International Scientific Conference on “Global commodity chains from a risk assessment perspective”

FROM DATA TO DECISION: LEVERAGING WGS AS A TOOL FOR PRECISION RISK ASSESSMENT ALONG THE FOOD PRODUCTION CHAIN UP TO CONSUMPTION

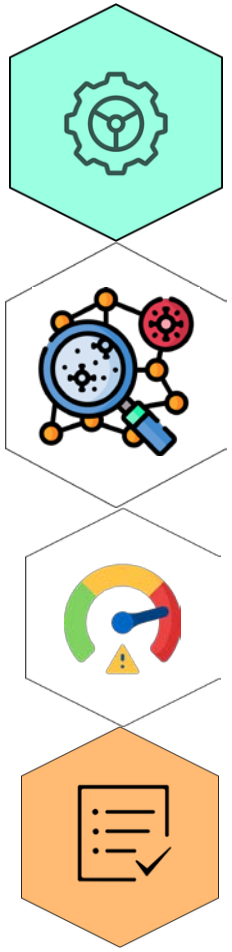
27TH MAY 2024

LAURENT GUILLIER

RISK ASSESSMENT DEPARTMENT

Outline

1. Context
2. Epidemiological investigations and source attribution
3. Risk assessemnt
4. Conclusion



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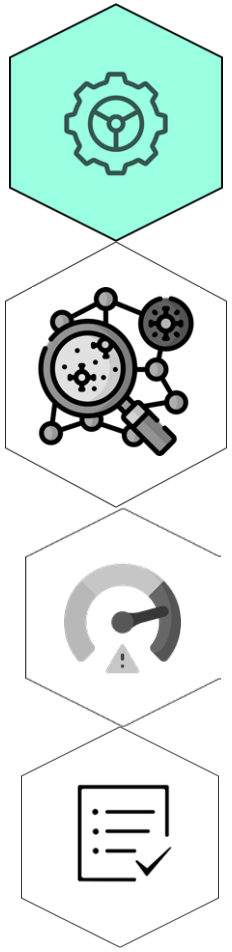
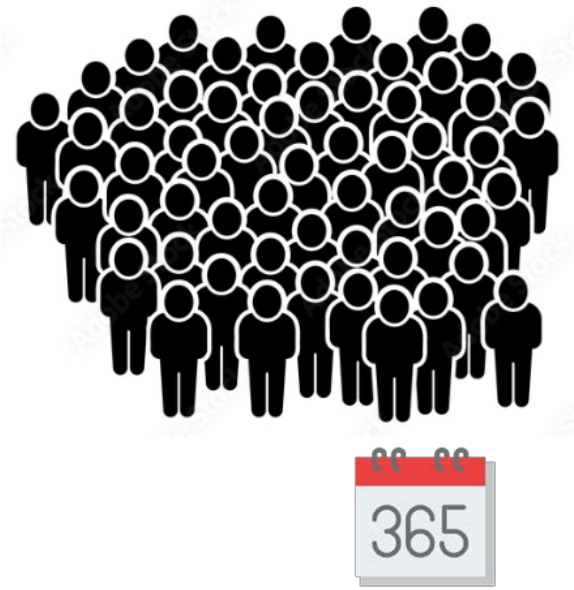


