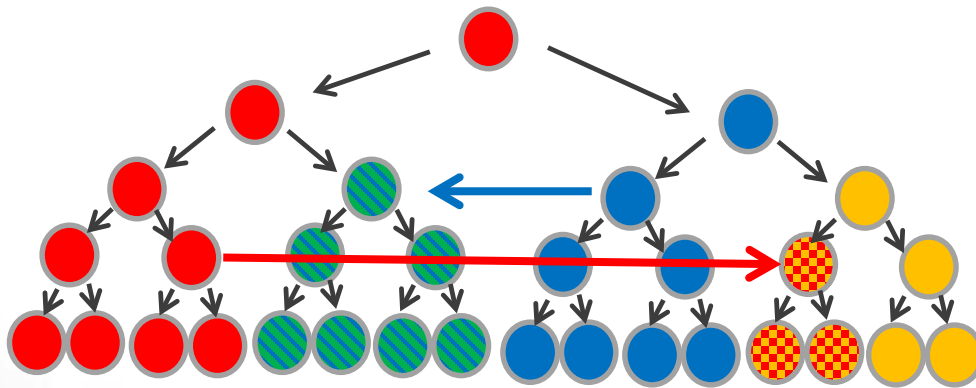
Each geologic epoch is defined by a unique marker in the rock strata, the sharper and more global the marker, the better. Markers can be fossils of new forms of life, or a chemical signal—like the high concentration of the element iridium produced when an asteroid hit Earth 66 million years ago, leading to a mass extinction.



Scientists are considering what the most useful markers for the beginning of the Anthropocene will be. Candidates include roads, microplastics, mercury from air pollution, and radionuclides from nuclear weapons testing. Various markers would put the start of the Anthropocene as early as the beginning of the industrial revolution or as recently as the beginning of widespread globalization in the 1950s.

What will characterize the fossil record of the Anthropocene? Our actions could determine whether the epoch is marked by diminishing biodiversity or even a mass extinction.

C. jejuni evoluzione

C. jejuni si caratterizza per la sua diversità genomica e per possedere una struttura di popolazione non clonale dovuta alla presenza di elevata ricombinazione genetica



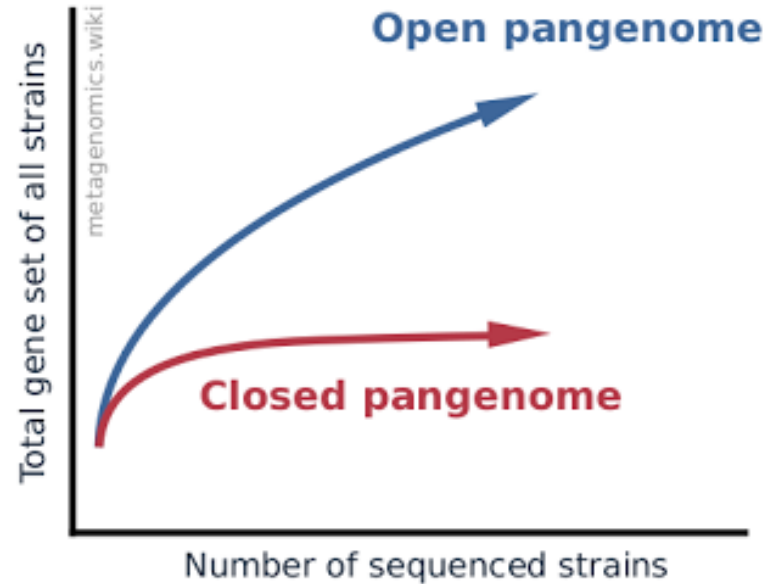
-  Genotipo non ricombinato
-  Genotipo ricombinato
-  Genotipo ricombinato

I microrganismi che manifestano ricombinazione sono più impegnativi per l'epidemiologia in quanto difficilmente possono essere associati in modo unico ad un reservoir. In pratica, bisognerebbe analizzare solo le parti del genoma informative per la loro evoluzione

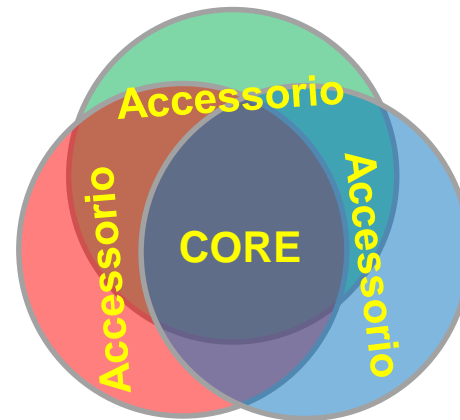
- **Alta diversità**
- **Ricombinazione omologa**



Non tutti i *Campylobacter* sono uguali



PANGENOMA



CORE: Geni conservati nella specie

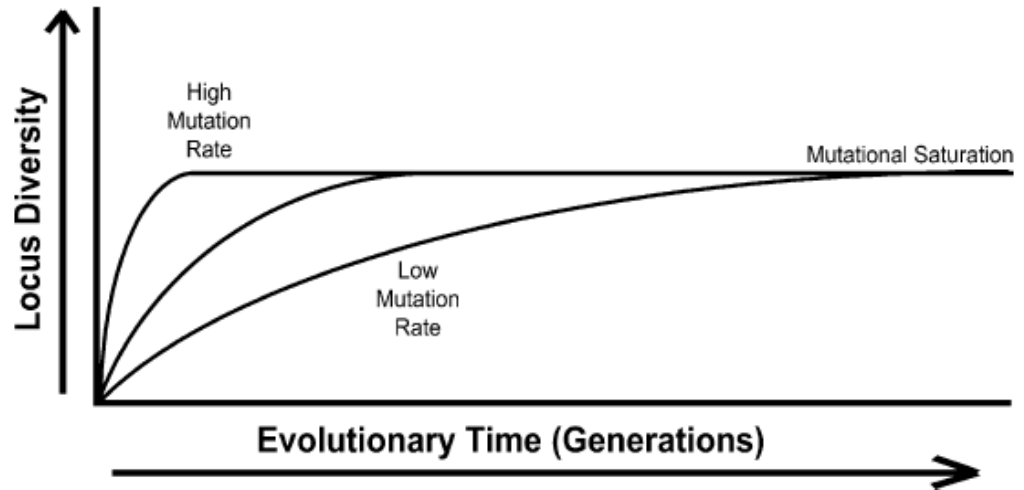
Accessorio: Geni presenti solo in uno o più ceppi

