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Poultry Supply Chain Impact on Campylobacteriosis Surveillance in Italy Over One Year

Giuliano Garofolo

The 19th EURL-Campylobacter workshop 21-23 October 2024

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ICEBERG effect

Human cases

Notification rate (per 100,000 population) 41.1

Trend (2017-2021) — Increasing, Decreasing, Stable

127,840 Cases of illness

81,311 Infections acquired in the EU

10,469 Hospitalisations

704 Infections acquired outside the EU

26 Deaths

45,825 Unknown travel status or unknown country of infection

Human cases in foodborne outbreaks

249 Foodborne outbreaks

20 Strong-evidence outbreaks

229 Weak-evidence outbreaks

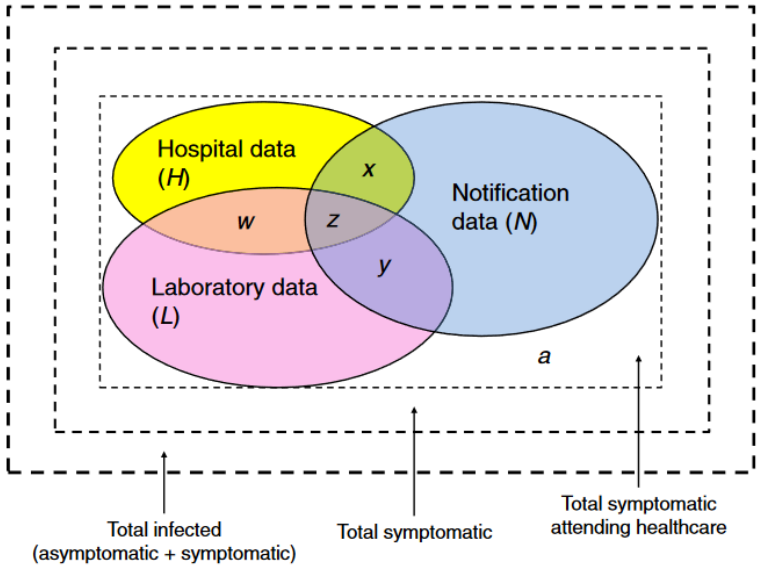
1,051 Cases of illness

134 Hospitalisations

6 Deaths

Campylobacter	Italy
Estimated incidence	586 x 100.000
Estimated cases	352.000
Cases reported	1,539
Reduction factor	250

Asyntomatic
Self-limiting



under-ascertainment (UA)
Underreporting

Underestimation

Gibbons, Cheryl L., et al. "Measuring underreporting and under-ascertainment in infectious disease datasets: a comparison of methods." *BMC public health* 14 (2014): 1-17.
Authority, European Food Safety. "The European Union One Health 2022 Zoonoses Report." *EFSA Journal* 21.12 (2023).

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Campylobacter – Animals

Animal reservoirs/Amplifying hosts

Healthy animals

Campylobacter is often found in warm-blooded animals, which are normally considered natural hosts

Prevalence*

71% *C. jejuni/C. coli* in broilers
45% *C. jejuni* in dogs
36% *C. jejuni* in cattle
42% C.jejuni/C. coli sheep
42% *C. coli* in pings

*The data presented are derived from NRL-IT Campylobacter research projects. They require caution in interpretation and careful evaluation of the epidemiological context

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frontiers in Microbiology

ORIGINAL RESEARCH
published: 13 June 2016
doi: 10.3389/fmicb.2016.00887

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

*Elisabetta Di Giannatale[1], Giuliano Garofolo[1], Alessandra Alessiani[1], Guido Di Donato[1], Luca Candeloro[2], Walter Vencia[3], Lucia Decastelli[3] and Francesca Marotta[1]**

Source attribution of 44 human Campylobacter jejuni isolates Piedmont, August 2012.

MLST

Asymmetric island assignment model

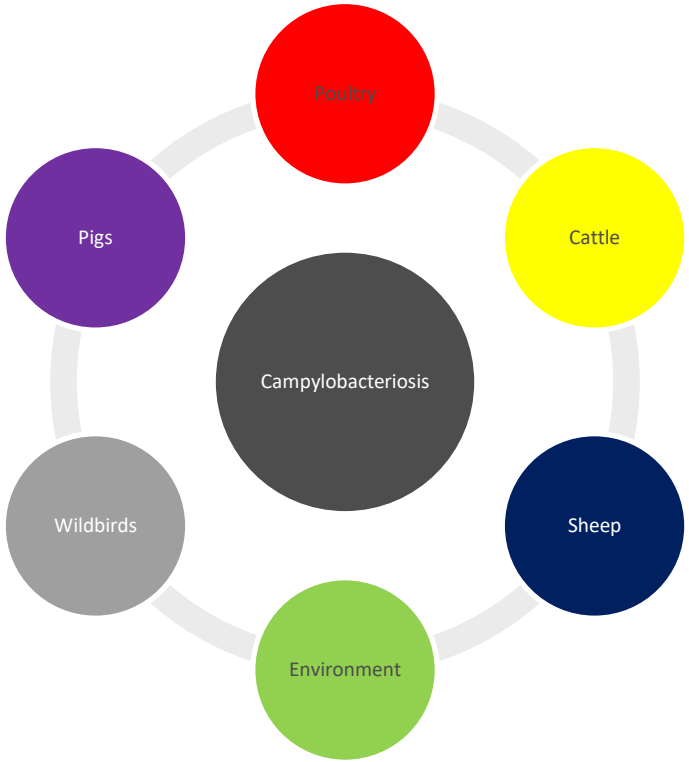
Phylogenetic approach with probabilistic assignment of cases to the considered sources of infection.

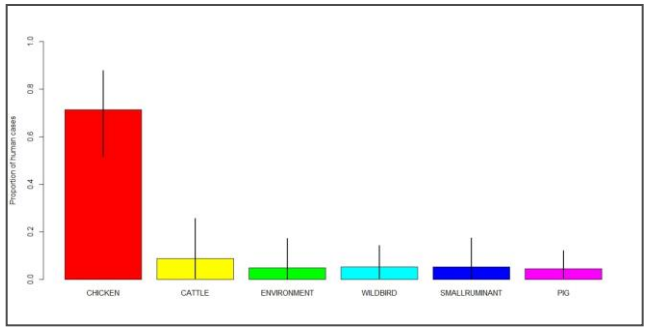
Input model

-Italy No.**687** C. jejuni strains

-PubMLST Nu. **6911** ù
DA 6 RESERVOIR

CROSS-VALIDATION
97% Pollame,
98% Ruminanti
77% uccelli selvatici,
62% ambiente





69,7 % 8,2% 7,3% 6,2% 5,3% 2,9%

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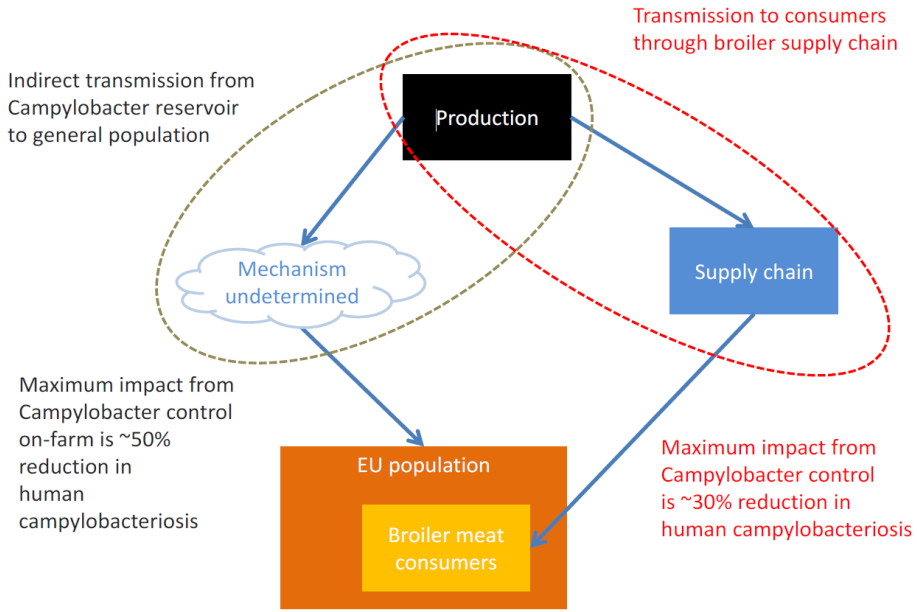
Source



- **Poultry as source 50-80%**

Wilson, Daniel J., et al. "Tracing the source of campylobacteriosis." *PLoS genetics* 4.9 (2008): e1000203.