Entering the world of genomics: the past 20 years

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

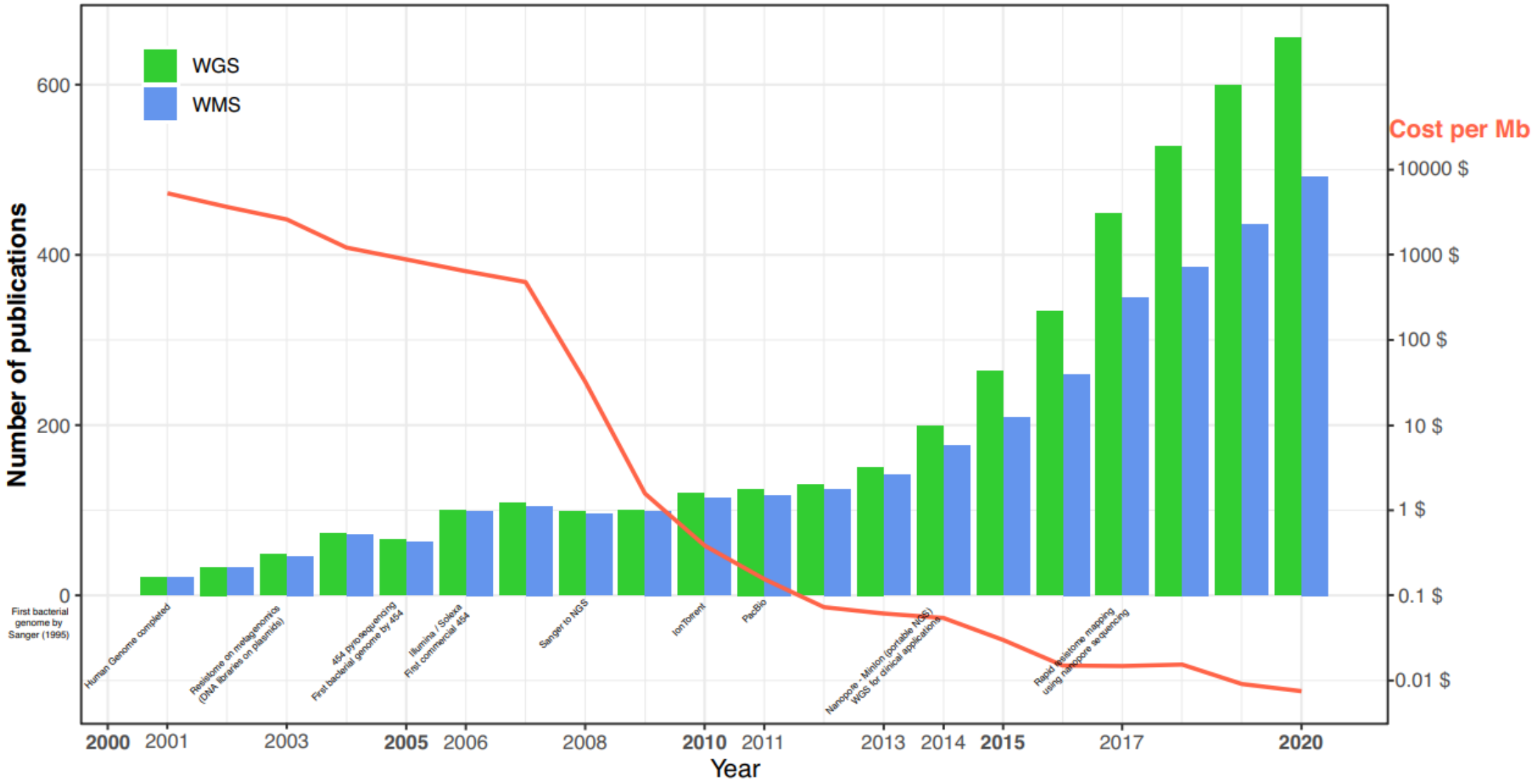
- International consortium of thousands of researchers
- 10 years project



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)

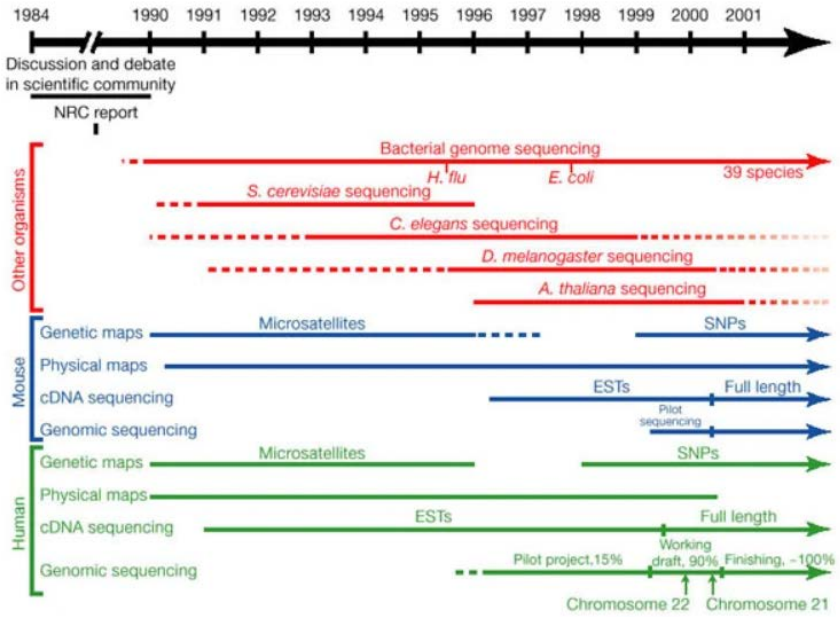
Source: https://nanoporetech.com/products/minion

