



anses

GENOMIC INSIGHTS FOR SAFER FOOD: EXPLORING THE POTENTIAL OF WHOLE GENOME SEQUENCE-BASED DATA WITHIN THE ONE HEALTH CONTEXT

7TH NOVEMBER 2023

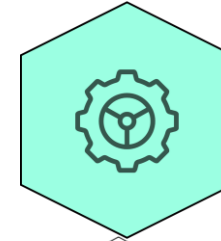
LAURENT GUILLIER

RISK ASSESSMENT DEPARTMENT

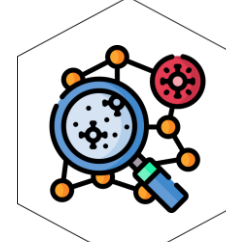
Food Safety
a key pillar of One Health approach

Outline

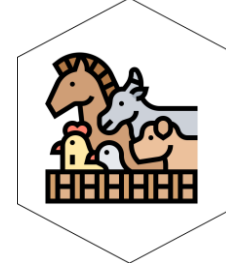
1. Context



2. Epidemiological investigations



3. Source attribution



4. Risk assessemnt

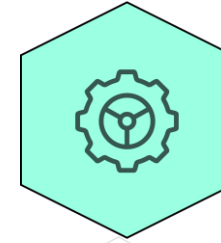


5. Conclusion

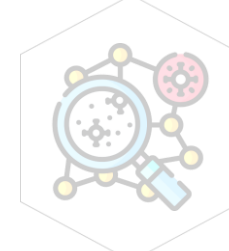


Outline

1. Context



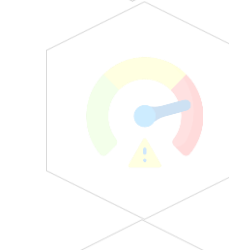
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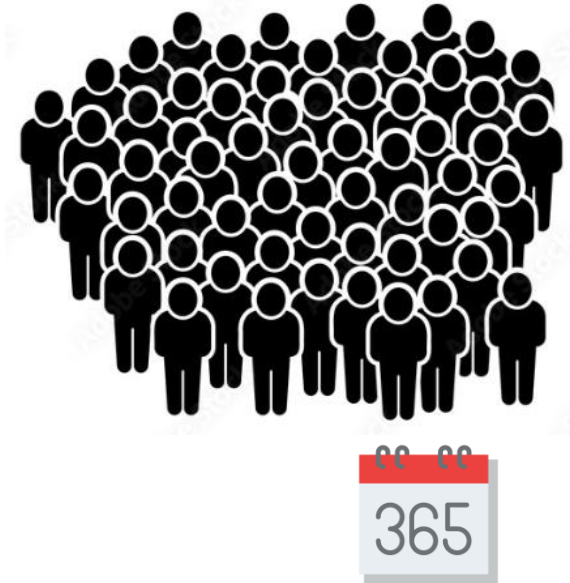


Entering the world of genomics: the past 20 years

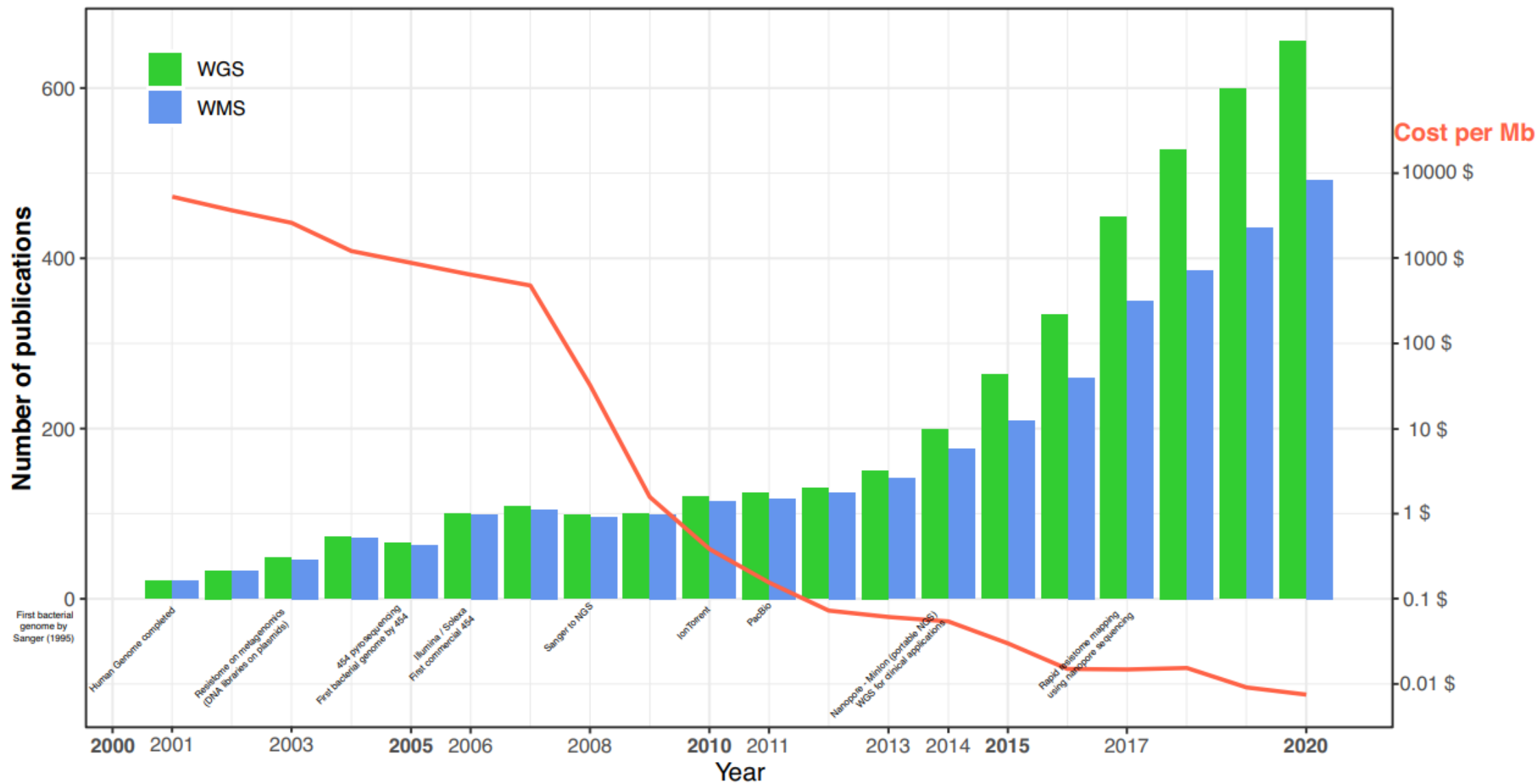
In early 2000s, the **Human Genome Project** produced a genome sequence that accounted for over 90% of the human genome

- International consortium of thousands of researchers
- **10 years** project

Today, it's a matter of **days** (**hours** to **weeks** depending on the specific NGS platform, sample preparation methods, and the desired coverage or depth of sequencing)