Mullner, Petra, 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.

Gras, Lapo 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.

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

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Slaughterhouse in Italy

- 2015-2016
- 450 batches

	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		

*Positive carcasses 69.2% (64.9-73.9%)

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Campylobacter at retail in Italy

Chicken	Neg	Pos	P %
Breast and Tight	1032	216	17,37

Bovine	Neg	Pos	P %
Hamburger Italian traditional preparations	1196	7	0.58

17.37% > 0,58%

p<0,0001; X2TEST

Di Giannatale, E., Calistri, P., Di Donato, G., Decastelli, L., Goffredo, E., Adriano, D., ... & Migliorati, G. (2019). Thermotolerant Campylobacter spp. in chicken and bovine meat in Italy: Prevalence, level of contamination and molecular characterization of isolates. *PLoS One*, *14*(12), e0225957.

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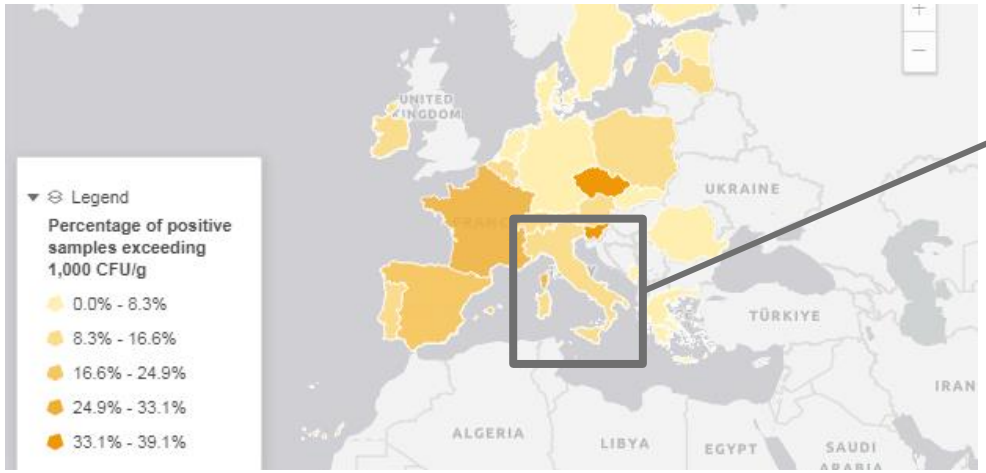
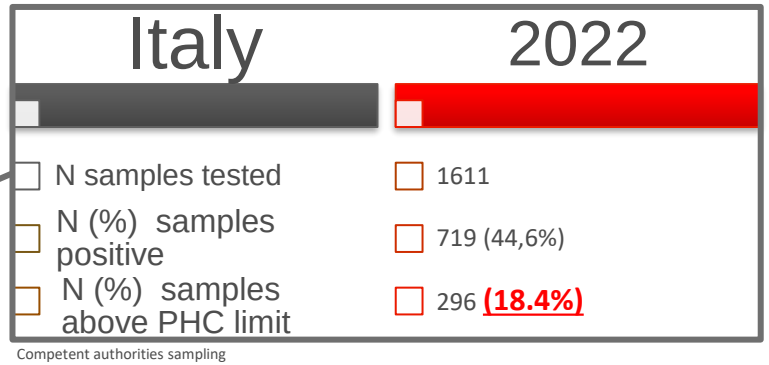
CAMPYLOBACTER IN THE CONTEXT OF REGULATION (EC) NO 2073/2005

It establishes a regulatory limit (process microbiological hygiene criterion - PHC) of 1,000 CFU/g of *Campylobacter* on the neck skins of chilled chicken carcasses. Compliance with the PHC indicates that the production process is functioning correctly



Food busisness operator sampling, 2022



Competent authorities sampling

Corrective measures required!

EFSA EUROPEAN FOOD SAFETY AUTHORITY

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Campylobacter

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SCOPE

- Studies for the identification of the origin of *Campylobacter* infection in humans are poorly undertaken in Italy and other international settings
- Surveillance of other foodborne pathogens using high-resolution typing methods has proven effective in detecting outbreaks and monitoring trends in epidemic strains
- A similar system for *Campylobacter* has not been widely adopted or made significant contributions

This study aims to understand the dynamics of *Campylobacter* strains circulating in poultry meat and their correlation with human case clusters occurred in Italy in 2023, using whole genome sequencing (WGS)

150 HUMAN ISOLATES	
127 - *C. jejuni*	23 – *C. coli*

109 POULTRY ISOLATES	
66 - *C. jejuni*	43 - *C. coli*

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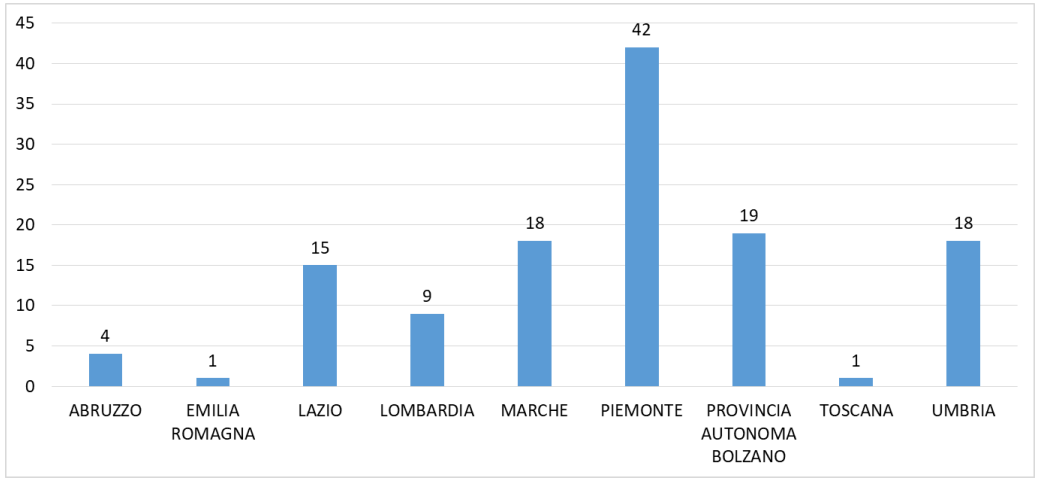
Humans – Enternet