Entering the world of genomics: the past 20 years

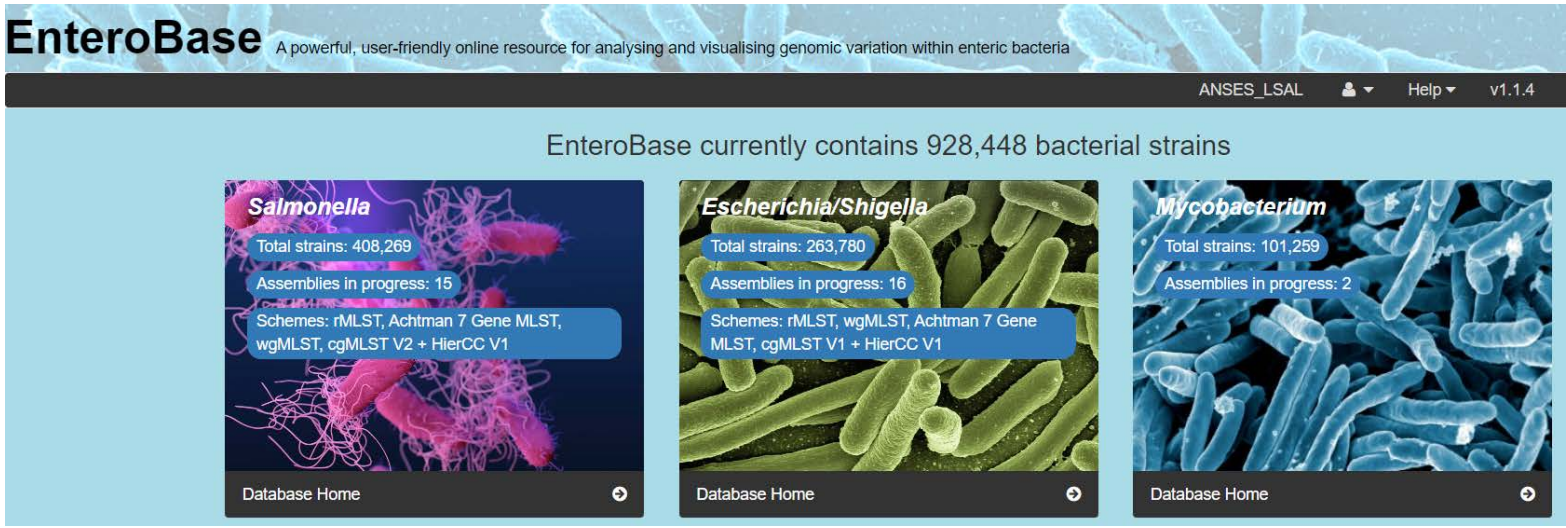


Source: Pennone et al COFS (2022)