Pangenoma: il pool di geni della specie



Diversità genetica

La genotipizzazione batterica cerca di valutare i vari meccanismi genetici che sono alla base della diversità genetica (Ochman & Davalos, 2006)



La diversità genetica è una misura composta che tiene in considerazione le varianti alleliche e le loro distribuzioni in una popolazione ed è influenzata da quattro processi:

- 1- mutazione
- 2- selezione
- 3- deriva genica
- 4- ricombinazione



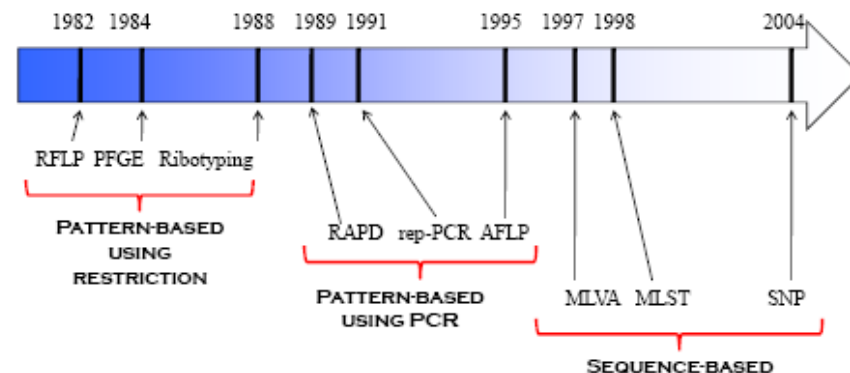
MLST

cgMLST - WGS

Metodi su base genomica:

Diventati disponibili dal momento
che sono state disponibili le
prime sequenze complete

1. dati generati da tali approcci sono facilmente conservabili e comparabili laboratoriali
2. Approccio multi locus mitiga gli errori che potrebbero generarsi con le metodiche a locus singolo
3. Vantaggiosi perché utilizzano marker a diversa velocità di mutazione



Campylobacteriosi

Studi precedenti per identificare la fonte di infezione nell'uomo basati sulla caratterizzazione genomica:

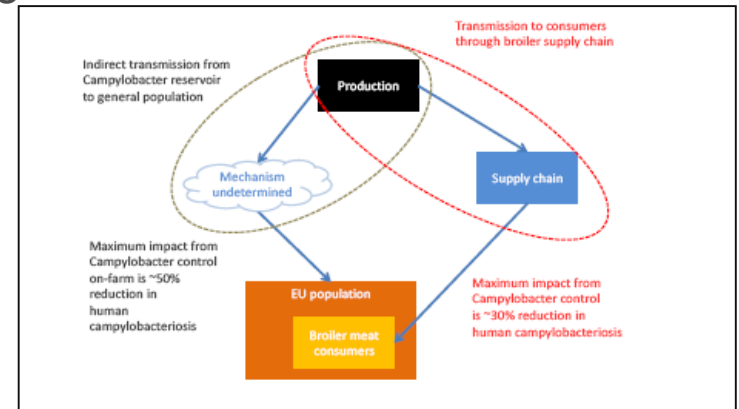
Nuova Zelanda

Inghilterra

Olanda

Danimarca:

- **Pollame 50-80%**
- **Bovino 15-30%**
- **Ovini, Maiali, Animali selvatici, acqua e ambiente contano Non più del 15%**



Quali sono le misure per ridurre il rischio ?

1. Ridurre la sua presenza in produzione primaria
2. Migliorare l'igiene della carne con trattamenti specifici





Il pollo è il principale serbatoio di *C. jejuni* che lo rende la principale fonte di infezione

• **Pollo 50-80%**

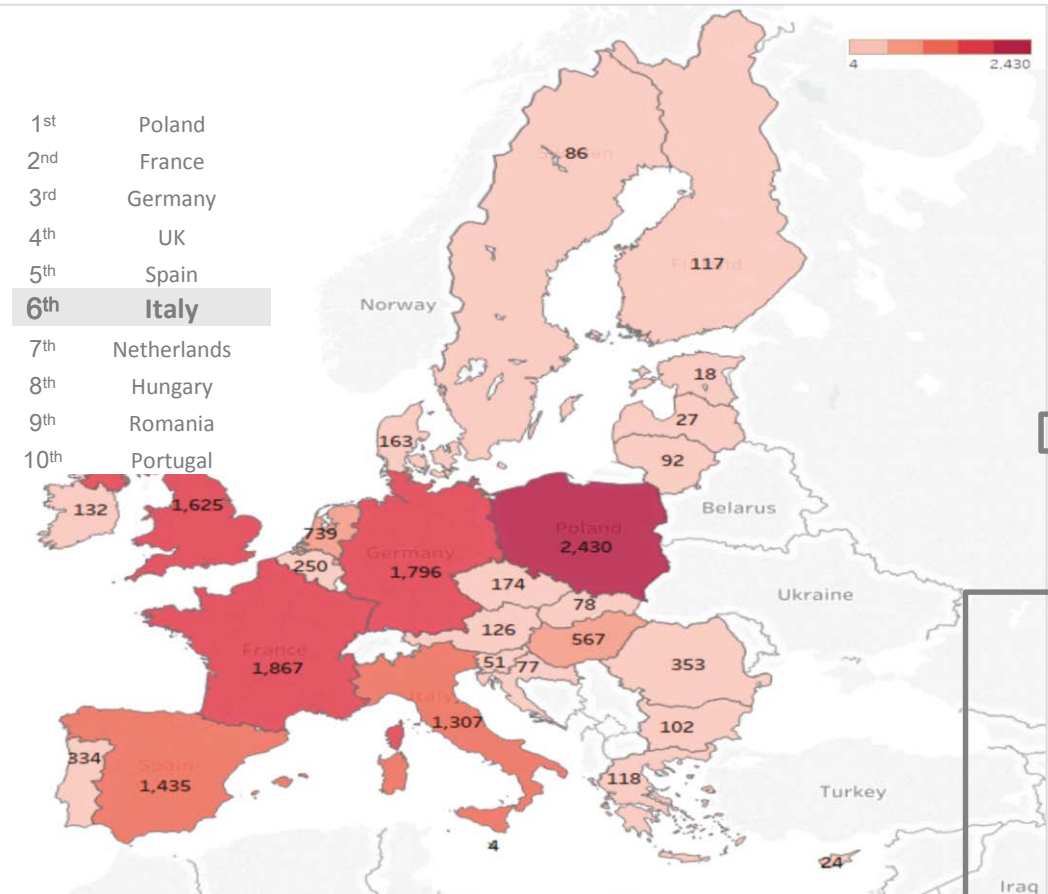
In Italia??

niel J., et al. "Tracing the source of campylobacteriosis." *PLoS genetics* 4.9 (2008): e1000303.