150 – *Campylobacter*

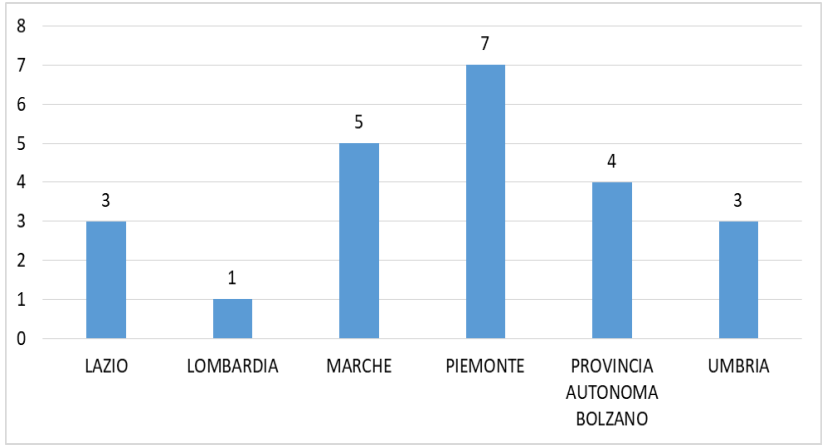
127 – *C. jejuni*

Isolated from stools



23 – *C. coli*

22 isolated from stools
1 isolated from blood



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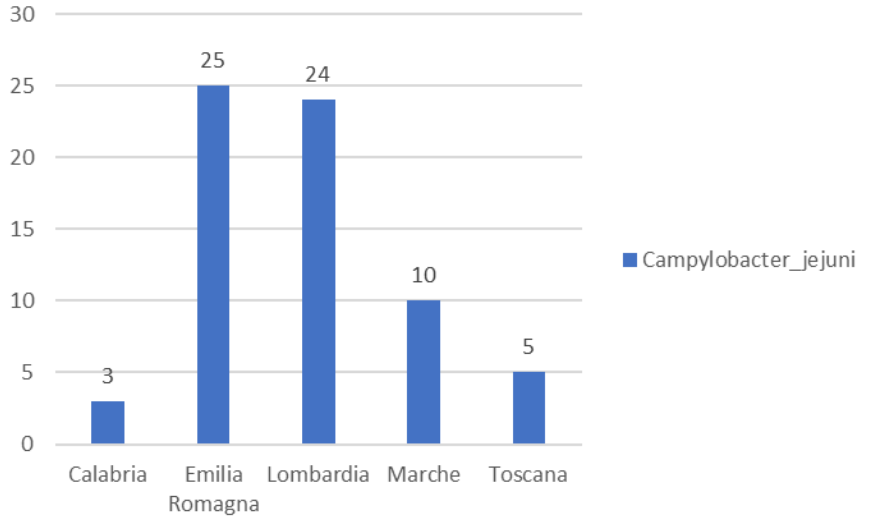
Food – PHC

109 – Campylobacter

66 – C. jejuni

Isolati – Alimenti

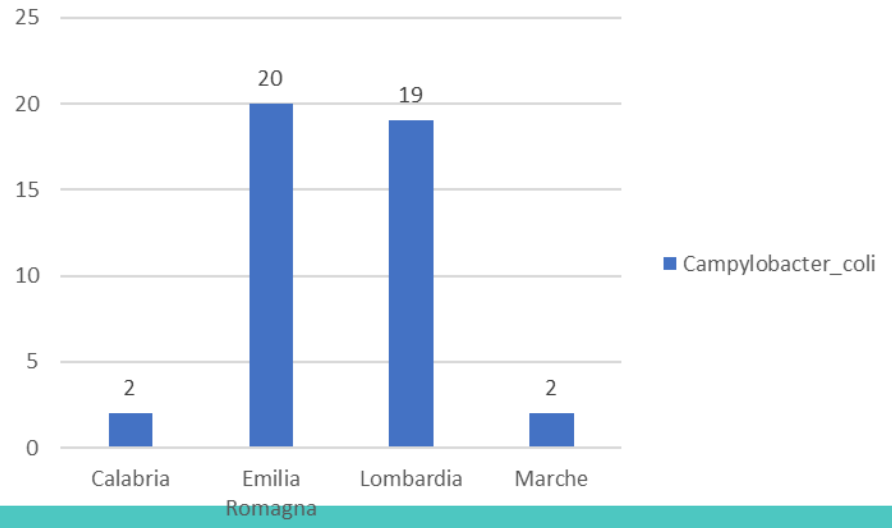
17 - Aziende



43 – C. coli

Isolati – Alimenti

10 - Aziende



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Year 2023 Surveillance C. jejuni

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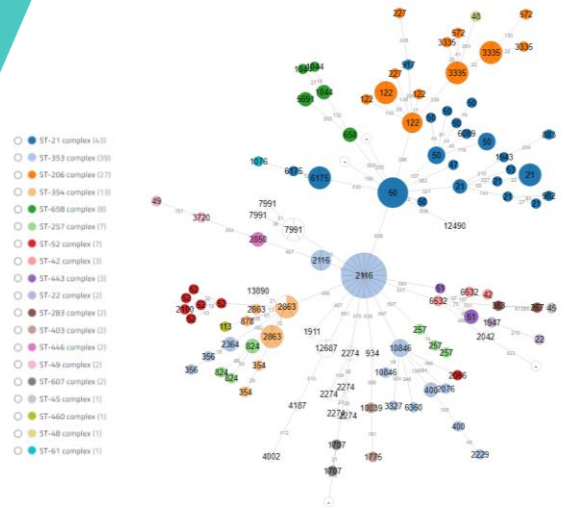
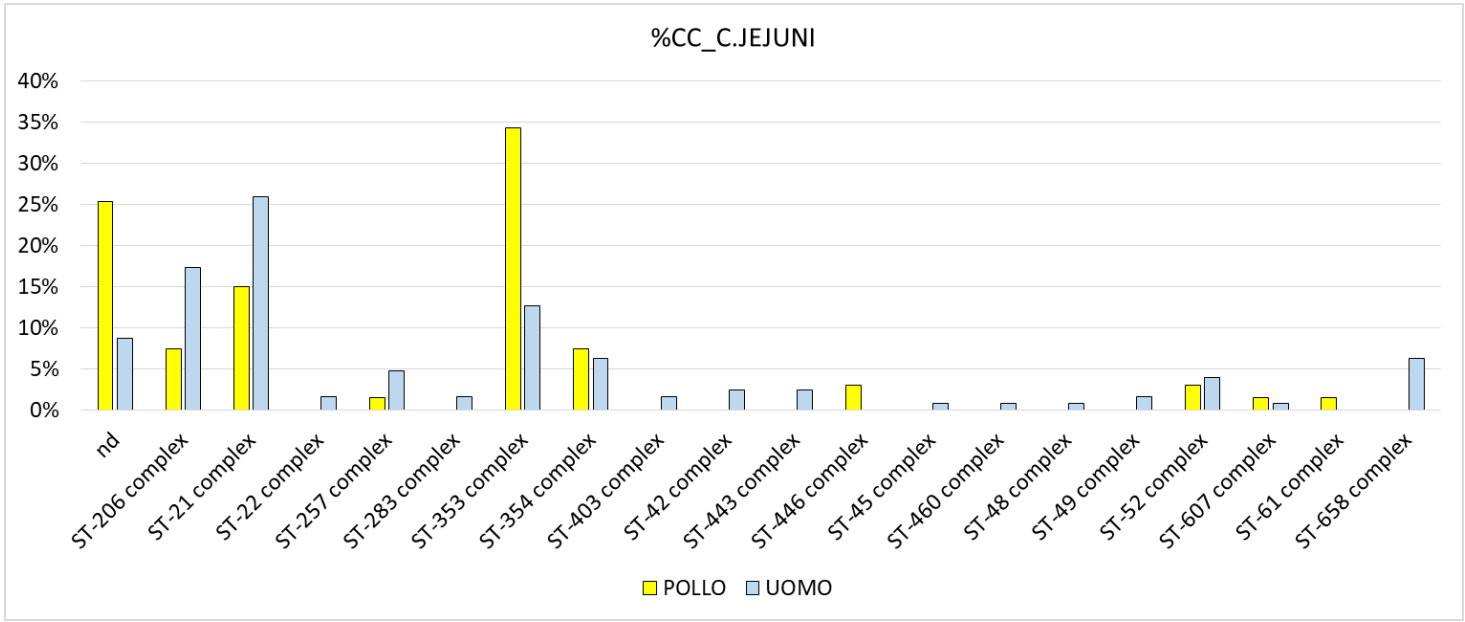
MLST C.JEJUNI