Entering the world of genomics: the past 20 years

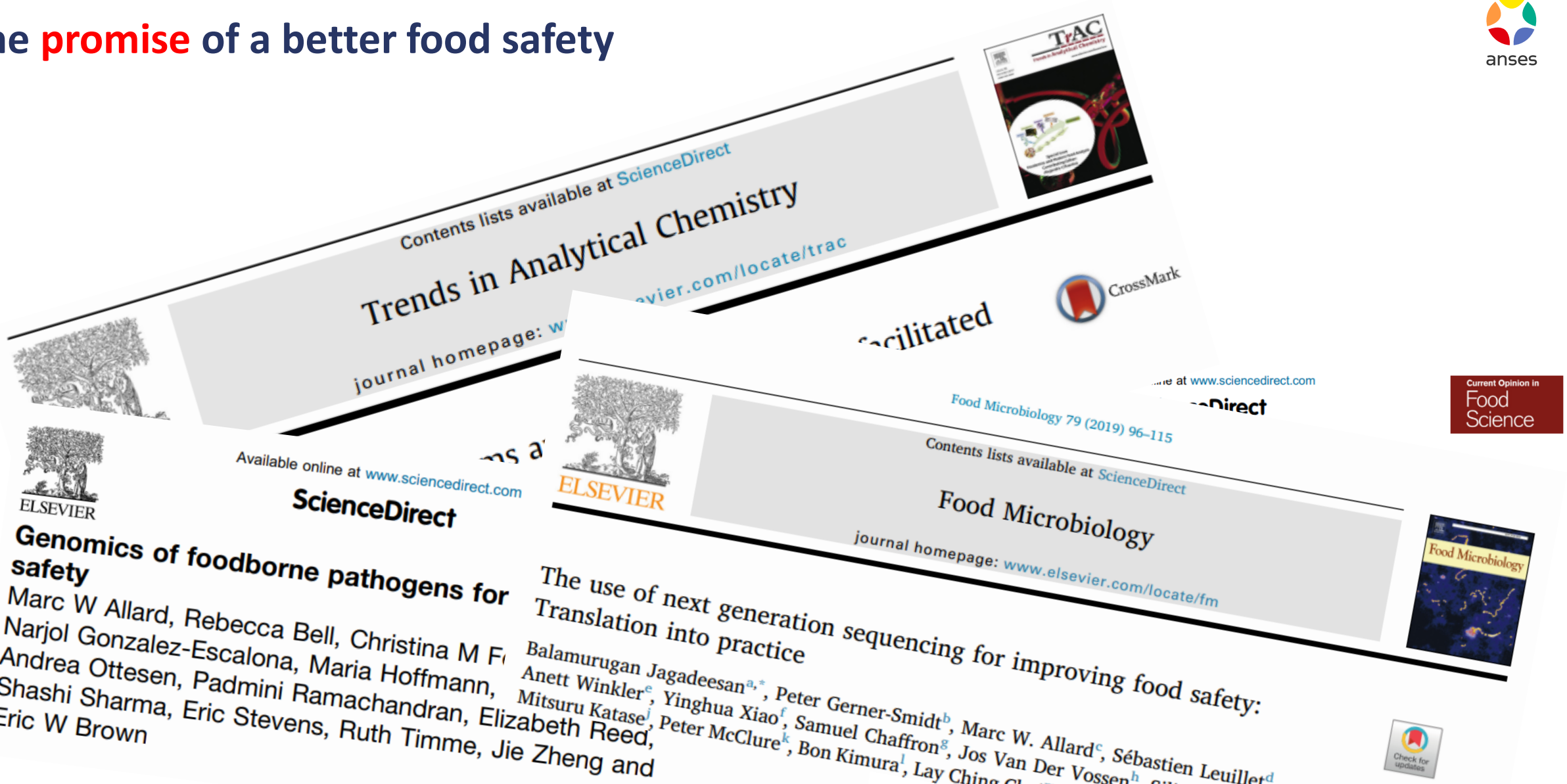


Source: International Human Genome Sequencing Consortium, Nature (2001)



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

The promise of a better food safety



EFSA BIOHAZ Panel's opinion

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 October 2019

doi: 10.2903/j.efsa.2019.5898

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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2. **Epidemiological investigations and source attribution**
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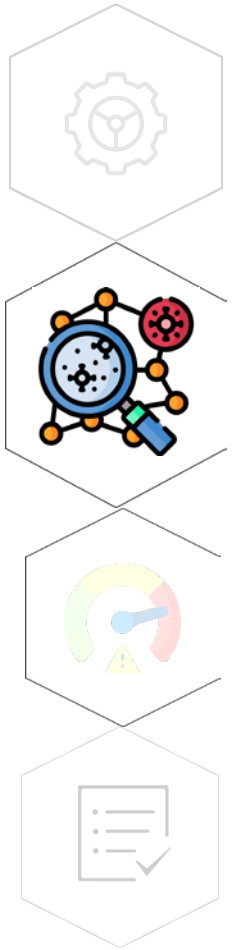


Outbreak investigation

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

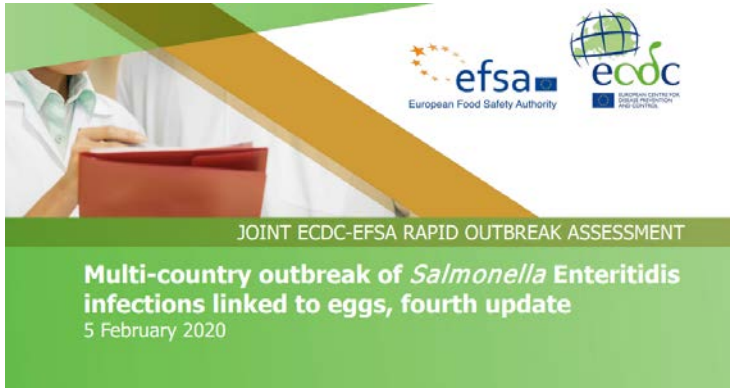
- Romaine lettuce *E. coli* O26 - USA (multiannual)
- Polony sausage *L. monocytogenes* South-Africa (2018)
- Melon *L. monocytogenes* Australia (2018)