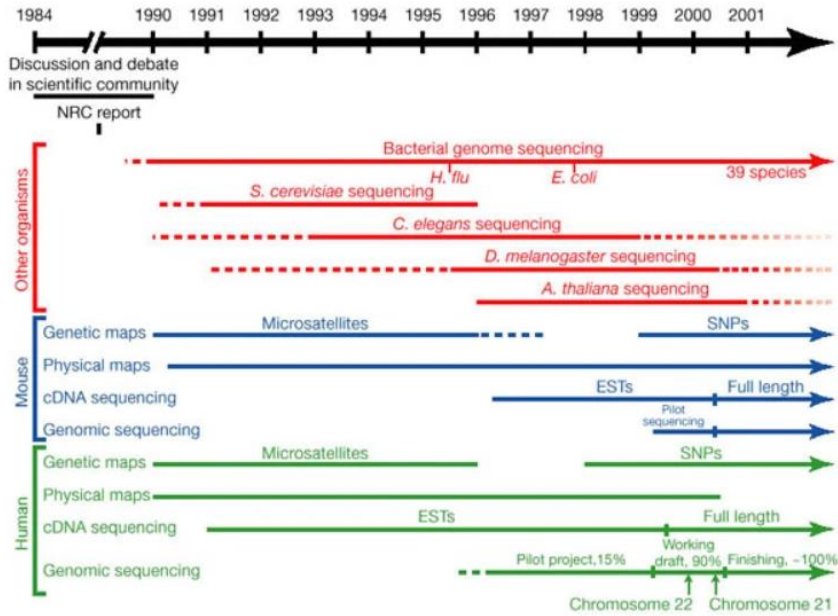


Entering the world of genomics: the past 20 years

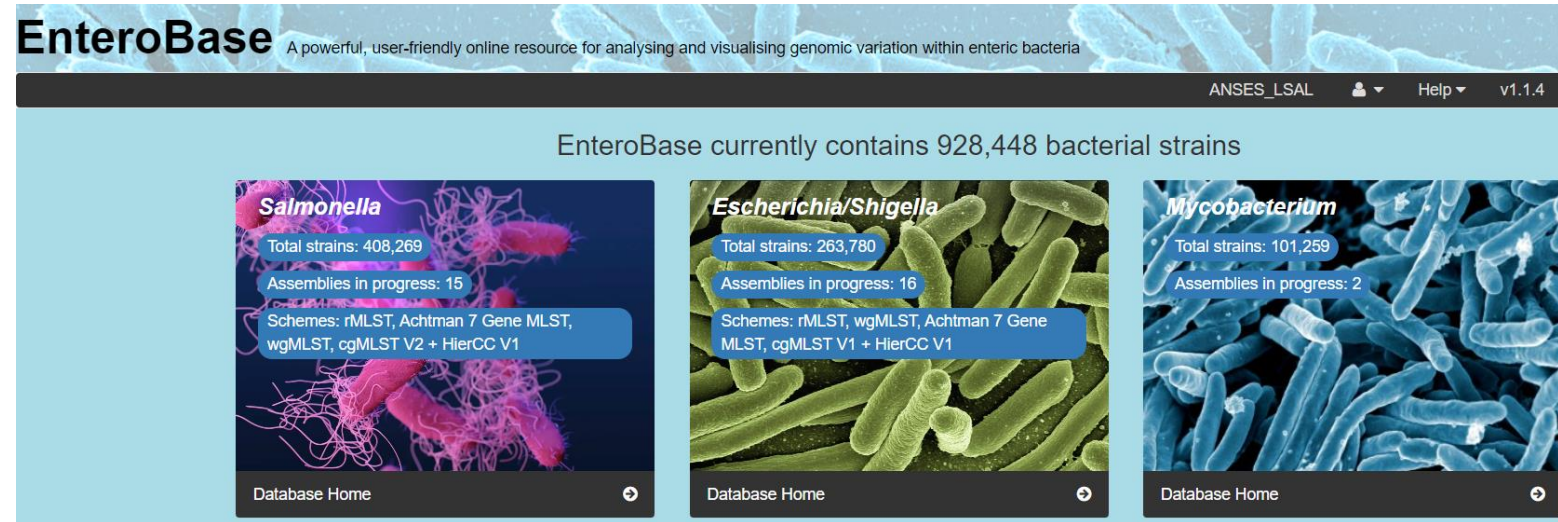


Source: Pennone et al COFS (2022)

Entering the world of genomics: the past 20 years



Source: International Human Genome Sequencing Consortium, Nature (2001)



EnteroBase A powerful, user-friendly online resource for analysing and visualising genomic variation within enteric bacteria

ANSES_LSAL Help v1.1.4

EnteroBase currently contains 928,448 bacterial strains

Organism	Total strains	Assemblies in progress	Schemes
<i>Salmonella</i>	408,269	15	rMLST, Achtman 7 Gene MLST, wgMLST, cgMLST V2 + HierCC V1
<i>Escherichia/Shigella</i>	263,780	16	rMLST, wgMLST, Achtman 7 Gene MLST, cgMLST V1 + HierCC V1
<i>Mycobacterium</i>	101,259	2	


Source: <https://enterobase.warwick.ac.uk/>

The promise of a better food safety


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Food Microbiology 79 (2019) 96–115

Contents lists available at ScienceDirect

Food Microbiology

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Check for updates

Genomics of foodborne pathogens for safety
Marc W Allard, Rebecca Bell, Christina M F Narjol Gonzalez-Escalona, Maria Hoffmann, Andrea Ottesen, Padmini Ramachandran, Elizabeth Reed, Shashi Sharma, Eric Stevens, Ruth Timme, Jie Zheng and Eric W Brown

The use of next generation sequencing for improving food safety: Translation into practice
Balamurugan Jagadeesan^{a,*}, Peter Gerner-Smidt^b, Marc W. Allard^c, Sébastien Leuillet^d, Anett Winkler^e, Yinghua Xiao^f, Samuel Chaffron^g, Jos Van Der Vossen^h, Silin Tangⁱ, Mitsuru Katase^j, Peter McClure^k, Bon Kimura^l, Lay Ching Chai^m, John Chapmanⁿ, Kathie Grant^{o,**}

SCIENTIFIC OPINION

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Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms

EFSA Panel on Biological Hazards (EFSA BIOHAZ Panel),
Kostas Koutsoumanis, Ana Allende, Avelino Alvarez-Ordóñez, Declan Bolton, Sara Bover-Cid,
Marianne Chemaly, Robert Davies, Alessandra De Cesare, Friederike Hilbert, Roland Lindqvist,
Maarten Nauta, Luisa Peixe, Giuseppe Ru, Marion Simmons, Panagiotis Skandamis,
Elisabetta Suffredini, Claire Jenkins, Burkhard Malorny, Ana Sofia Ribeiro Duarte,
Mia Torpdahl, Maria Teresa da Silva Felício, Beatriz Guerra, Mirko Rossi and Lieve Herman

Outline

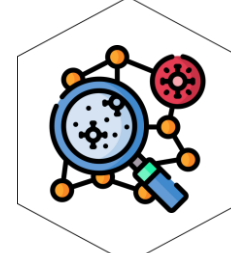
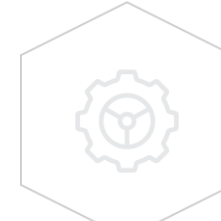
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Outbreak investigation

French examples

- Milk powder *Salmonella* ser. Agona (2018)
- Frozen pizzas *E. coli* STEC O26 (2022)
- Vegan cheese *L. monocytogenes* (2022)
- Chocolate *Salmonella* ser. Typhimurium (2022)
- ...



Recent media coverage in Europe

- Frozen corn *L. monocytogenes* (2018)
- Eggs *Salmonella* ser. Enteritidis (2017-...)
- ...

And in the world

- Romaine lettuce *E. coli* O26 - USA (multiannual)
- Polony sausage *L. monocytogenes* South-Africa (2018)
- Melon *L. monocytogenes* Australia (2018)
- ...



Outbreak investigation

A success for EU

- EFSA/ECDC Rapid outbreak assessment
- Role of EURLs
- Database of WGS
- ...