Poultry= 66
Humans= 127

19 CCs
9 CC - SHARED





RESULTS

LABORATORIO NAZIONALE DI RIFERIMENTO PER *CAMPYLOBACTER*

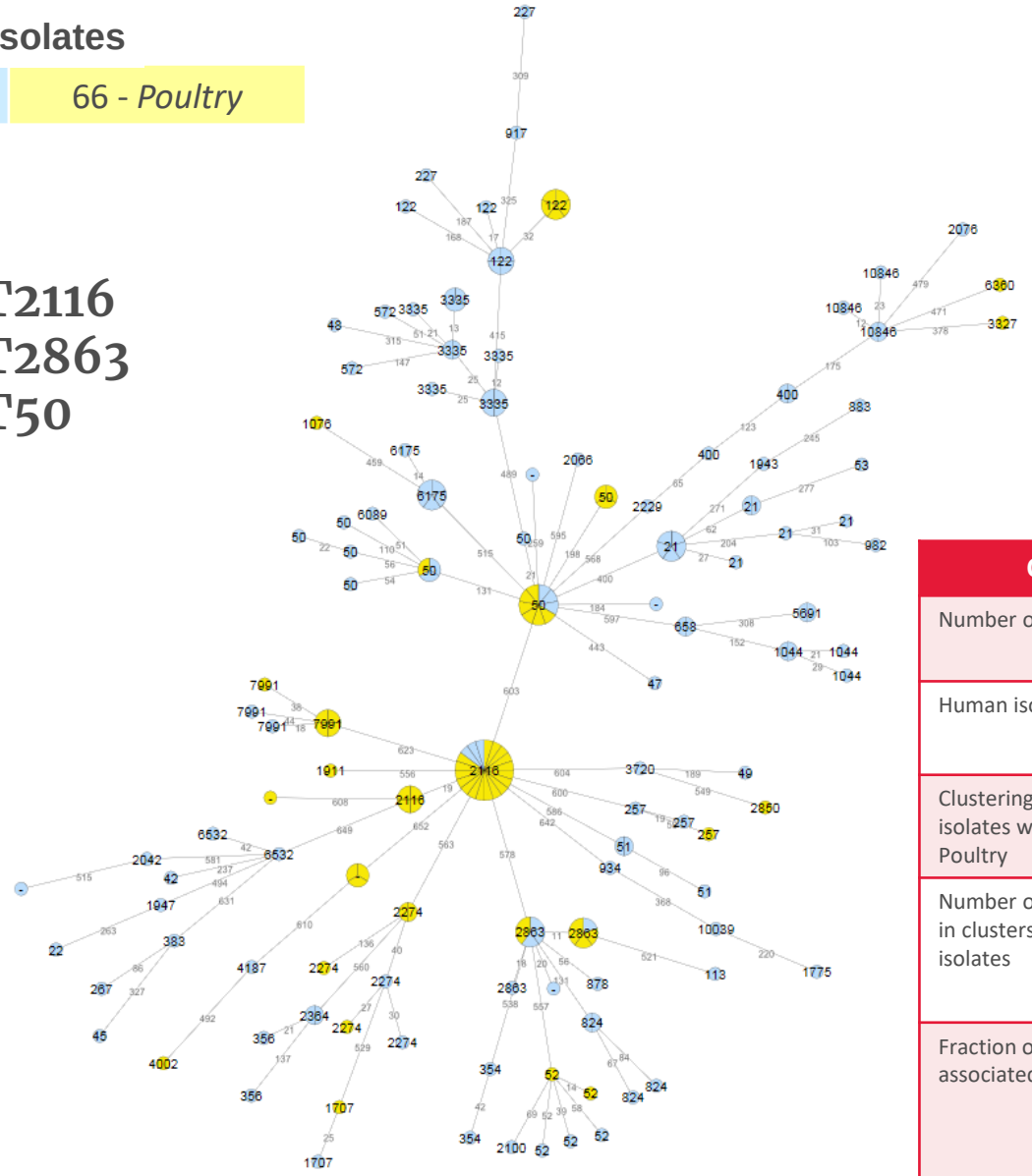
C. jejuni isolates

127- *Human*	66 - *Poultry*

23% ⇒

- **ST2116**
- **ST2863**
- **ST50**

cgMLST _*C. jejuni*_678



C. jejuni	10 alleles cut-off *
Number of clusters	25
Human isolates in clusters	53
Clustering index of human isolates with Human or Poultry	**42%**
Number of human isolates in clusters with poultry isolates	12
Fraction of human isolates associated with poultry	**23%**

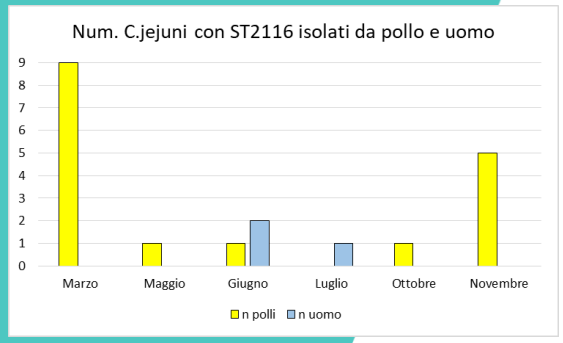
*Reference: Joseph, Lavin A., et al. "Evaluation of core genome and whole genome multilocus sequence typing schemes for *Campylobacter jejuni* and *Campylobacter coli* outbreak detection in the USA." *Microbial Genomics* 9.5 (2023): 001012.

Similar results obtained with *C. jejuni/coli* cgMLST (637 loci) RIDOM SEQSPHERE *and C. jejuni/coli*_cgMLST (1343 loci) OXFORDSHIRE

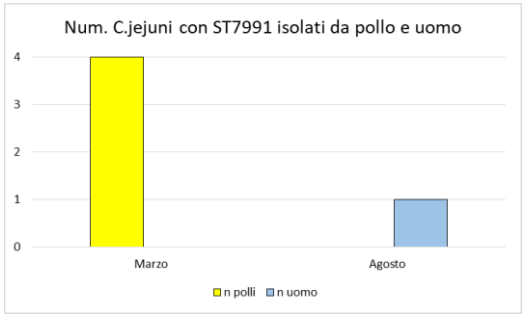
Cluster

Cluster	Campioni	Origine	ST	Giorni
	20	**Pollo (17) Uomo (3)**	**2116**	**257**
	4/5	**Pollo (1) Uomo (3)/Pollo (4) Uomo (1)**	**2863**	**316/190**
	9/3	**Pollo (6) Uomo (3)/Pollo (1) Uomo (2)**	**50**	**150/241**
	5	**Pollo (4) Uomo (1)**	**7991**	**110**
	5/5	Uomo (5)/Uomo (5)	3335	
	5/2	Uomo (5)/Uomo (2)	21	
	4	Uomo (4)	122	
	2	Uomo (2)	51	
	3	Uomo (3)	10846	
	2	Uomo (2)	2850	
	2	Uomo (2)	400	
	2	Uomo (2)	658	
	2	Uomo (2)	824	
	2	Uomo (2)	1044	
	2	Uomo (2)	2364	
	2	Uomo (2)	2274	
	2	Uomo (2)	5691	
	5	Uomo (5)	6175	

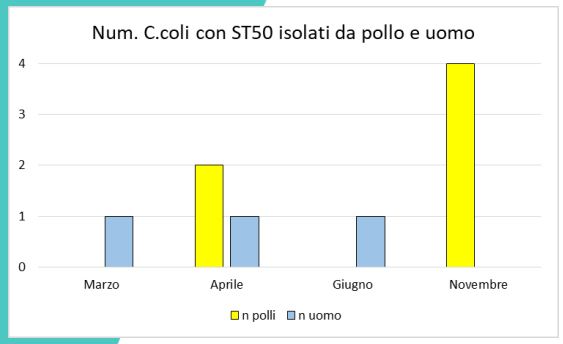
22 cluster - Humans



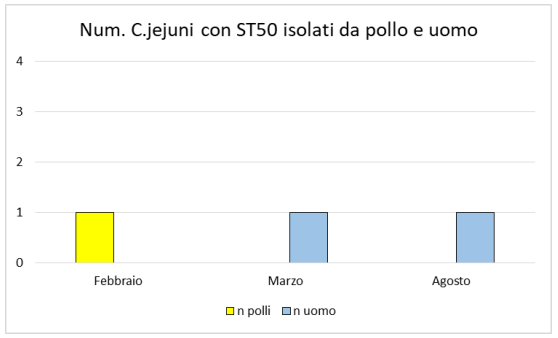
✓ 6 slaughterhouses – Emilia Romagna/Lombardia;
Cases – Piemonte.