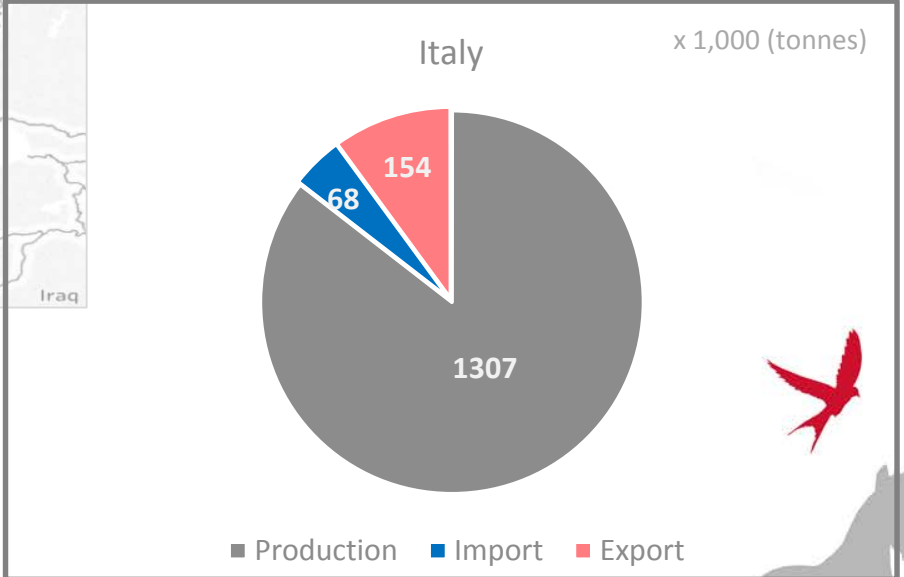
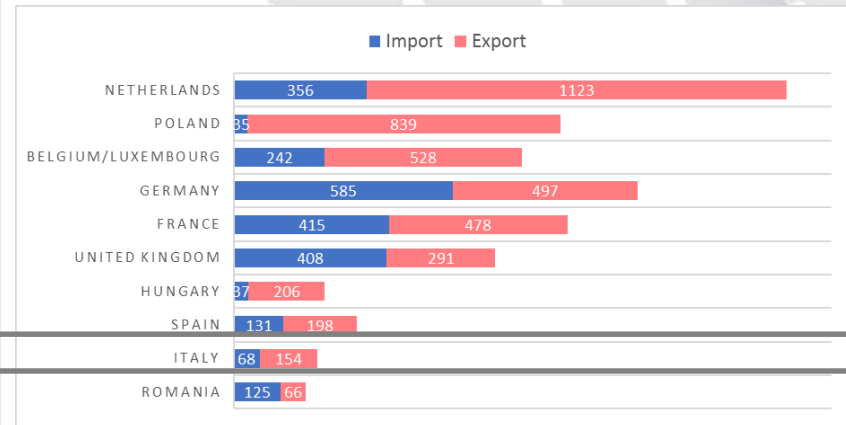
tra, et al. "Assigning the source of human campylobacteriosis in New Zealand: a comparative genetic and epidemiological approach." *Infection, Genetics and Evolution* 9.6 (2009): 1311-1319.

Mughini, et al. "Risk factors for campylobacteriosis of chicken, ruminant, and environmental origin: a combined case-control and source attribution analysis." *PloS one* 7.8 (2012): e42599.

Samuel K., et al. "Campylobacter genotyping to determine the source of human infection." *Clinical Infectious Diseases* 48.8 (2009): 1072-1078.



- 1st Poland
- 2nd France
- 3rd Germany
- 4th UK
- 5th Spain
- 6th Italy**
- 7th Netherlands
- 8th Hungary
- 9th Romania
- 10th Portugal



Produzione x 1,000 tonnellate in 2015

Data from: Association of Poultry Processors and Poultry Trade in the EU Countries, Annual Report 2016



Obiettivi

- a) Caratterizzare *C. jejuni* nella filiera avicola
- b) Studio comparativo con i genomi di *C. jejuni* pubblici



Ceppi analizzati 487

Pollo: 250

Carne di pollo: 193

Casi clinici: 44





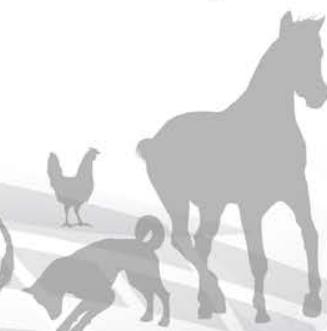
M&M Pollame

- a) 2015-2016 -
- b) 450 lotti di macellazione
- c) Northern, Central and Southern regions

	Italy		
	Conv.	f. r.	Org.
Broiler farms	6.088		
	5.812	100	176
percentage	95,5%	1,6%	2,9%
Broilers/farm	40.000	14.000	12.000
Breeding farms	208		
Broiler slaughterhouses	177		



Carcasse positive 69.2% (64.9-73.9%)