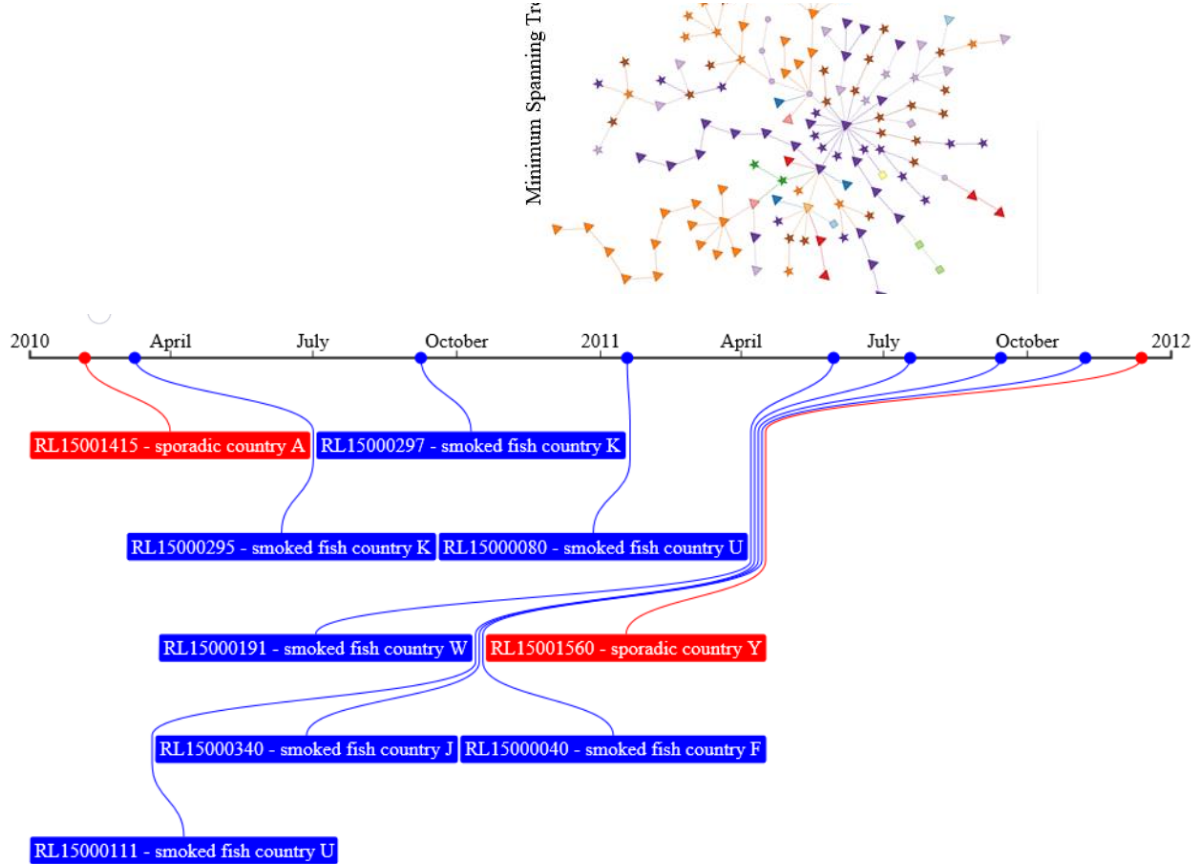
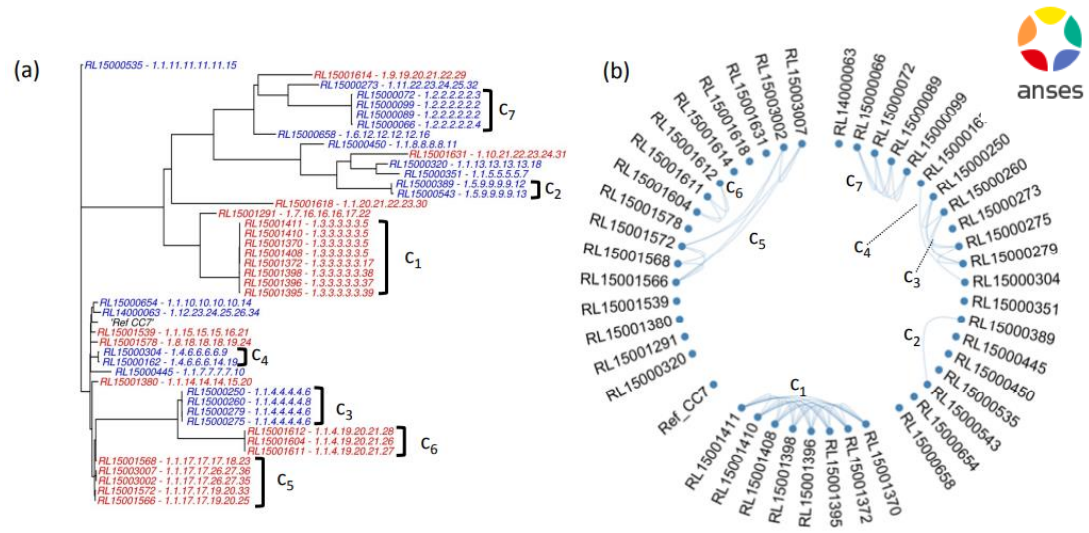
EFSA One Health WGS
System

Outbreak investigation

Principle is simple

- ① Clustering of strains based on SNP/cgMLST distance
- ② Use of epidemiological data (strains, patients,...)

Genomics alone is not sufficient to confirm an outbreak, epidemiologist still have to investigate

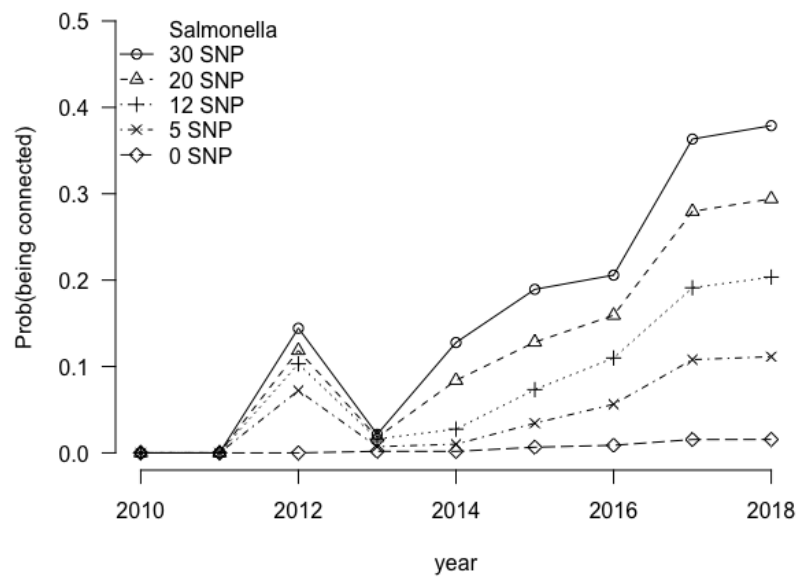
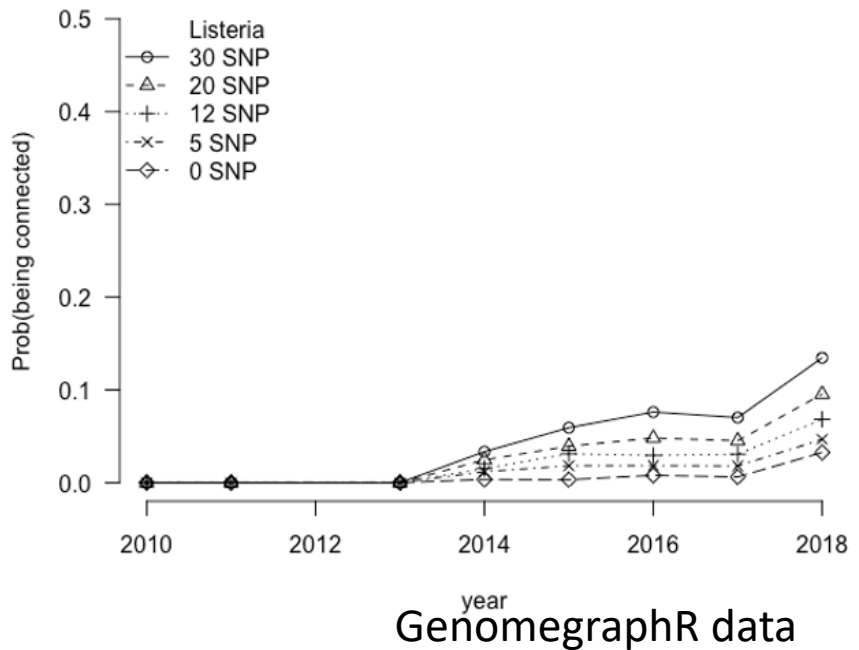


Outbreak investigation

A solution to solve every recorded cases ?

No

- Proportion of connected strains can be low (e.g. *Listeria*)
- Number of small genomic clusters is too important to be investigated in real life (e.g. *Salmonella* clusters in France)
- Somewhat a lack of well described food strains (metadata)
- Paths of contamination can be complex
- Strains can evolve rapidly (e.g. STEC)