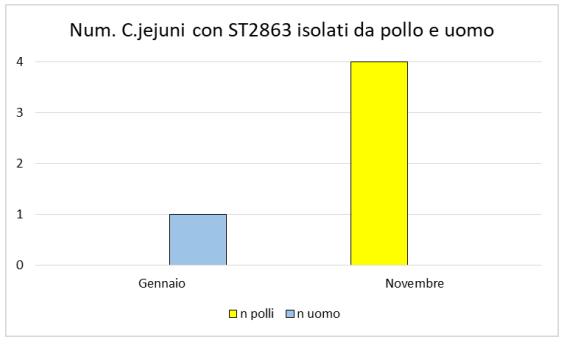
✓ 1 slaughterhouses – Marche;
Cases – Umbria.



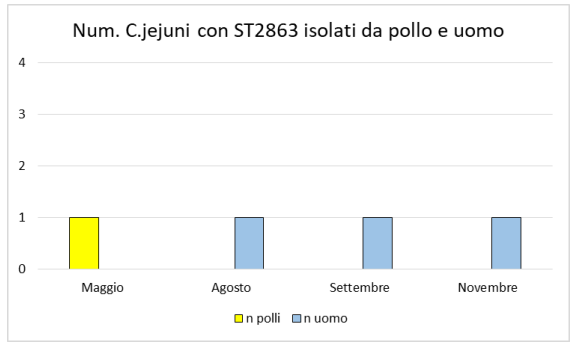
✓ 2 slaughterhouses – Emilia Romagna/Marche;
cases - Piemonte-Umbria-Marche.



✓ 1 Slaughterhouse – Lombardia;
Casi – Bolzano-Lombardia.

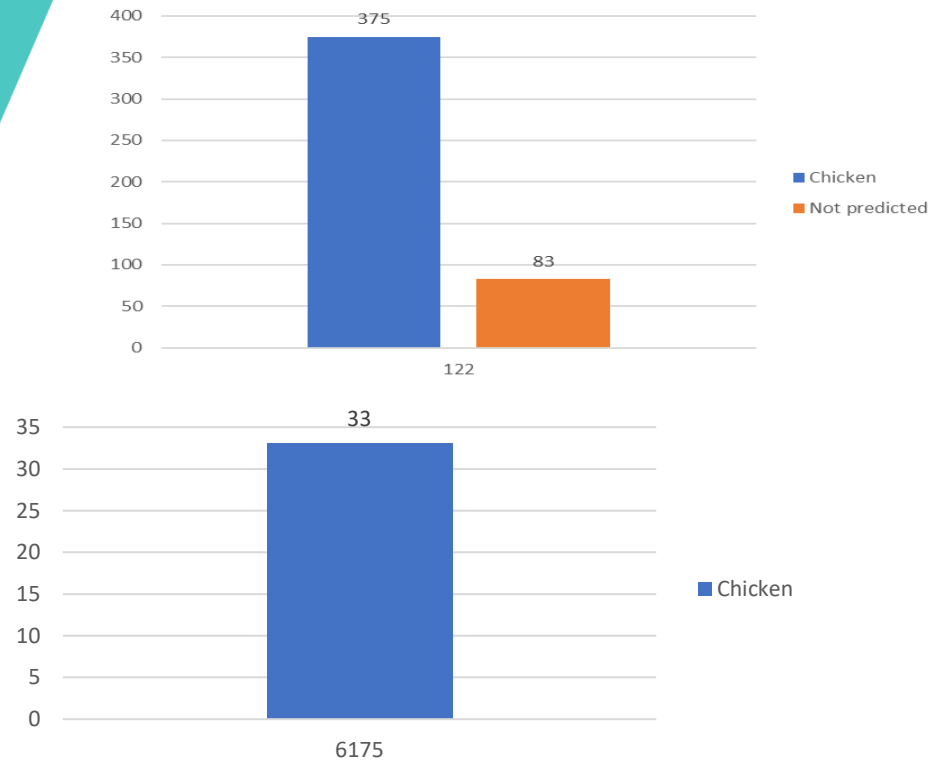


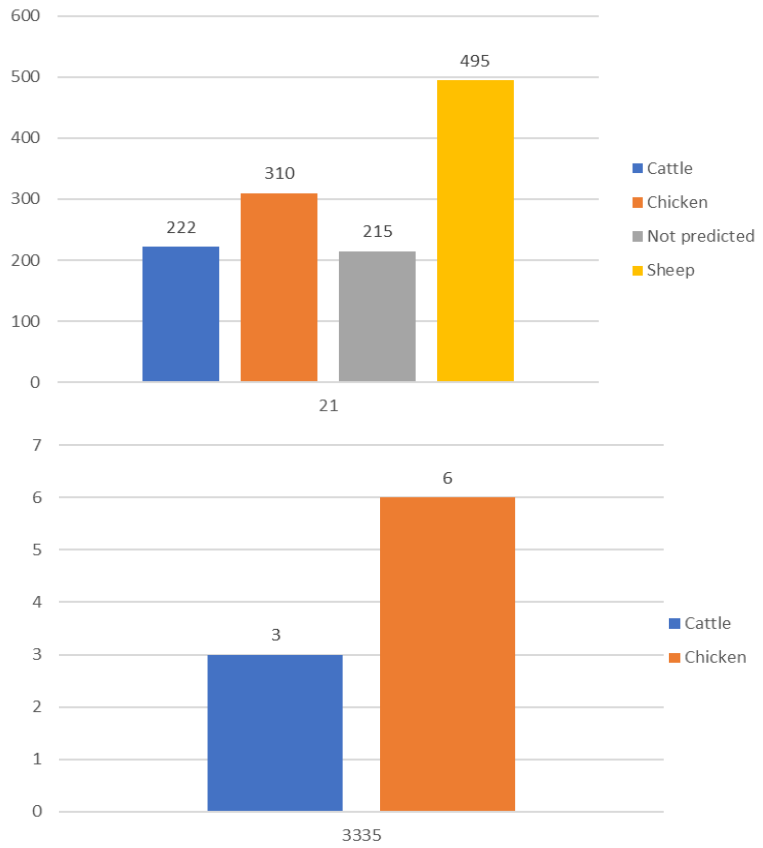
✓ 1 slaughterhouse – Lombardia;
Casi –Bolzano.



✓ 1 slaughterhouse – Lombardia;
Casi – Marche-Bolzano.

Machine learning & Source Attribution





	5	Uomo (5)	3335
	5	Uomo (5)	21
	4	Uomo (4)	122
	5	Uomo (5)	6175

Arning, N., Sheppard, S. K., Bayliss, S., Clifton, D. A., & Wilson, D. J. (2021). Machine learning to predict the source of campylobacteriosis using whole genome data. *PLoS genetics*, *17*(10), e1009436.