- …

Outbreak investigation

A success for EU

- EFSA/ECDC Rapid outbreak assessment
- EURL role
- Database of WGS
- …

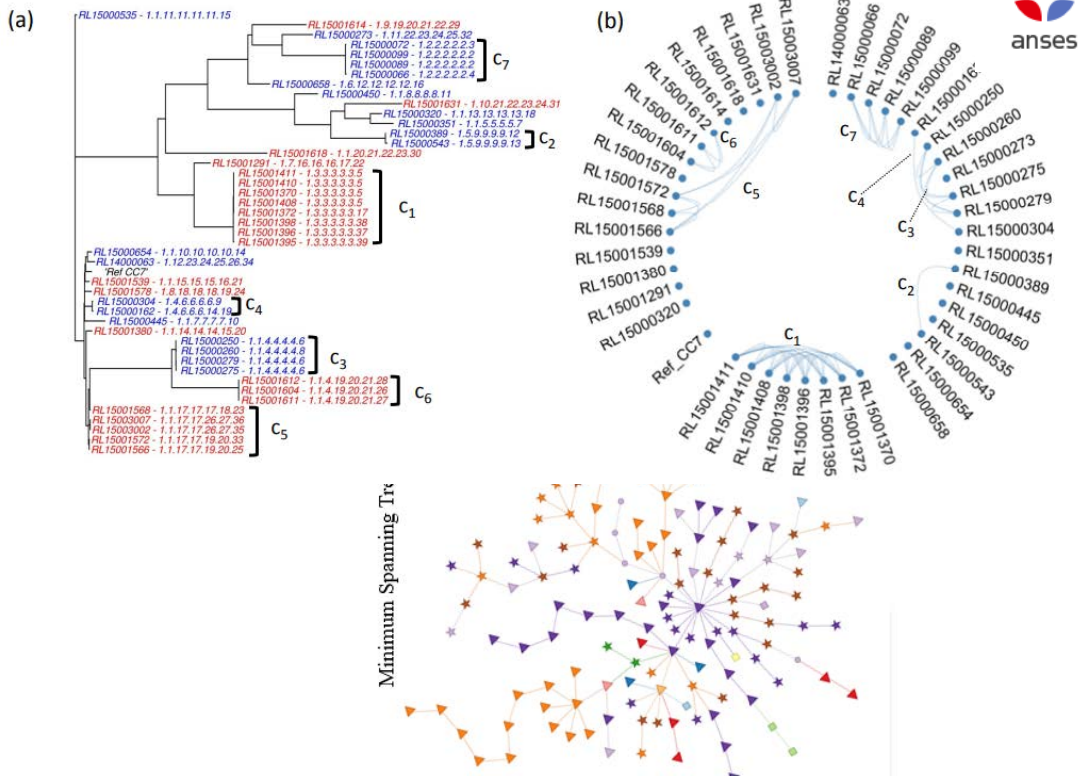


Outbreak investigation

Principle is simple

❶ Clustering of strains based on SNP/cgMLST distance

❷ Use of epidemiological data (strains, patients,…)



Genomics alone is not sufficient to confirm an outbreak, epidemiologists still have to **investigate** to support **decision making**

Food Chain Lab
Created by BfR

Outbreak investigation

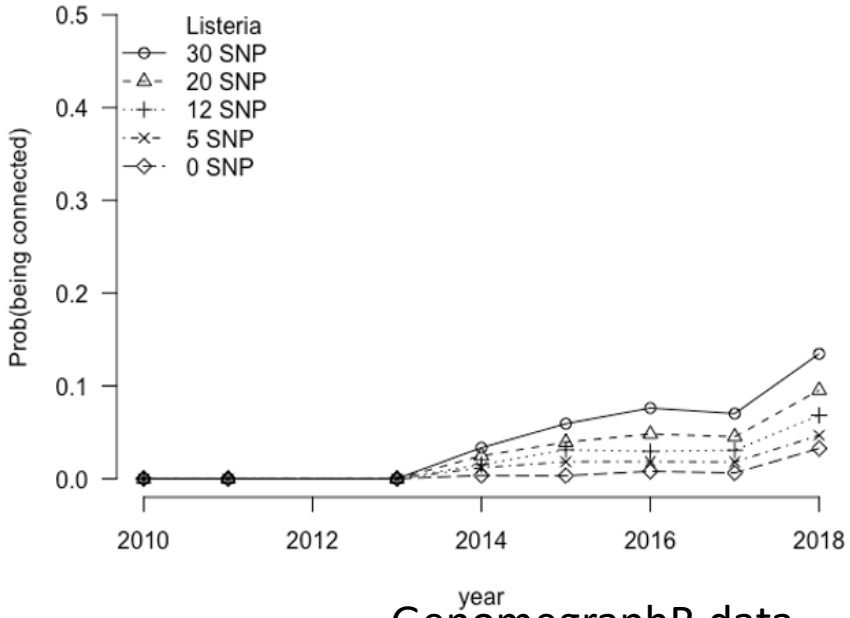
A solution to solve every recorded cases ?

No

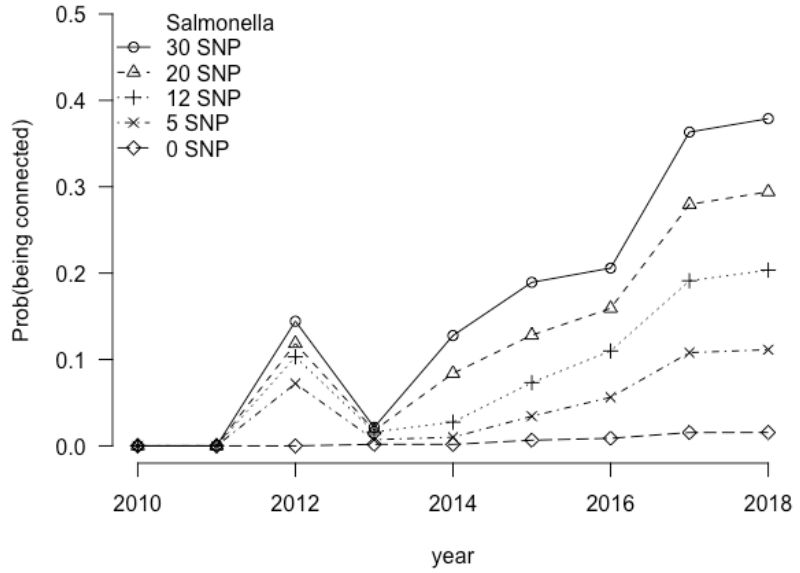
- Proportion of connected strains can be low (e.g. *Listeria*)

- 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)