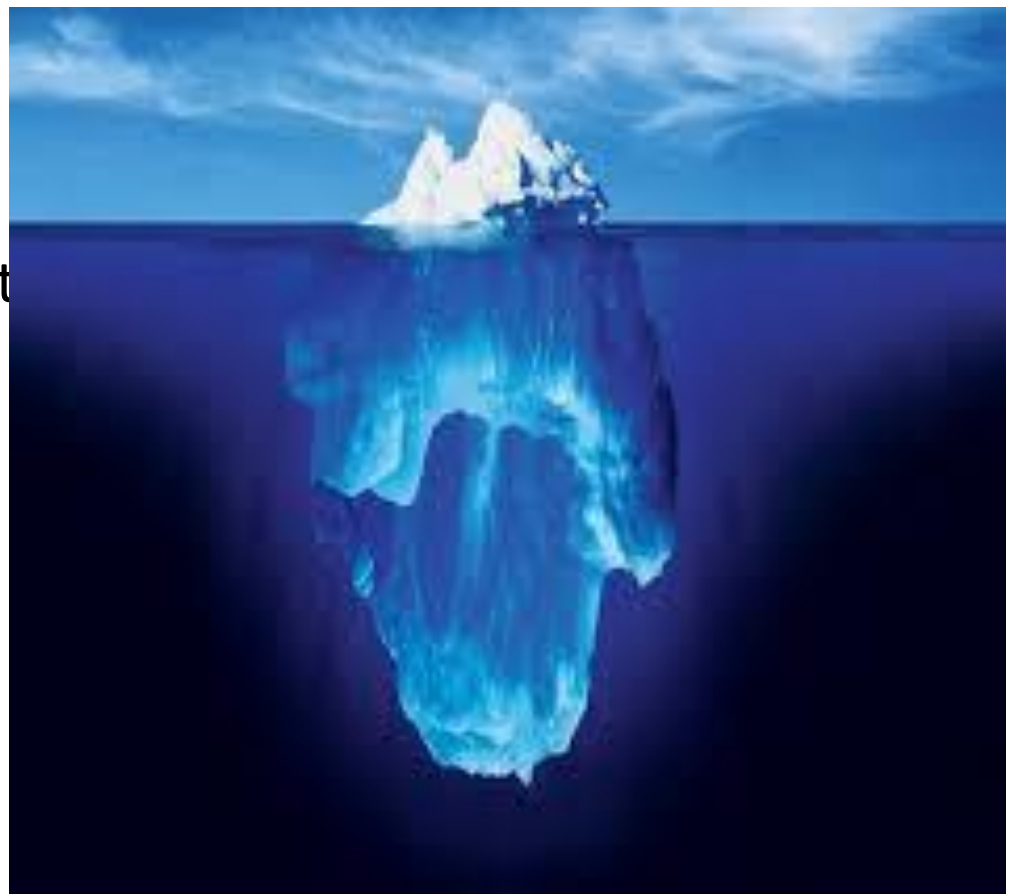
Beyond outbreaks

Outbreaks but also

... sporadic cases

(sporadic case = isolated case with no identified link to other cases of the same disease)

... unrecorded cases

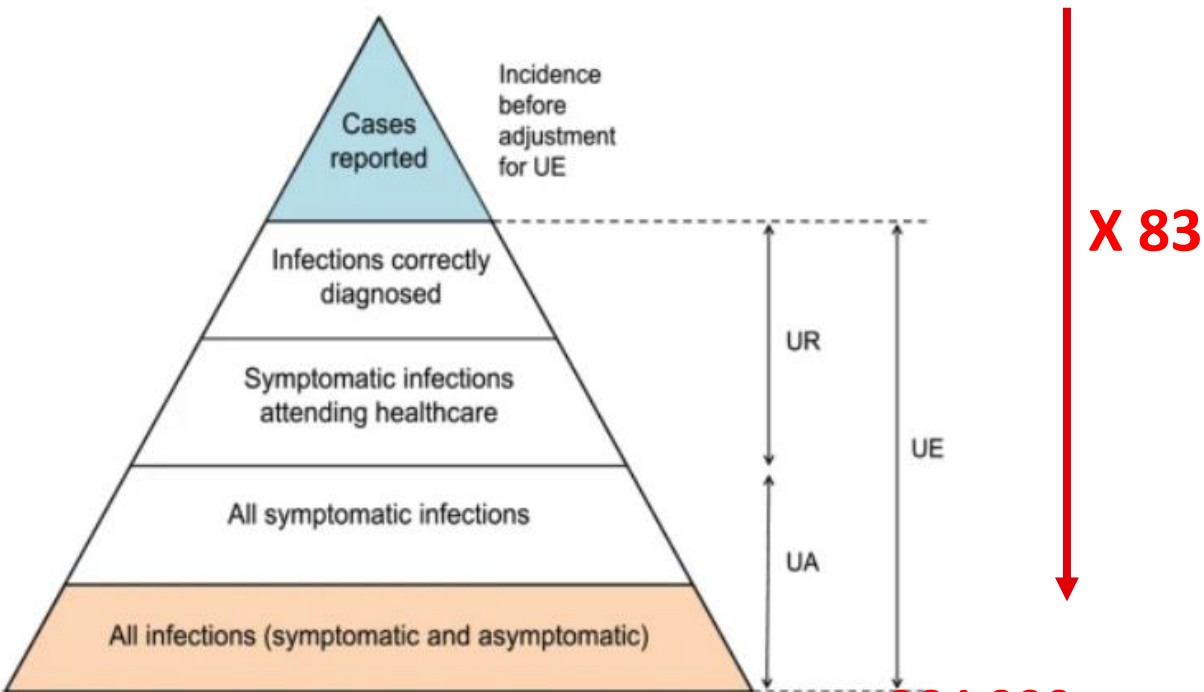


Beyond outbreaks

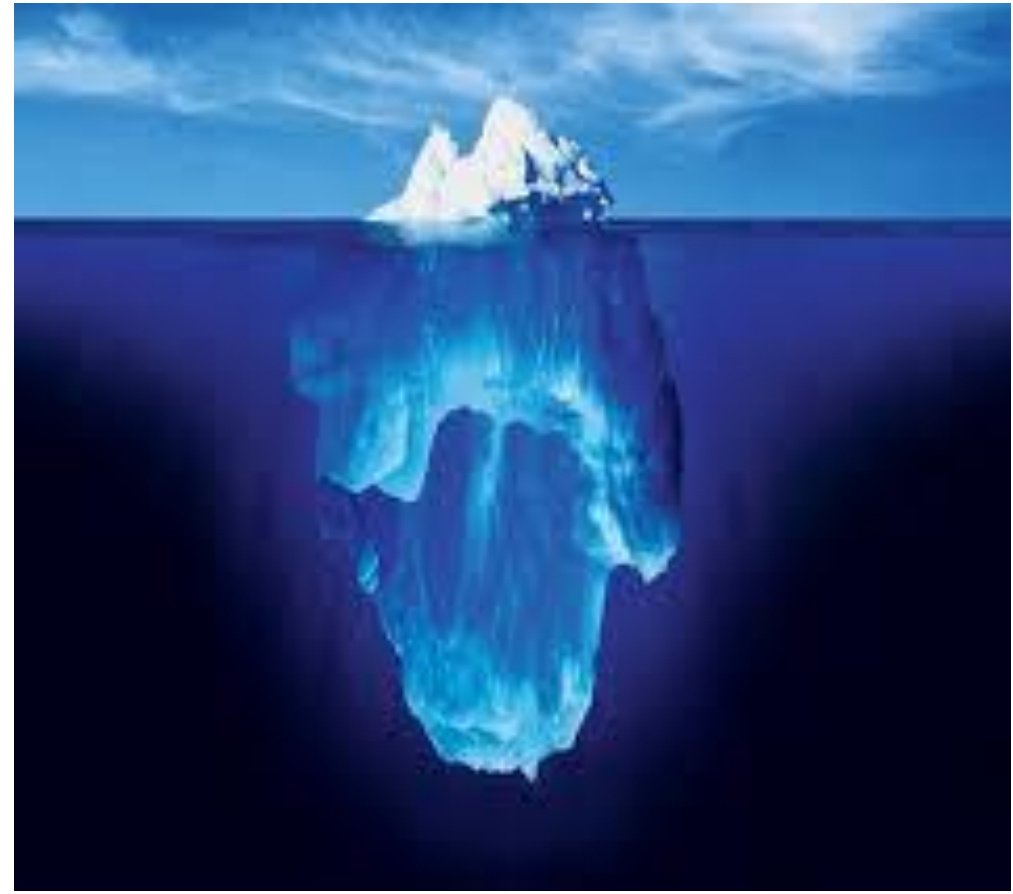
Estimated number of actual cases

« Pyramidal approach » (Van Cauteren et al., 2017)

Campylobacteriosis: N=4600



384 000 symptomatic
IC90% [240 000 – 790 000]