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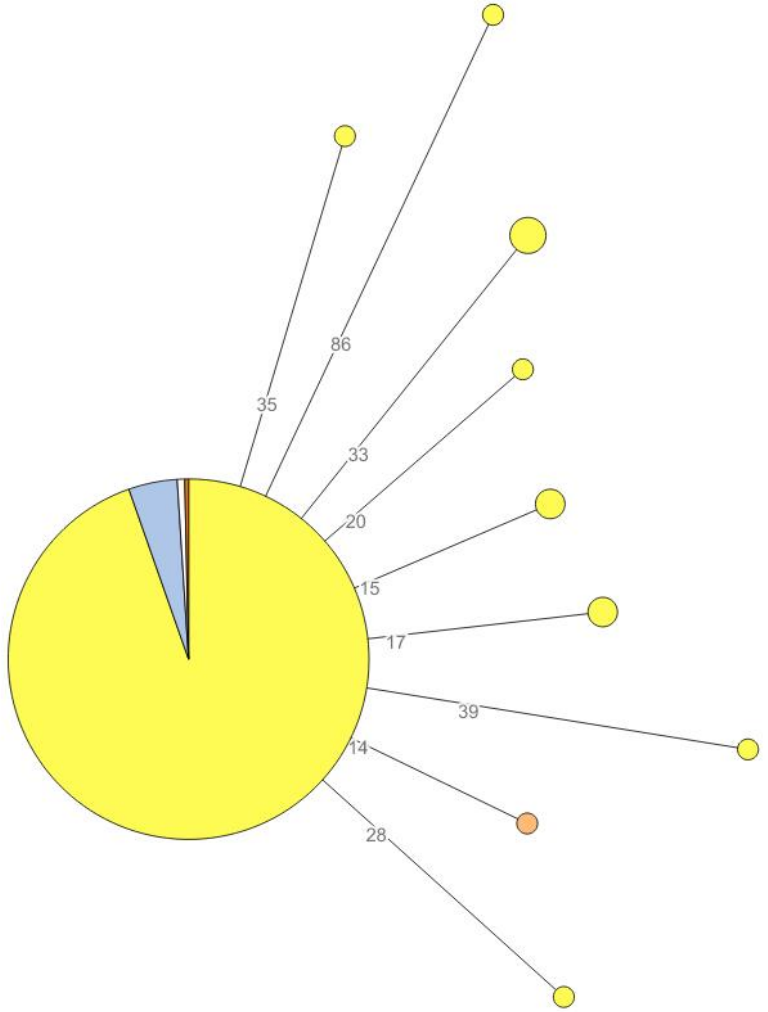
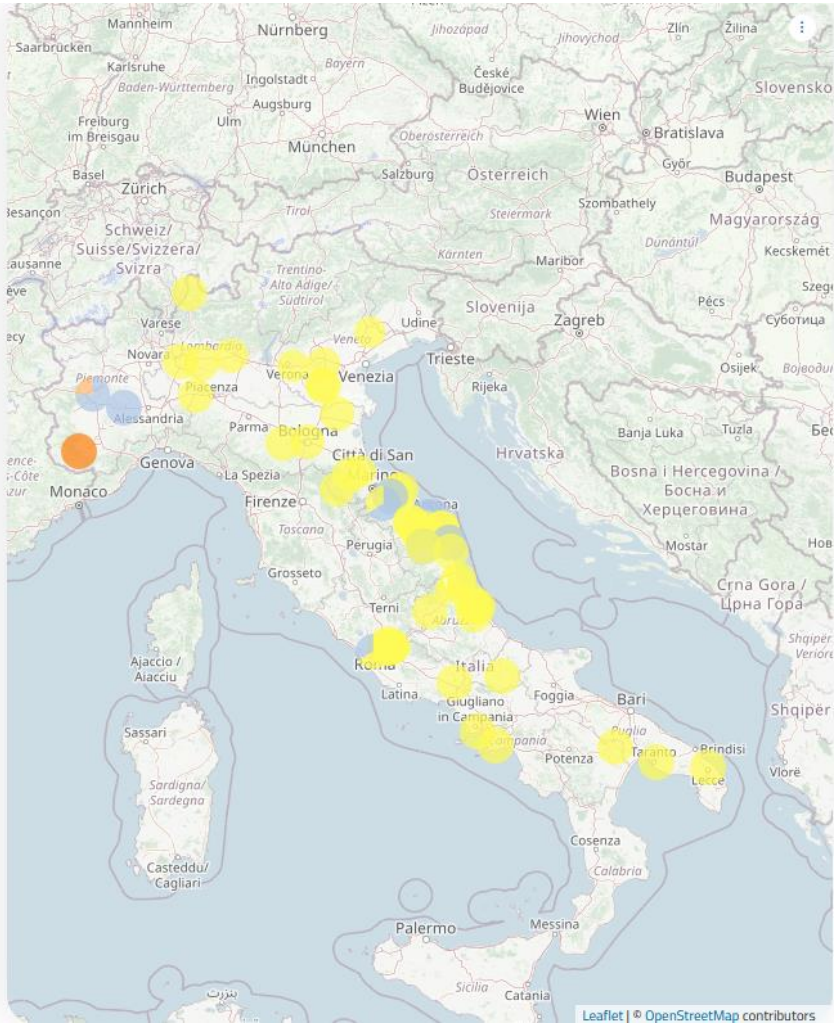
cgMLST C.jejuni ST2116 (num.311)

Data prelievo: 2008-2023

Num. strains =298





Host origine

- POLLO (294)
- UOMO (13)
- BALLERINA BIANCA (1)
- PICCIONE SELVATICO (1)

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Year 2023 Surveillance C. coli

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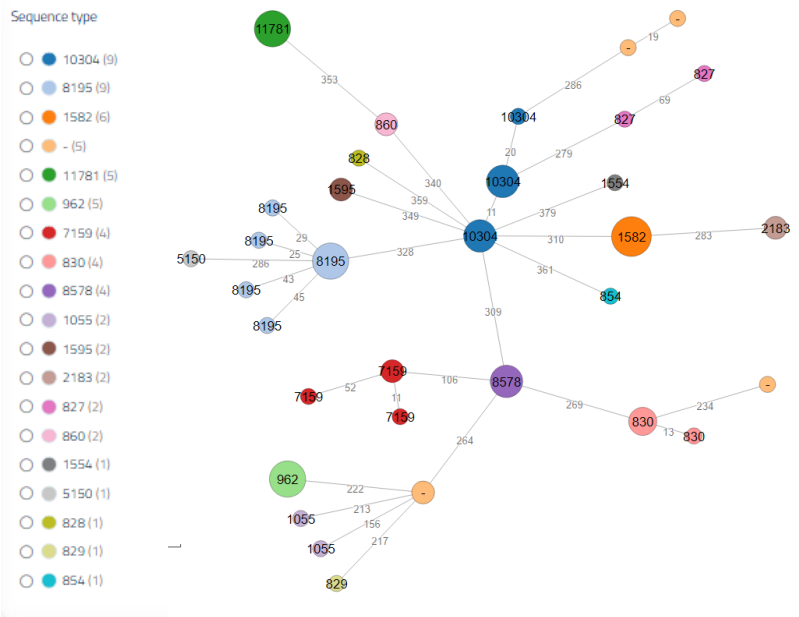
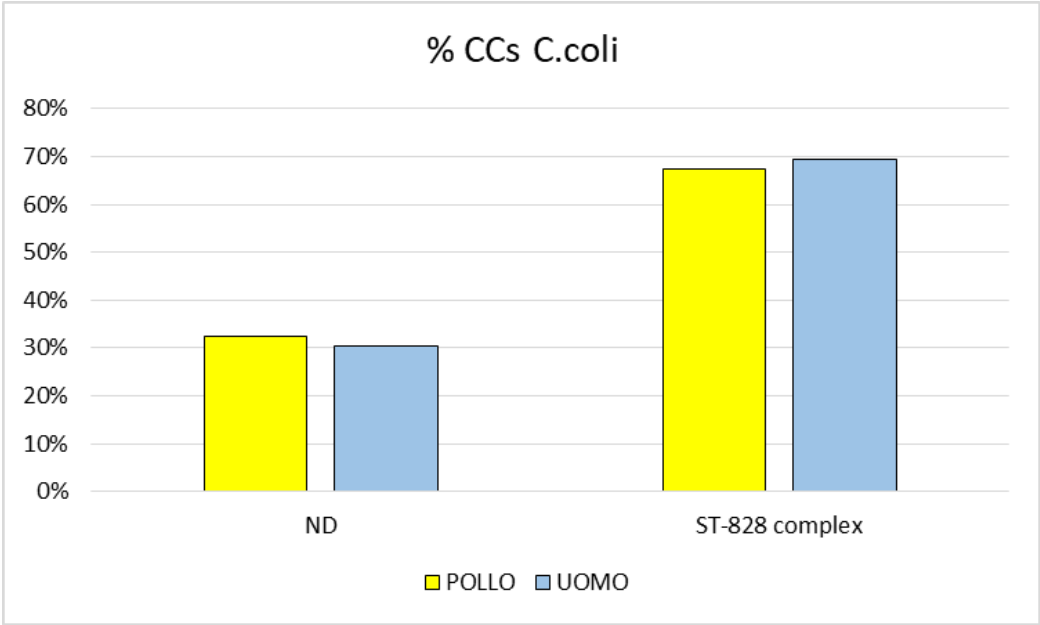
Poultry= 43
Humans= 23