GenomegraphR data



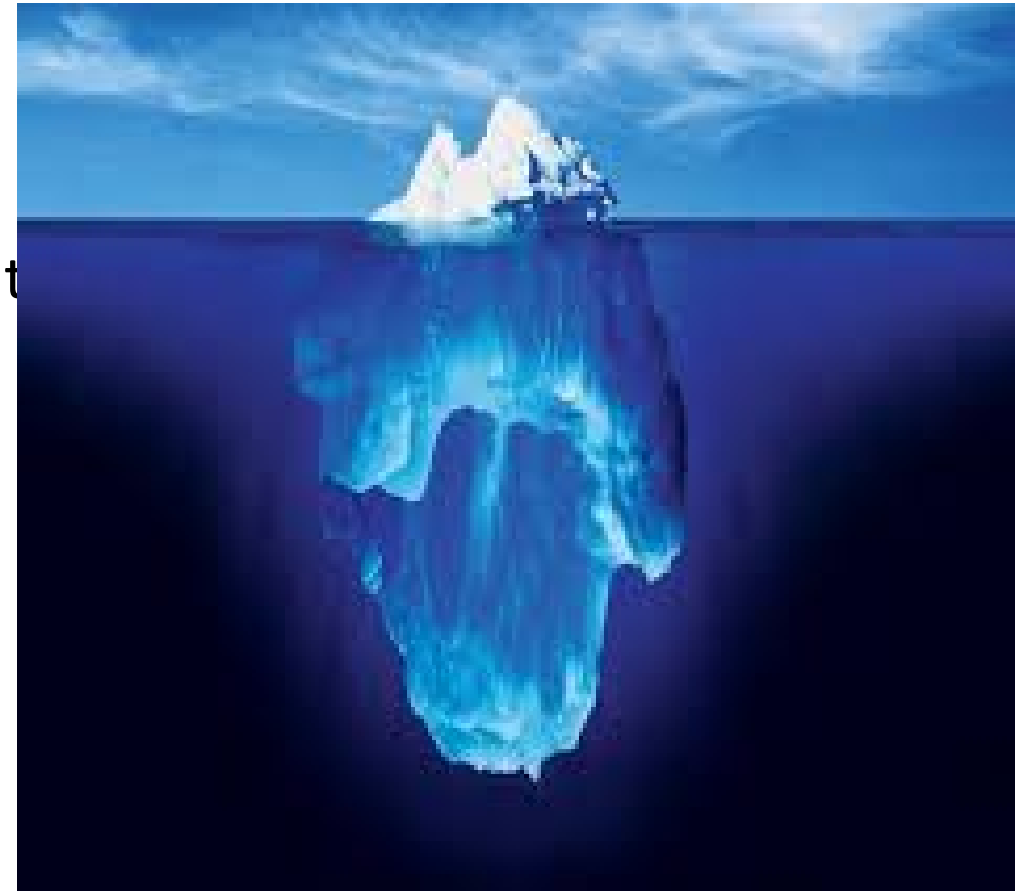
Beyond outbreaks

Outbreaks but also

... sporadic cases

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

... unrecorded cases

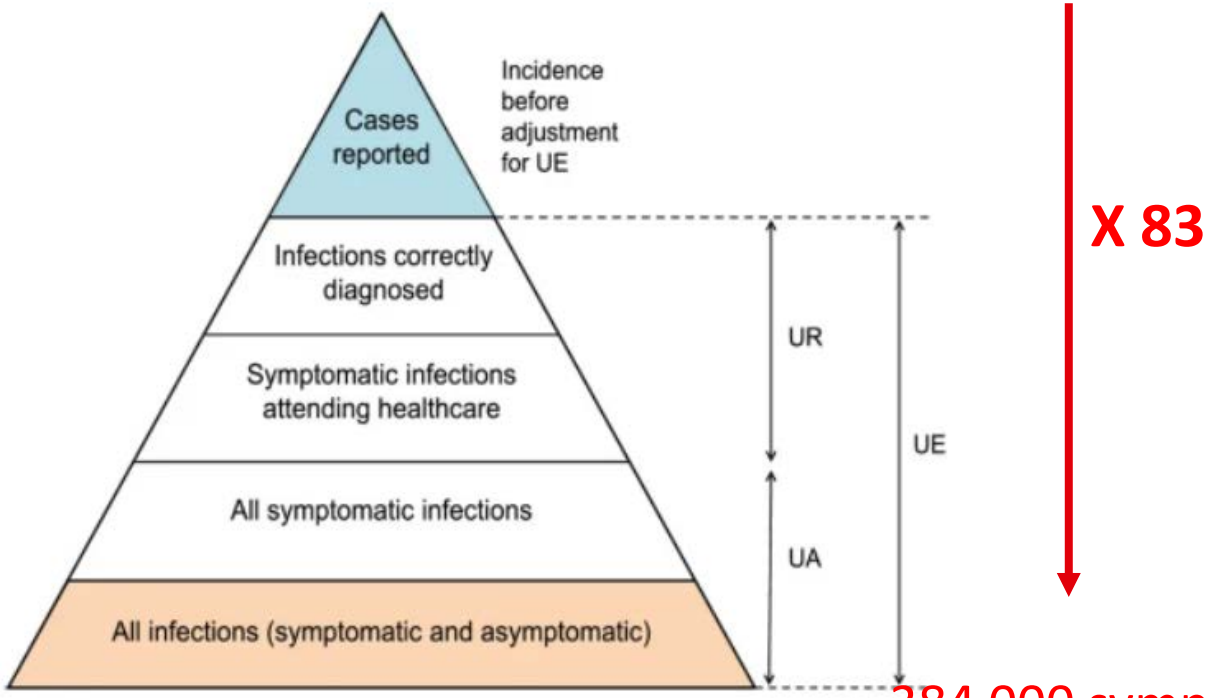


Beyond outbreaks

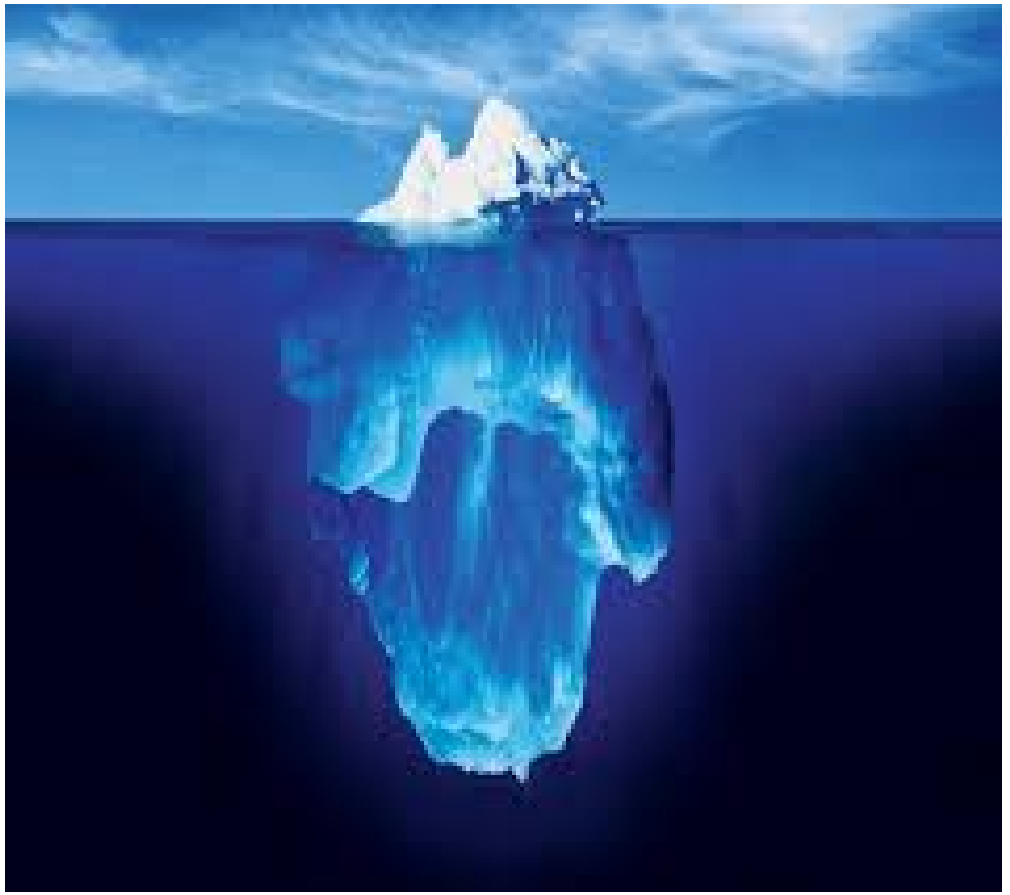
Estimated number of actual cases

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