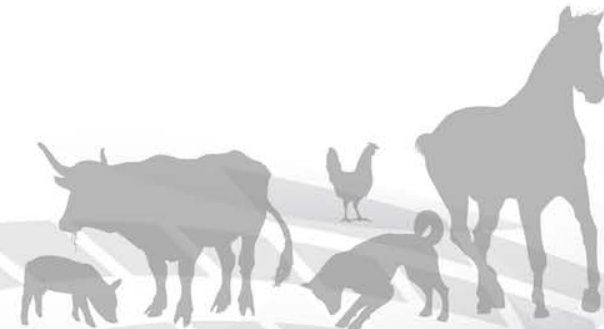



M&M Alimenti

- a) 2015-2016
- b) Vendita al dettaglio (Supermarket, convenience and traditional markets)
- c) Nord, Sud e Centro
- d) Alimenti– 1,243 (petto/coscia)

Pollo	Neg	Pos	P %
Petto e coscia	1032	216	17,37

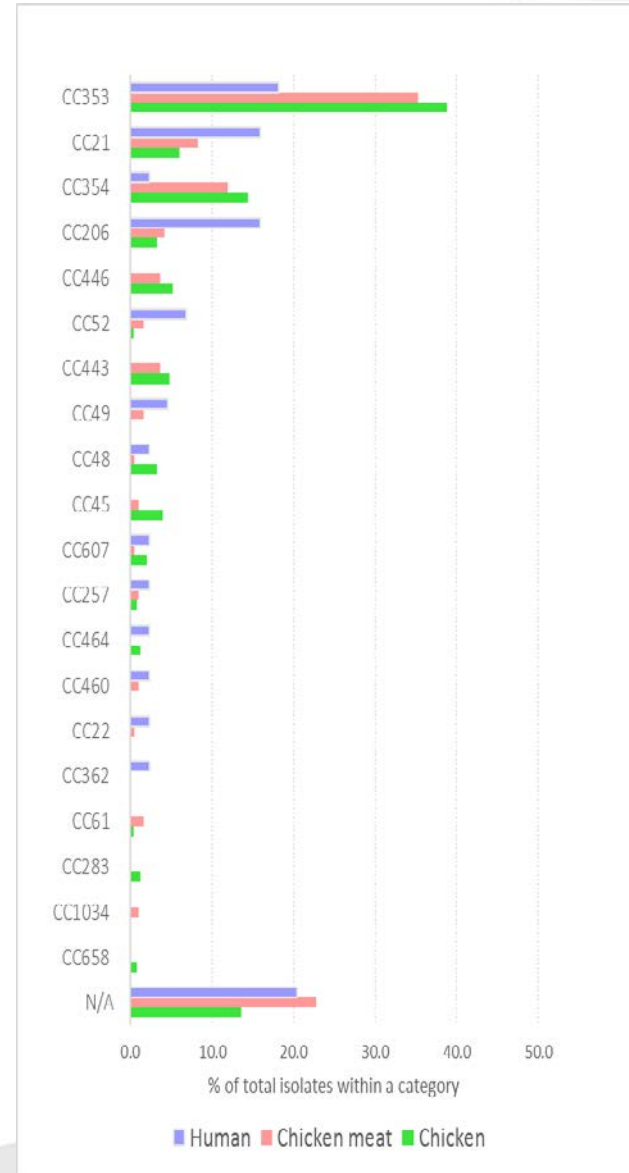
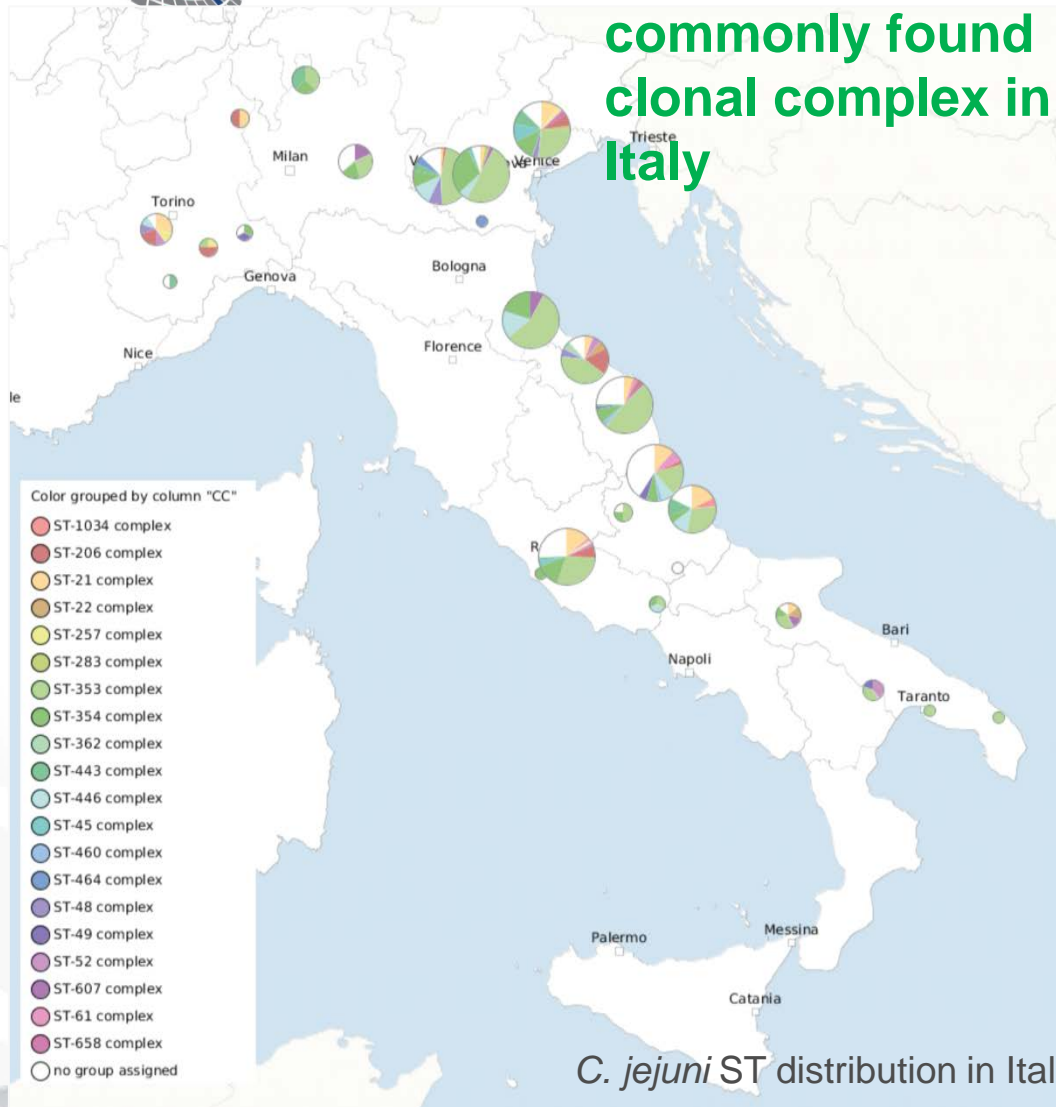
17.37%