Outline

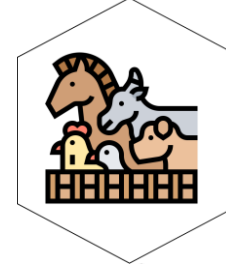
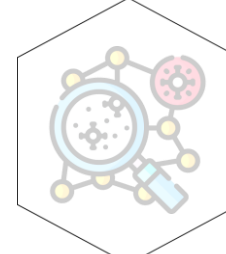
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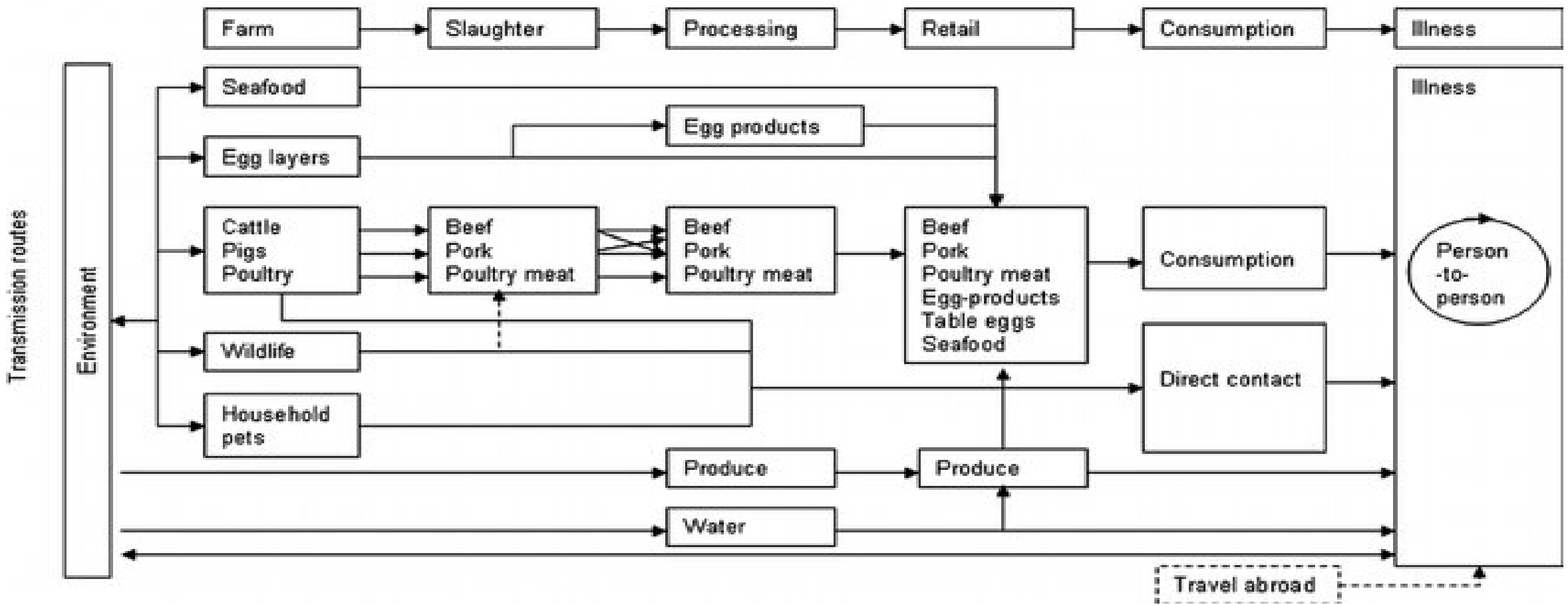
4. Risk assessemnt

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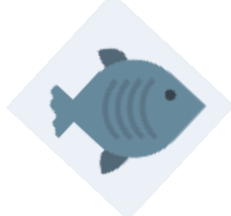






Source attribution

What is the food (or other) origin of the cases?



Source attribution methods

Typing/genomic approaches

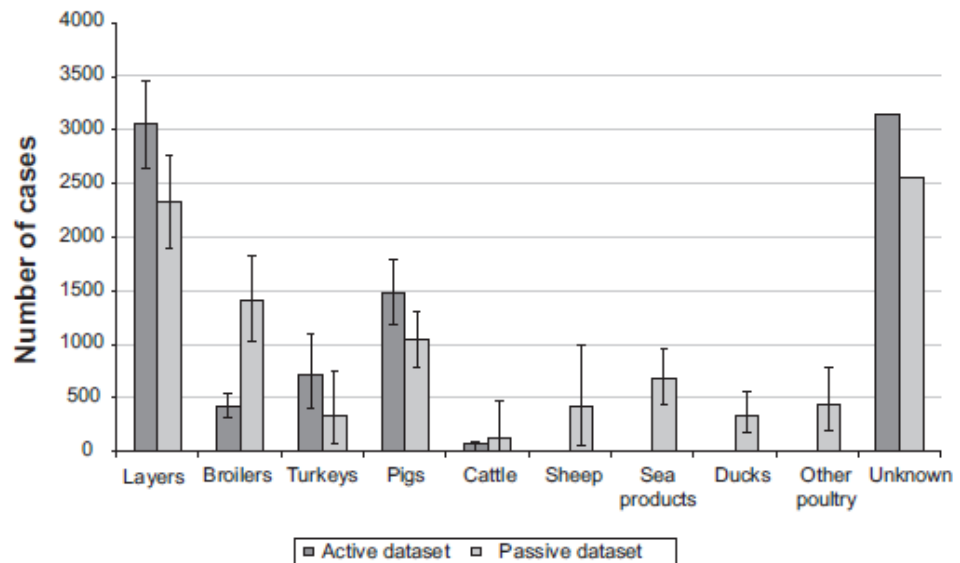
Origin	Strain	locus 1	locus 2	locus 3	locus 4	Membership coefficients to sources 1/2/3
 Source 1	Strain 1	28	31	32	7	1/0/0
	Strain 2	28	31	4	8	1/0/0
	Strain 3	28	12	32	7	1/0/0
	Strain 4	28	12	4	7	1/0/0
 Source 2	Strain 5	35	12	15	7	0/1/0
	Strain 6	35	42	15	7	0/1/0
	Strain 7	35	42	4	7	0/1/0
	Strain 8	35	42	15	8	0/1/0
 Source 3	Strain 9	7	15	22	7	0/0/1
	Strain 10	14	17	22	11	0/0/1
	Strain 11	7	17	22	7	0/0/1
	Strain 12	7	17	27	7	0/0/1
Strains to attribute 	Strain 13	35	42	4	8	0,07/0,92/0,01 
	Strain 14	28	31	32	8	0,97/0,02/0,01 
	Strain 15	7	15	32	7	0,22/0,04/0,74 
	Strain 16	14	17	2	11	0,04/0,04/0,92 

Some source attribution results

Typing/genomic approaches

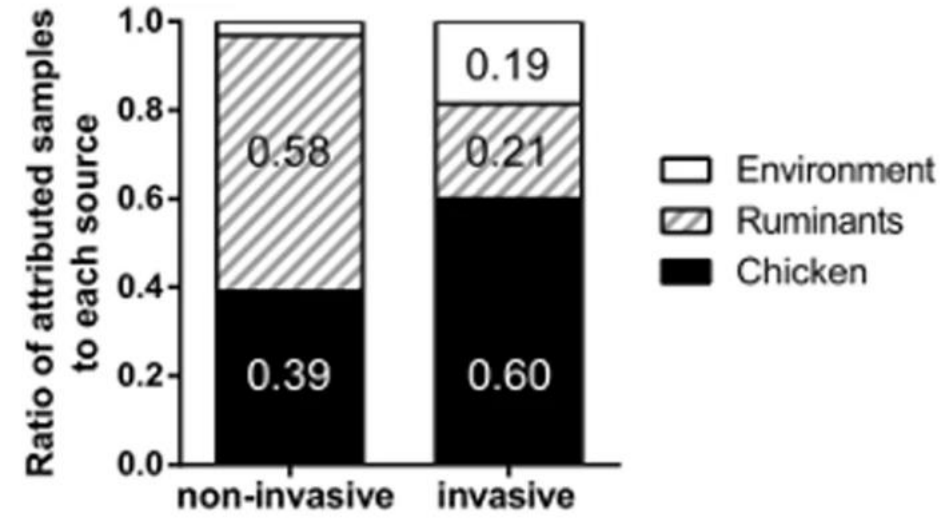
Frequency comparison models

Ex. Salmonellosis in France
(David et al., 2013)



Models based on population genomics

e.g. Campylobacteriosis in France
(Berthenet et al., 2019)