Campylobacteriosis: N=4600



X 83

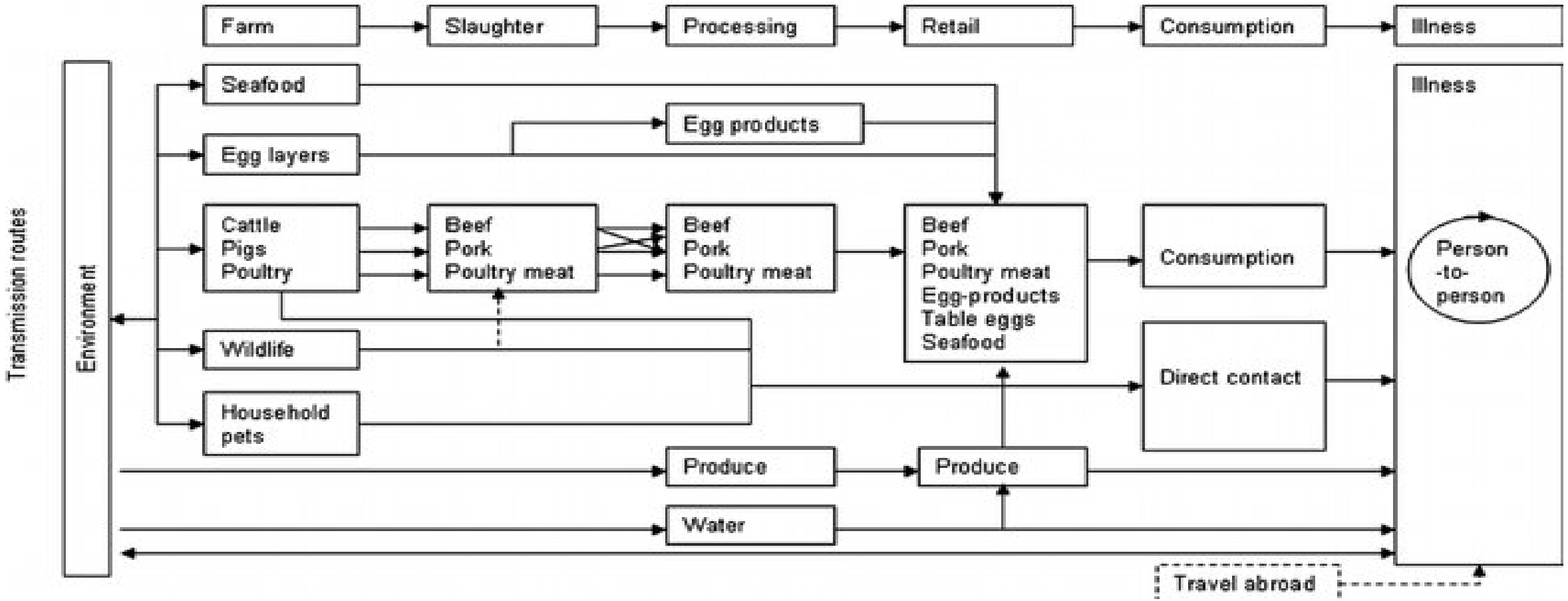


384 000 symptomatic

IC90% [240 000 – 790 000]

Source attribution

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



Pires et al https://doi.org/10.1089/fpd.2008.0208

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

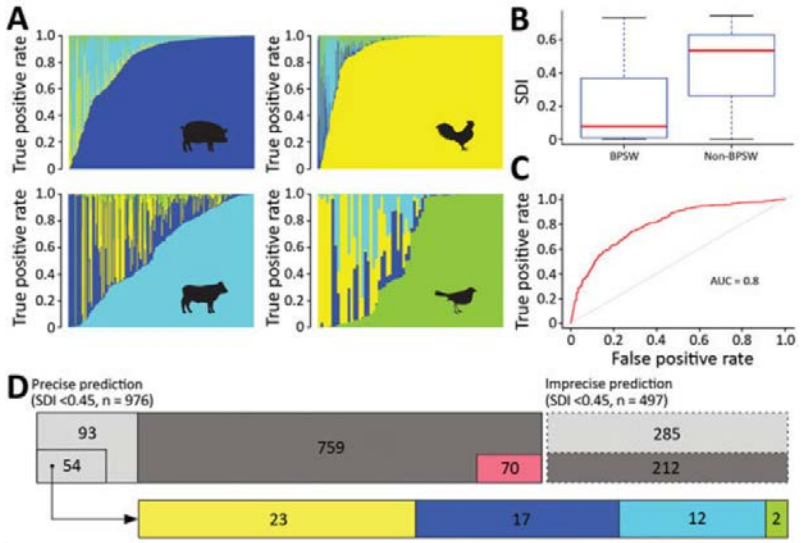