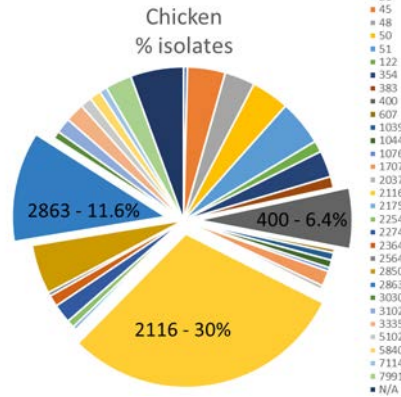
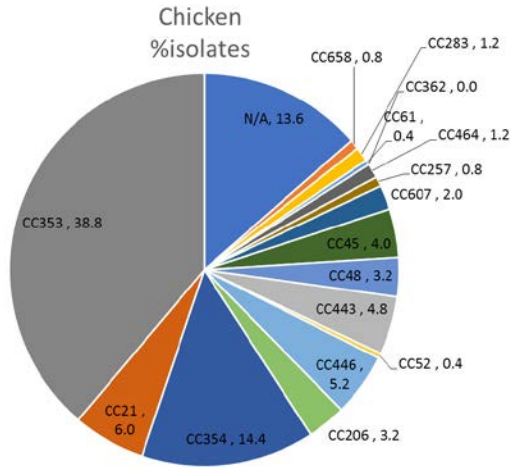
- 
- a) *C. jejuni* sono stati sequenziati con Illumina NextSeq 500 e assemblati denovo con SPAdes 3.8.1.
 - b) ~5600 genomi sono stati scaricati da ENA (November 2016) e assemblati con INNUca (QA/QC) <https://github.com/B-UMMI/INNUca>
 - c) MLST typing è stato ricavato dai WGS
 - d) Abbiamo usato un pangenoma ricavandolo con il metodo di analisi **chewBBACA: BSR-Based Allele Calling Algorithm** 2810 loci <https://github.com/mickaelsilva/chewBBACA/wiki>
 - e) cgMLST 99% → 989 loci
 - f) Abbiamo definito un Clusters quel gruppo che possedeva non più del 5% di diversità allelica (I gruppi differiscono per non più di 50 loci per tutto il cgMLST over 989 loci)
 - g) Per ogni gruppo abbiamo applicato uno schema di cgMLST specifico (approccio dinamico di cgMLST)



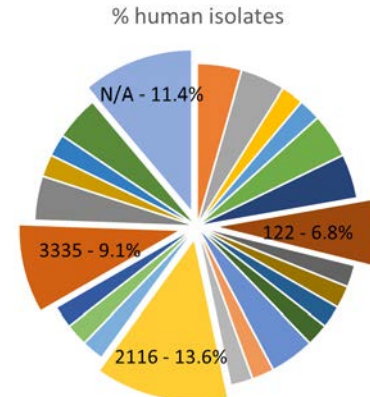
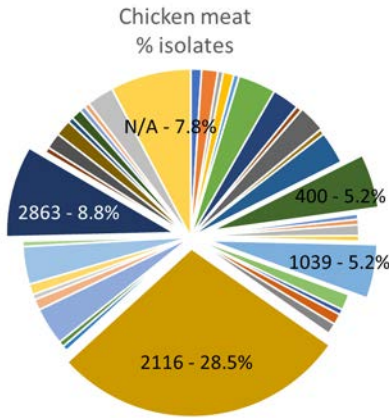
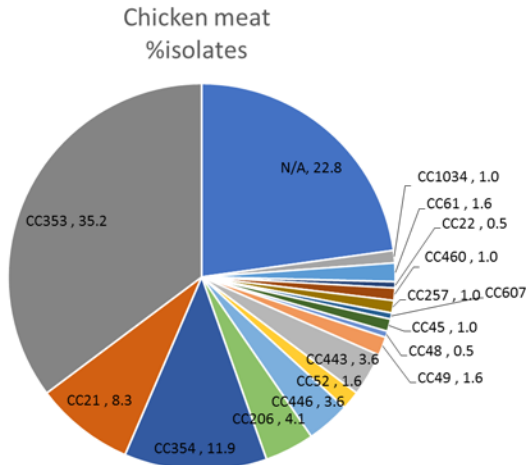
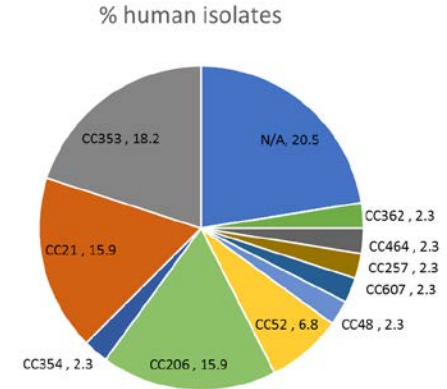
CC353 is the most commonly found clonal complex in Italy



MLST



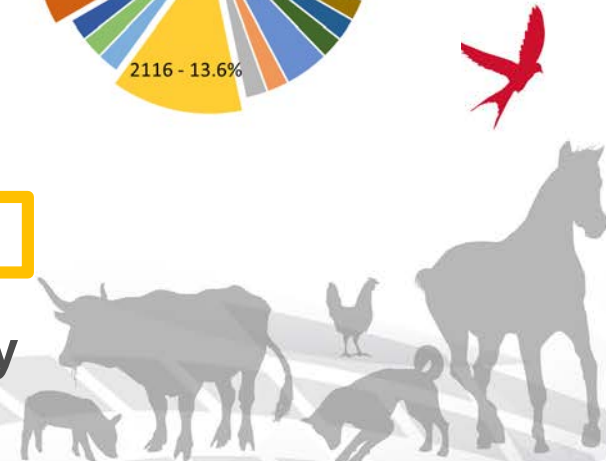
- 21
- 45
- 48
- 50
- 51
- 122
- 354
- 383
- 400
- 607
- 1039
- 1044
- 1076
- 1707
- 2037
- 2116
- 2175
- 2254
- 2274
- 2364
- 2564
- 2850
- 2863
- 3030
- 3102
- 3335
- 5102
- 5840
- 7114
- 7991
- N/A