1 CCs
1 CCs - shared





RESULTS

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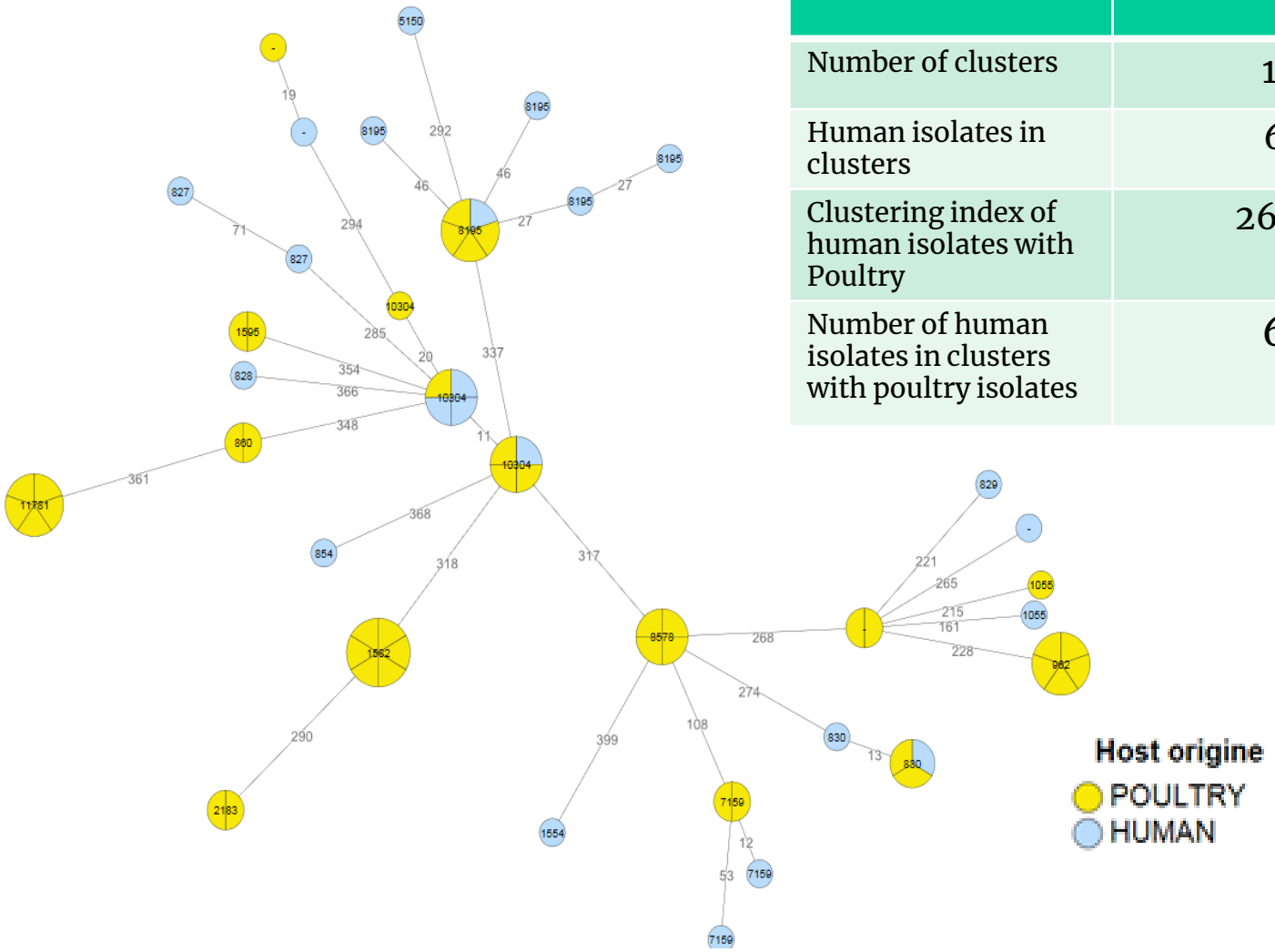
***C. coli* isolates**

23 - *Human*	43 - *Poultry*

cgMLST _*C. coli*_528

- **ST1034**
- **ST8195**
- **ST830**

C. coli	10 alleles cut-off
Number of clusters	13
Human isolates in clusters	6
Clustering index of human isolates with Poultry	26%
Number of human isolates in clusters with poultry isolates	6

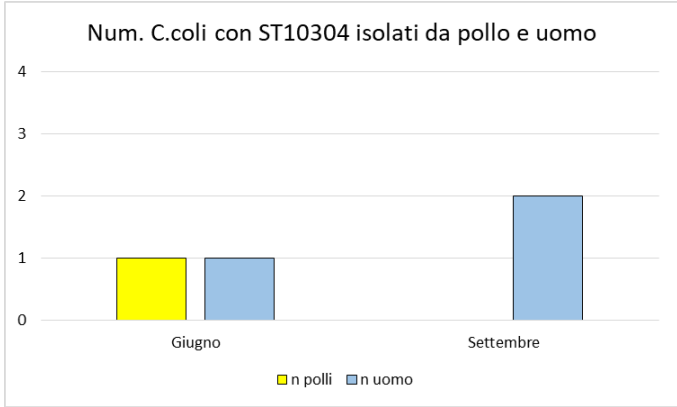


Similar results obtained with *C. jejuni/coli* cgMLST (637 loci) RIDOM SEQSPHERE *and C. jejuni/coli*_cgMLST (1343 loci) OXFORDSHIRE

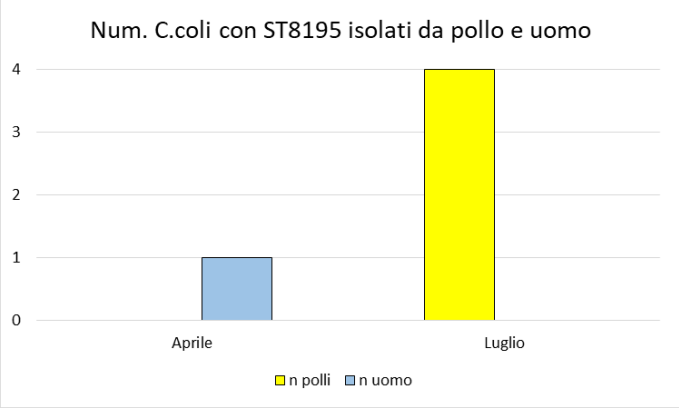
Cluster

Cluster	Campioni	Origine	ST	Giorni
1	3	Pollo (2) Uomo (2)	830	220
2	5	Pollo (4) Uomo (1)	8195	80
3	4/5	Pollo (1) Uomo (3)/Pollo (4)Uomo (1)	10304	90/90