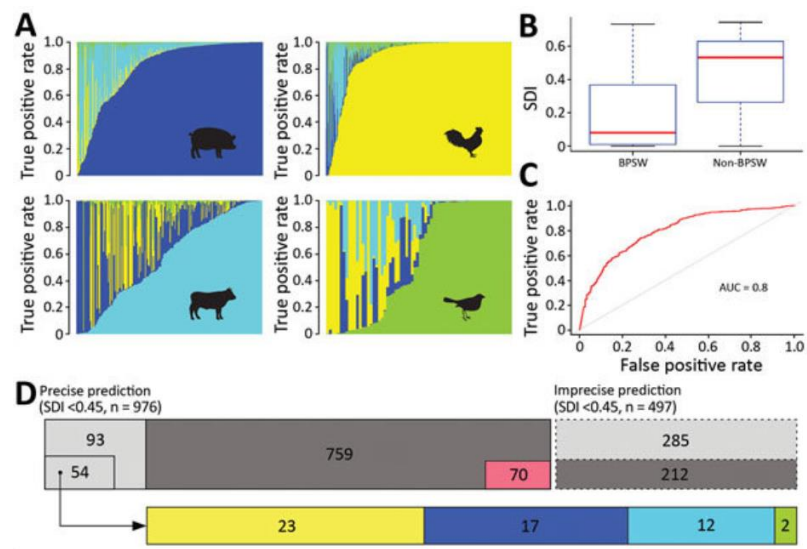


Some source attribution results

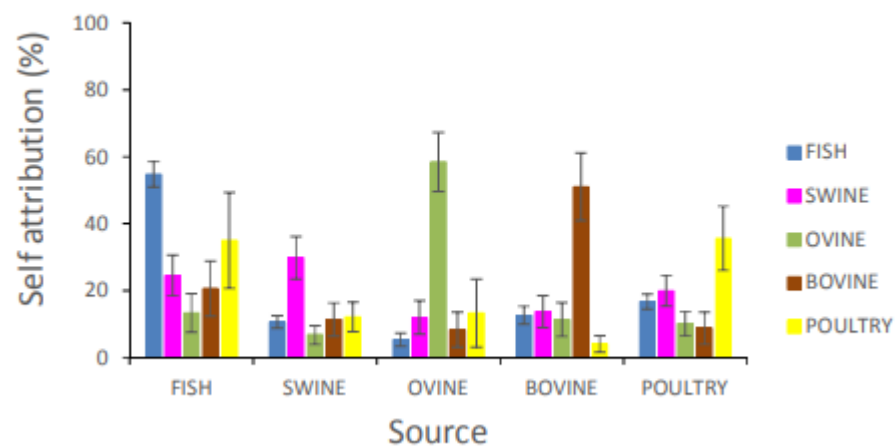
Typing/genomic approaches

With WGS, the accuracy of the model is slightly improved compared with simple types (like 7-MLST)

Need for complex model (accounting for possibility of transfer between sources)



STRUCTURE - self attribution



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Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms

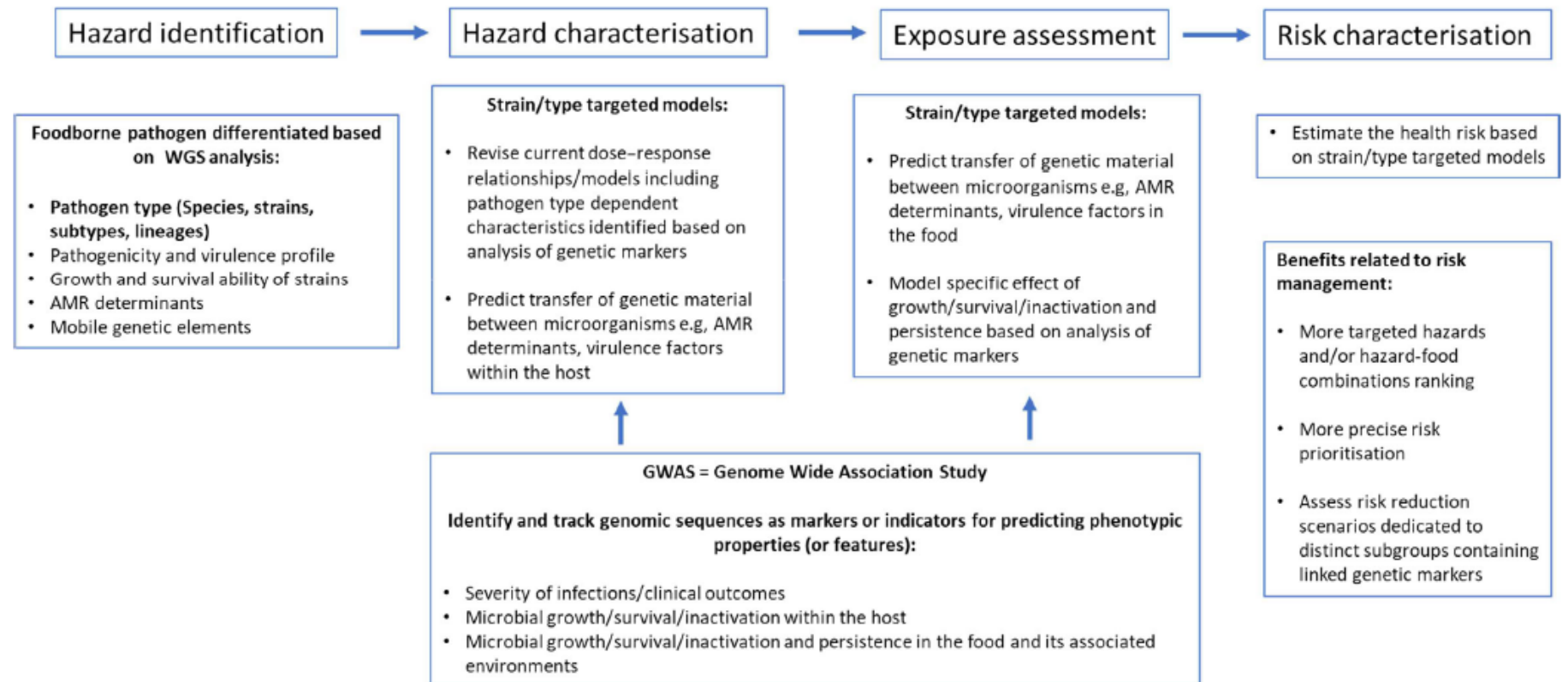
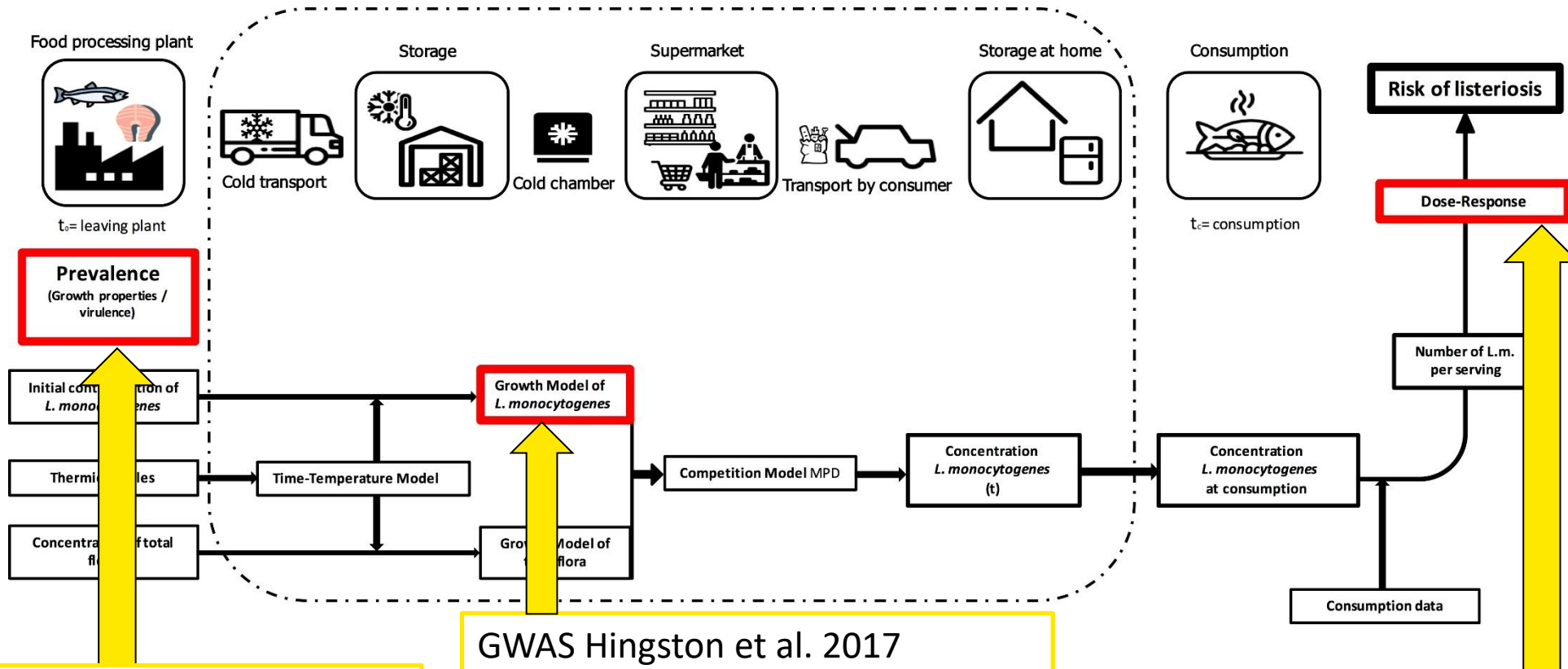


Figure 2: Future perspectives for WGS to add value to microbial risk assessment

How to do in practical terms?