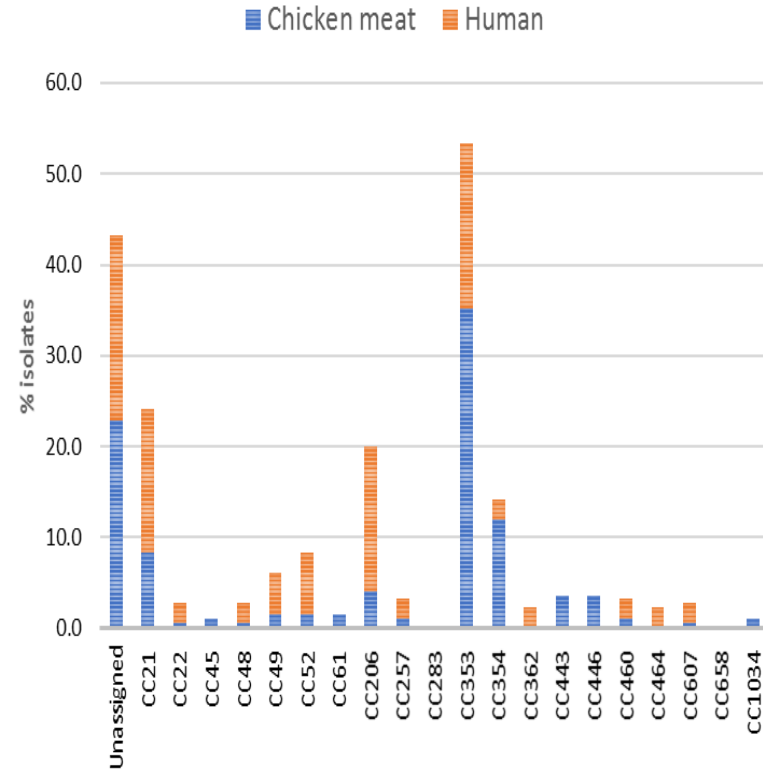
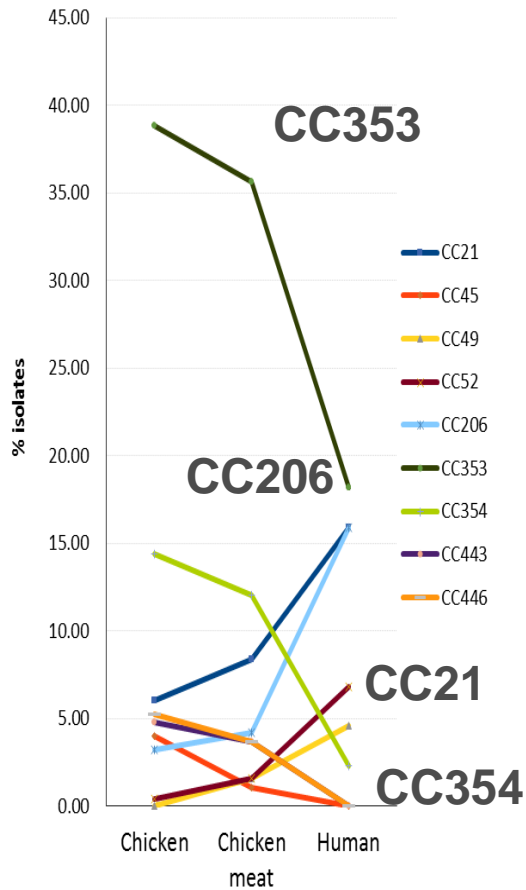


CC 353

ST-2116


Prevalence of *C. jejuni* isolates isolated in Italy





Cambiamento della prevalenza dei complessi clonali nella filiera del pollo da carne



- 
- a) I nostri genomi (~500) sono stati divisi in 86 cgMLST99 (989 loci) gruppi (senza singletons);
 - b) 42 (~50%) comprendono solo isolati Italiani
 - c) Il gruppo più grande (ST21) comprende 5 ceppi Italiani
 - d) La maggior parte dei ceppi italiani (24% of the samples; 138) clusterizzavano in un singolo gruppo (ST2116)
 - e) Il secondo gruppo più grande (ST2863) comprendeva anche 2 genomi provenienti da UK