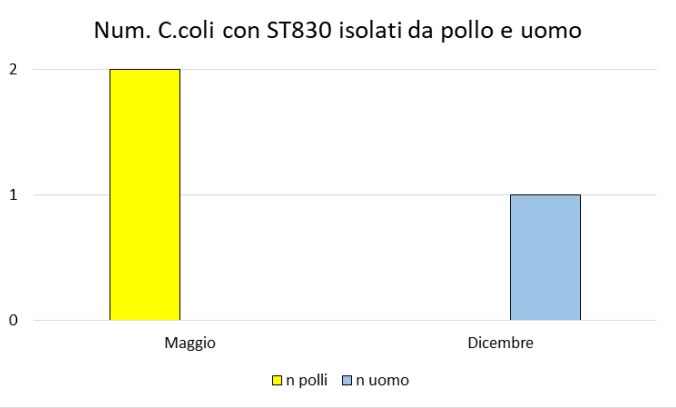
4 cluster - UOMO



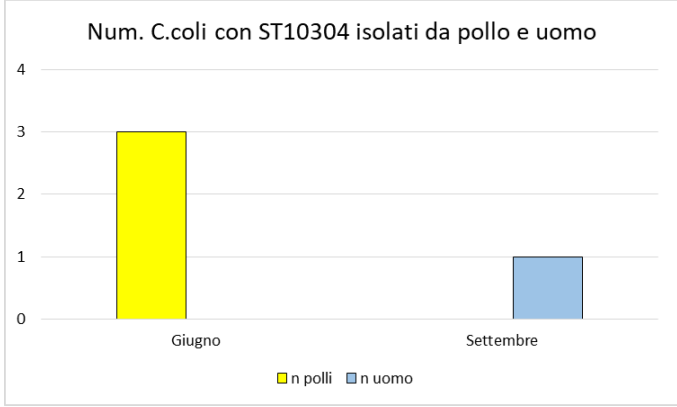
✓ 1 mattatoio –Emilia Romagna;
Casi –Piemonte, Bolzano.



✓ 1 mattatoio – Emilia Romagna;
Casi – Marche.



✓ 1 mattatoio – Emilia Romagna;
Casi – Piemonte-Umbria.



✓ 2 mattatoi –Emilia Romagna;
Casi –Marche.

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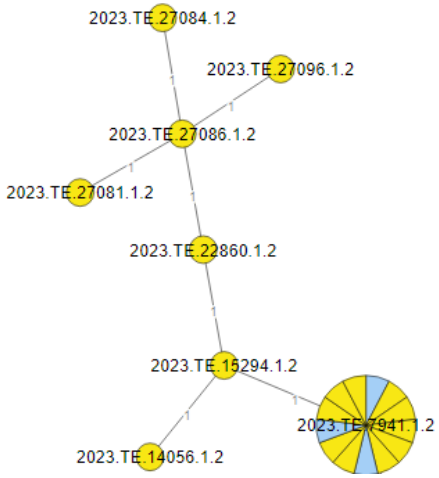
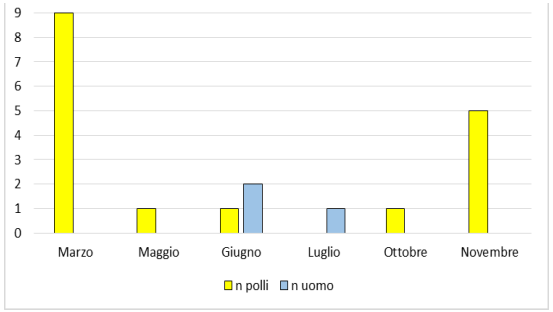
FOCUS ON CLUSTERS

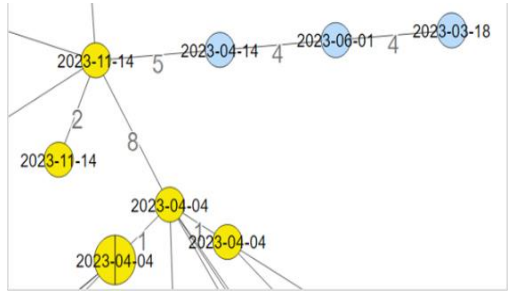
C. jejuni ST2116

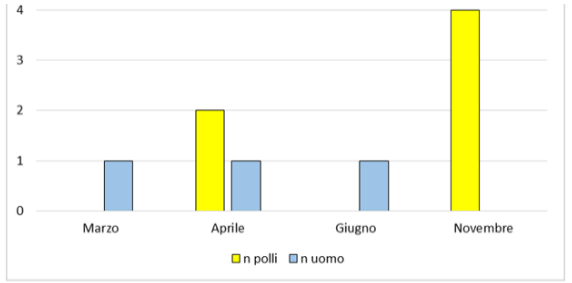




- 17 poultry isolates (Emilia Romagna/Lombardia)
- 3 human isolates (Piemonte)

C. jejuni ST50





- 6 poultry isolates (Emilia Romagna/Marche)
- 3 human isolates (Piemonte/Marche/Umbria)

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Annex 1 – Working in progress

	C.JEJUNI					C.COLI			
SCHEMI (cgMLST)	CLUSTER 1 ST-2116	CLUSTER 4 ST-2863	CLUSTER 5 ST-2863	CLUSTER 2 ST-50	CLUSTER 3 ST-50	Cluster 1 ST-830	Cluster 4 ST-10304	Cluster 3 ST-10304	Cluster 2 ST-8195
	H=no. 3 P=no. 17	H= no. 1; C= no. 4	H= no. 3; P= no. 1	H= no. 3; P= no. 6	H= no. 2; P= no. 1	H= no. 1; P= no. 2	H= no. 1; P= no. 3	H= no. 3; P= no. 1	H= no. 1; P= no. 4
RIDOM (637 loci)	X (0-9 AD)	X (0-10 AD)	X (0-6 AD)	X (0-9 AD)	X (0-2 AD)	X (0-4 AD)	X (0-6 AD)	X (0-15 AD)	/
GENPAT (678 loci)	X (0-4 AD)	X (0-12 AD)	X (0-12 AD)	X (0-10 AD)	X (0-5 AD)	X (0-7 AD)	X (0-12 AD)	X (0-12 AD)	X
OXFORDSHIRE (1,343 loci)	X (0-1 AD)	X (0-11 AD)*	X (0-6 AD)	X (0-10 AD)	X (1-2 AD)	X (0-2 AD)	X (3-11 AD)**	X (2-7 AD)	X (0-5 AD)
SNPS	X AD (1-6)	X AD (0-16)	X AD (1-6)	X AD (1-10)	X AD (2-7)	X AD (3-23)	/ AD (14-21)	X AD (0-6)	/ AD(1-13)

166631
112188

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CONCLUSIONS

Campylobacter Cases in Italy - 2023 Findings

Outbreaks overview: **39%** of Campylobacter strains from clinical cases clustered together using WGS typing methods, suggesting hidden outbreaks.

Estimated Cases from Hidden Outbreak:
Range: **112,188 - 166,631** *Confidence Interval (C.I.): 95%*

Poultry-Linked Human Isolates: **12%** of human isolates clustered with poultry isolates.
Likely linked to poultry meat.

Estimated Poultry-Linked Cases
Range: **23,246 - 54,552** *Confidence Interval (C.I.): 95%*

- Strategic implications and future outlook in Europe:

1. ***Can we use focused investments in genomic surveillance to control Campylobacter?***
2. ***Can Real-time application of WGS drive targeted interventions?***

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NRL-IT

Francesca Marotta
Anna Janowicz
Roberta Di Romualdo
Teresa Romualdi
Federica DI Timoteo
Lisa Di Marcantonio
Katiuscia Zilli
Elisa Di Domenico

ISTITUTO SUPERIORE DI SANITÀ

L. Villa
A. Garcia-Fernandez
I. Artuso

ISTITUTO ZOOPROFILATTICO SPERIMENTALE DELL'UMBRIA E DELLE MARCHE "TOGO ROSATI"

M. Napoleoni

THANK YOU FOR YOUR ATTENTION!