Fritsch et al. 2018, MRA

Implementation for cold smoked salmon-related listeriosis



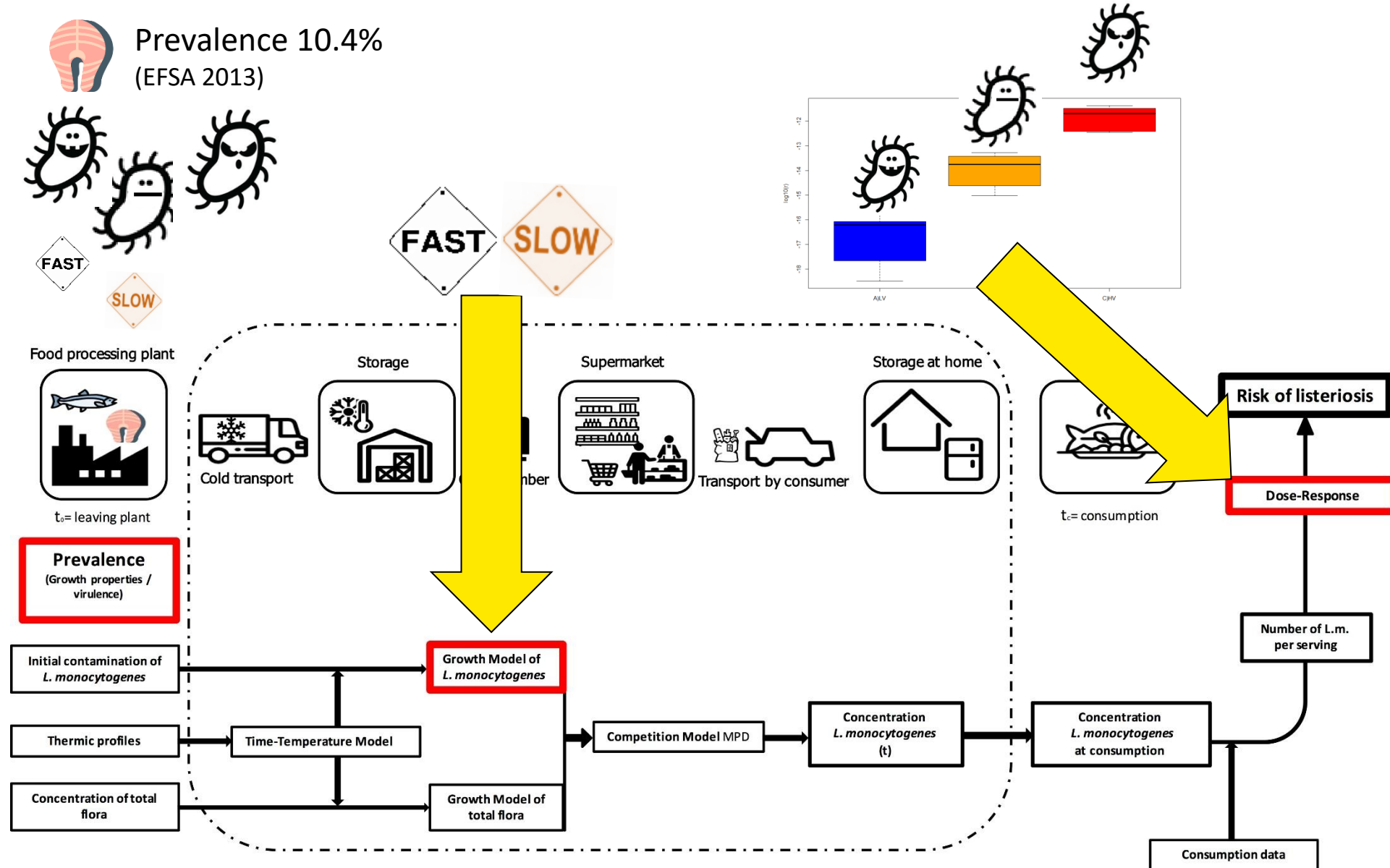
EFSA baseline +
Møller-Nielsen et al. 2017
294 sequenced strains

GWAS Hingston et al. 2017

Virulence
Maury et al. 2016

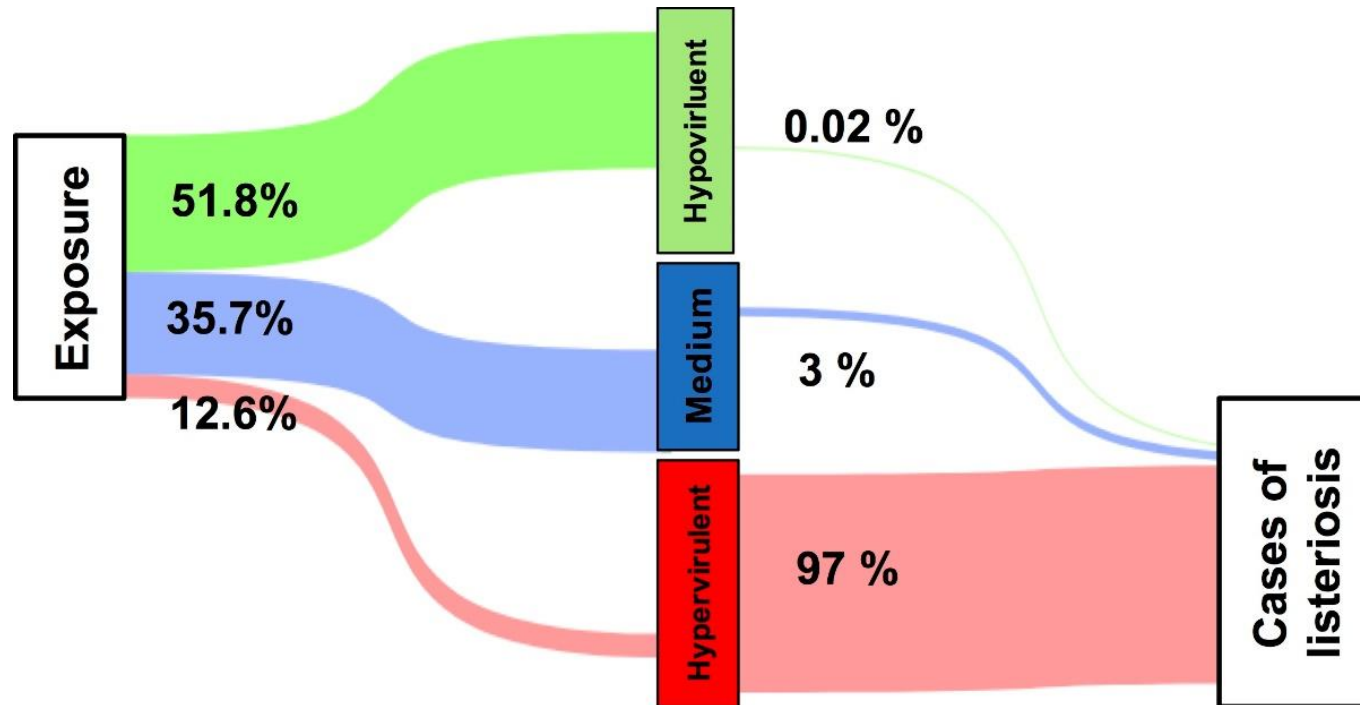
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How to do in practical terms?

Implementation for cold smoked salmon-related listeriosis



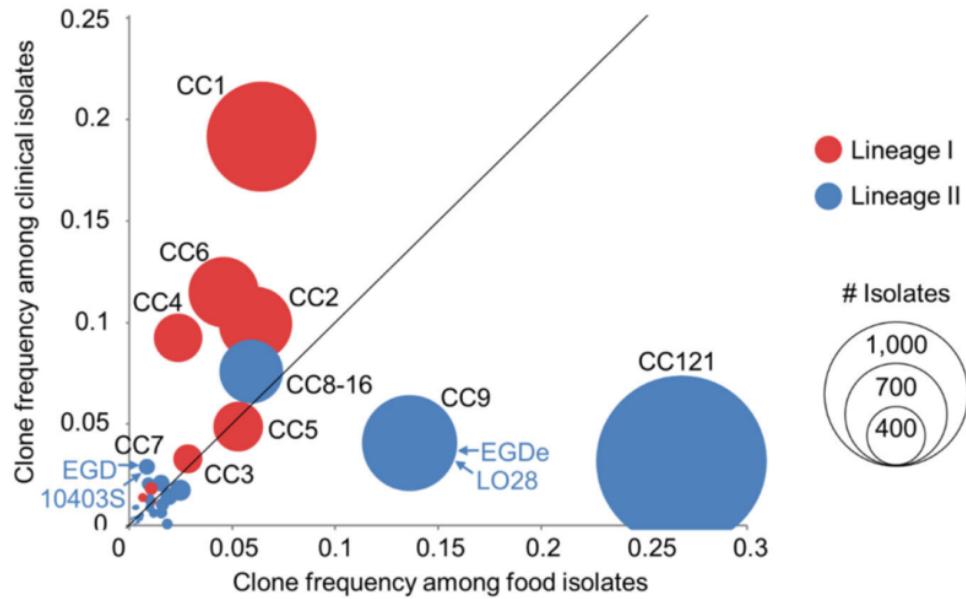
These results raise questions about the management measures associated with the different strains