Country

ST21

Source **cgMLST**

Gruppo più grande

5 from **Italia**

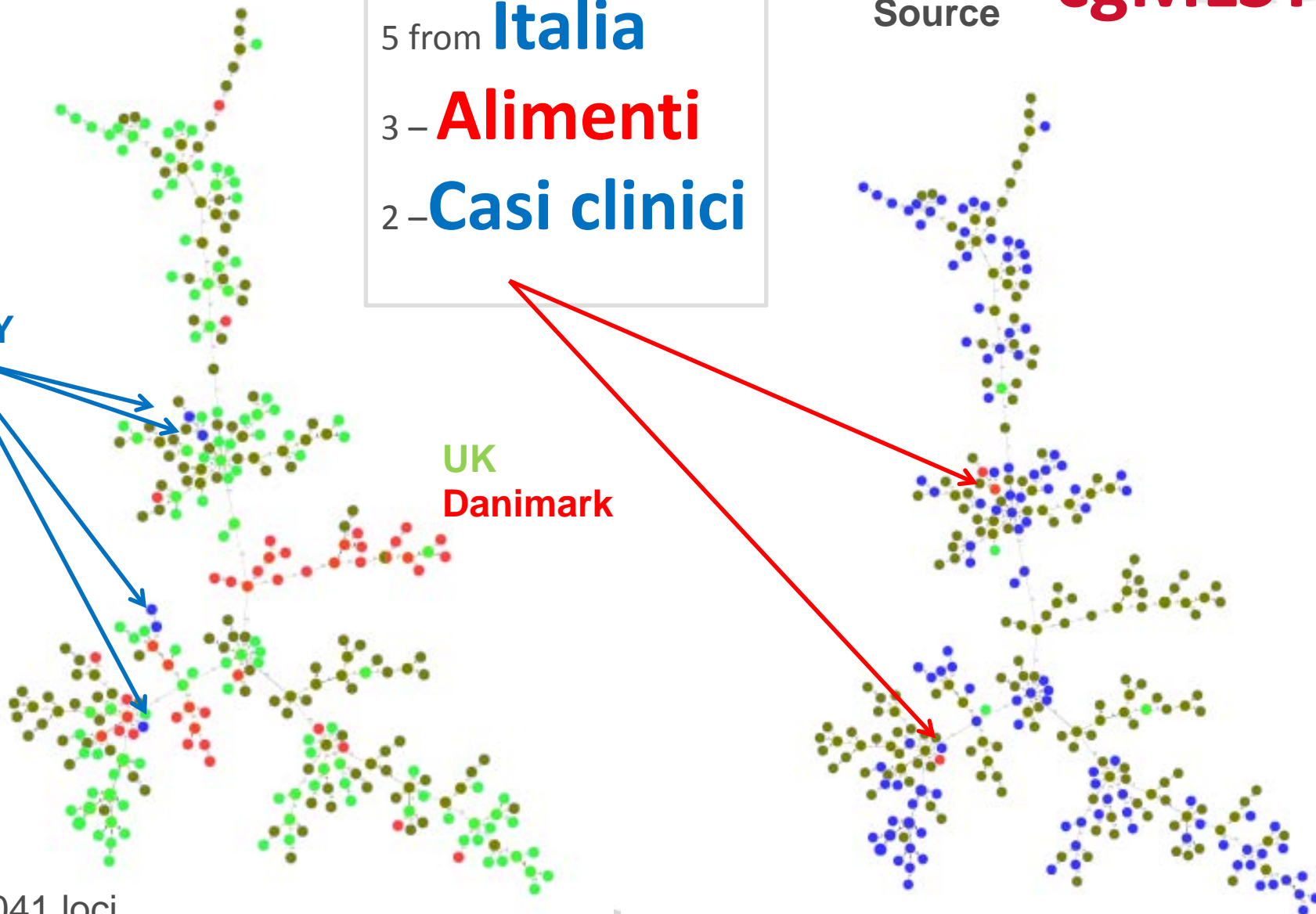
3 – **Alimenti**

2 – **Casi clinici**

ITALY

UK
Danimark

1,041 loci



ST2116

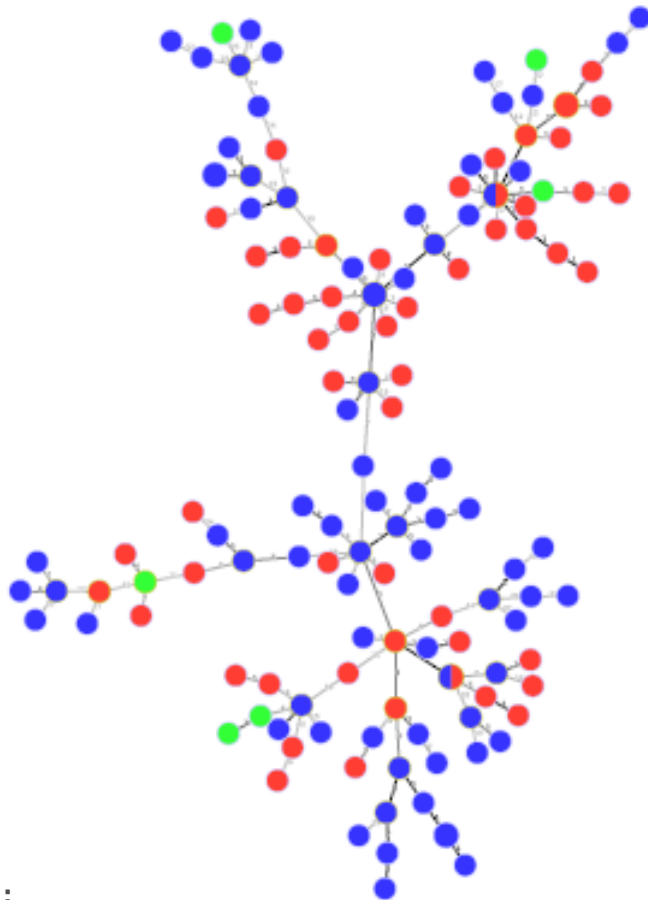
Il gruppo italiano più grande
138 ceppi

Tutti dall'**Italia**

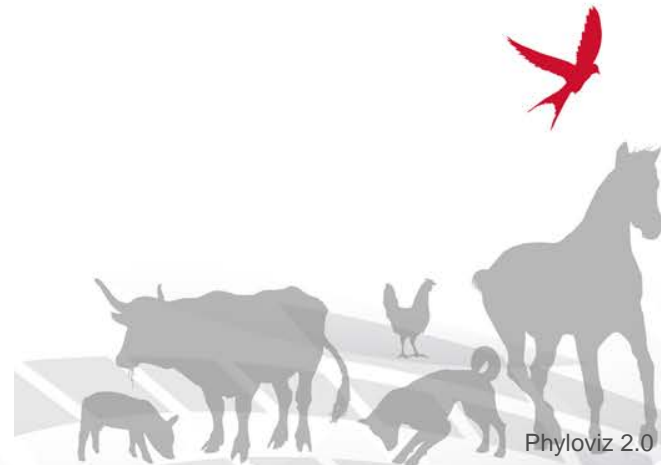
55 – **Alimenti**

77 – **Pollo**

6 – **Casi clinici**



1,088 loci





ST2863

Il secondo gruppo più grande
48 ceppi

46 from **Italia**

2 from **UK**

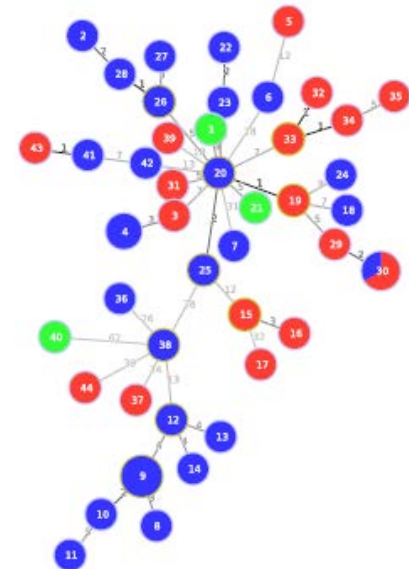
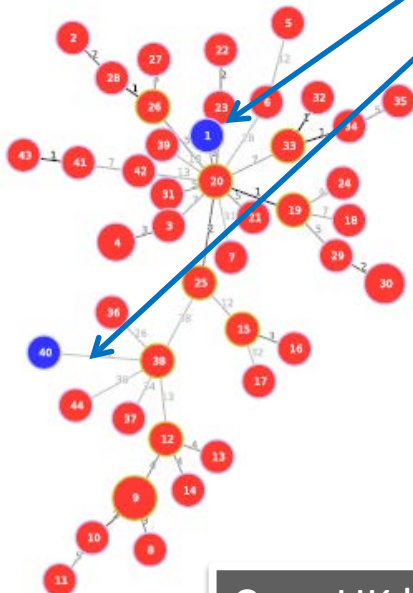
18 – **Alimenti**

29 – **pollo**

3 – **casi clinici**

Country

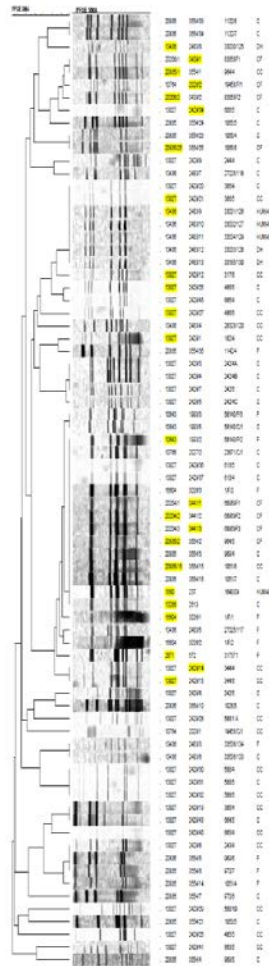
Source



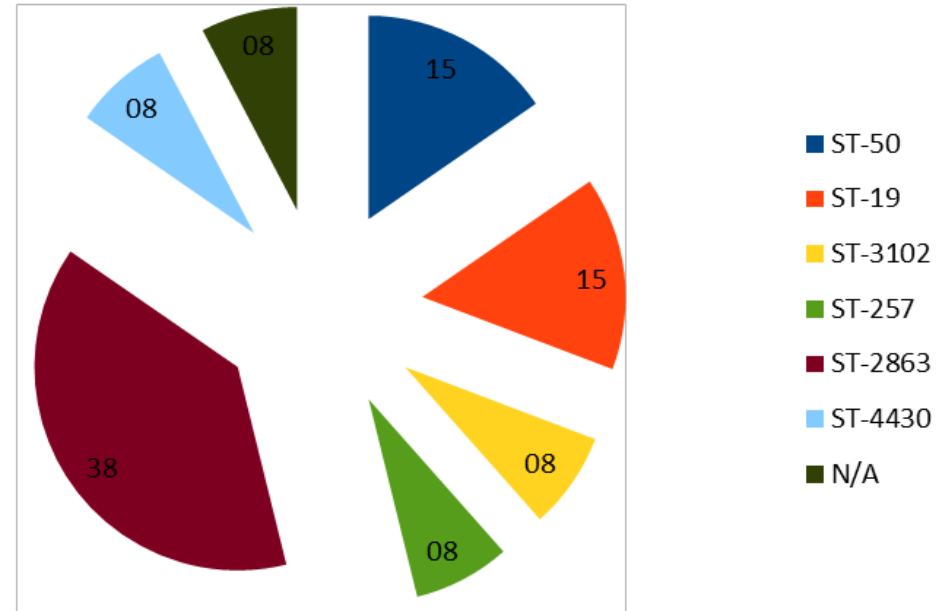
1,031 loci

Caso UK ha mostrato solo 4 alleli di differenza dai genomi italiani

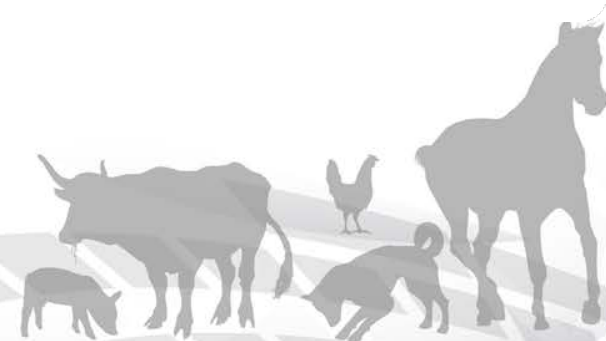
Work in progress



ST distribution *C. jejuni* -2008/2009



Abbiamo selezionato 30 isolati dai profili PFGE types per WGS and MLST profiling



Conclusioni

- a)** Il nostro studio ha messo in evidenza che nella filiera del pollo da carne esistono 2 cloni di *C. jejuni* (St 2116 e St2863) che sembrano essere geograficamente isolati nel nostro paese
- b)** La loro coevoluzione potrebbe suggerire la presenza di caratteristiche idonee alla sopravvivenza nella nostra filiera
- c)** Quali sono le ragioni dell'espansione relativamente recente del St2116?
- d)** Le nostre analisi hanno evidenziato una elevata identità nucleotidica tra ceppi della nostra filiera e casi clinici UK, il che potrebbe suggerire l'introduzione di tali ceppi tramite l'esportazione di prodotti avicoli o infezioni da viaggio....
- e)** Le nuove metodologie di tipizzazione su dati NGS si sono mostrate più efficaci rispetto ai metodi tradizionali





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Laboratorio Nazionale di Riferimento

Ringraziamenti



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