Tipping point

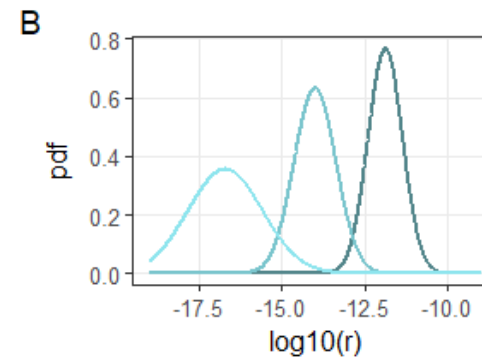
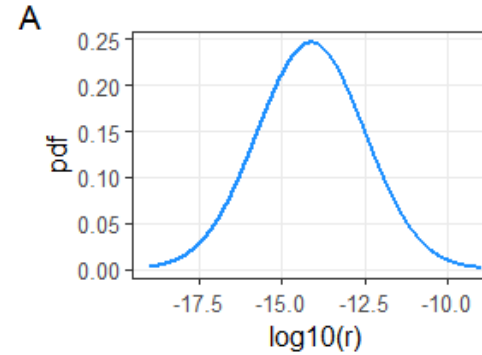


Is already there for dose response... driven by epidemiological data

L. monocytogenes



Maury et al. 2016



Fritsch et al. 2018



To be published in 2024

Tipping point

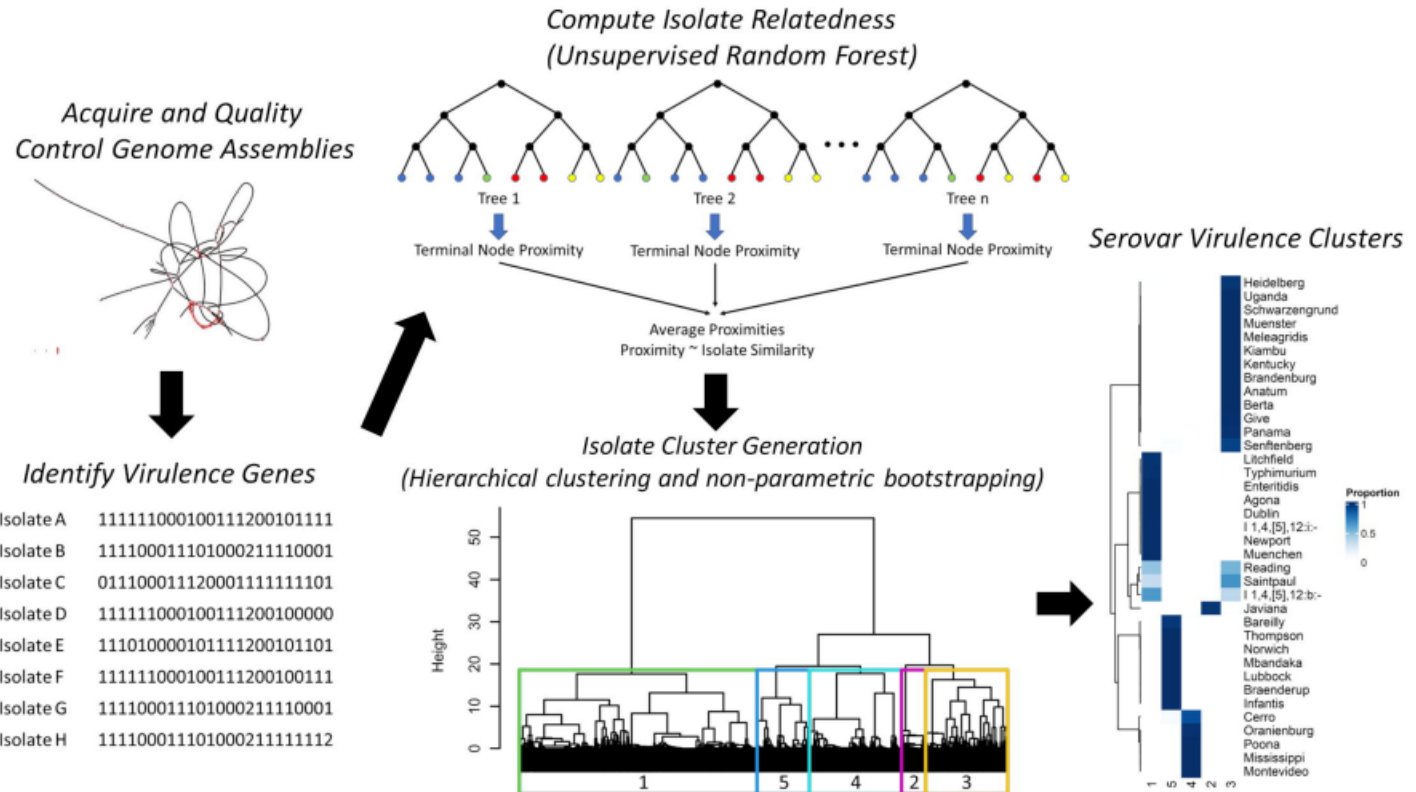
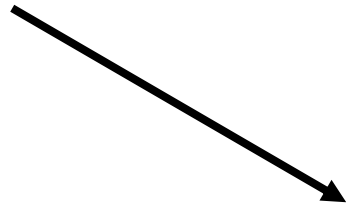
Is already there for dose response... driven by epidemiological data



Salmonella

Strikland et al. 2023 JFP

Fenske et al. 2022 MedRxiv



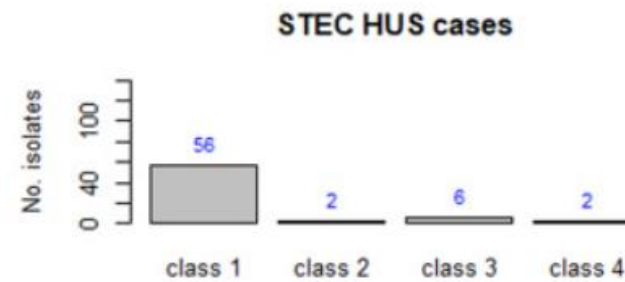
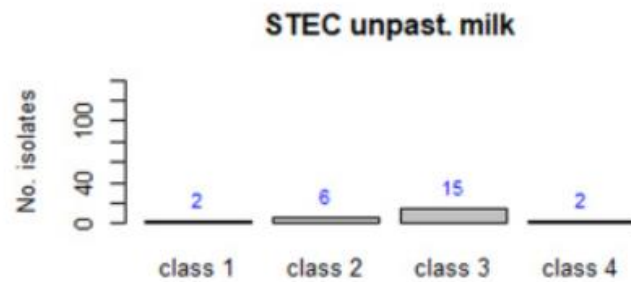
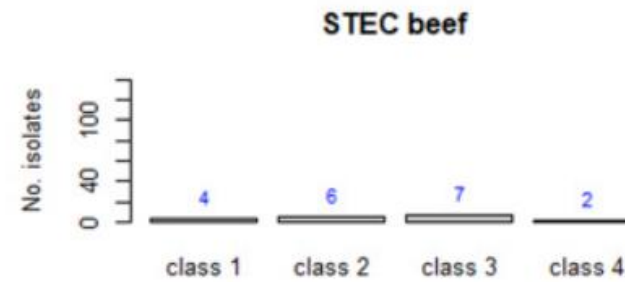
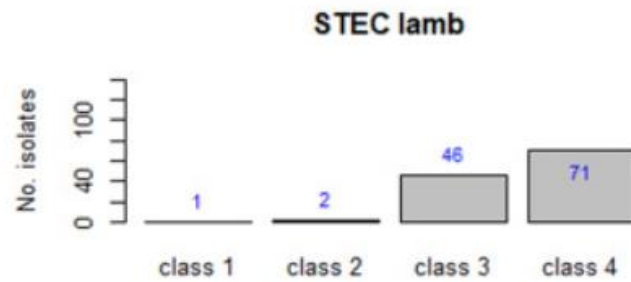
Tipping point



Is already there for dose response... driven by epidemiological data

STEC

Lindqvist et al. 2023



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Conclusions: What has WGS changes

1. Investigations of outbreaks

Real improvement

More to come with shared information at EU

Need to be aware that this will not solve everything

2. Source attribution

Not yet a revolution

3. Risk assessment

Methodologies are ready

Are people ready to change paradigm?



Thank you for your attention



Πολλές ευχαριστίες για την πρόσκλησή σας