Some source attribution results

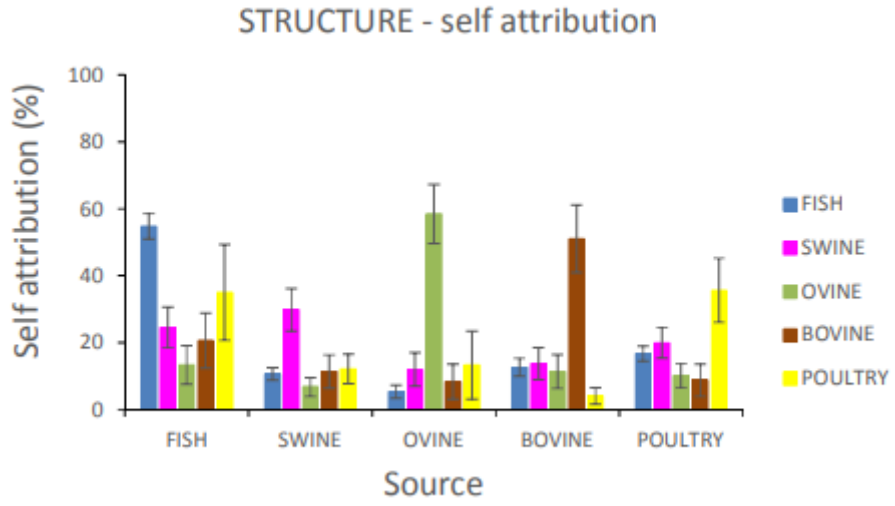
Typing/genomic approaches

With WGS, the accuracy of the model is slighly improved

Need for complex model (accounting for possibility of transfert between source)

WGS is not (yet) a revolution for quantification of the importance of sources





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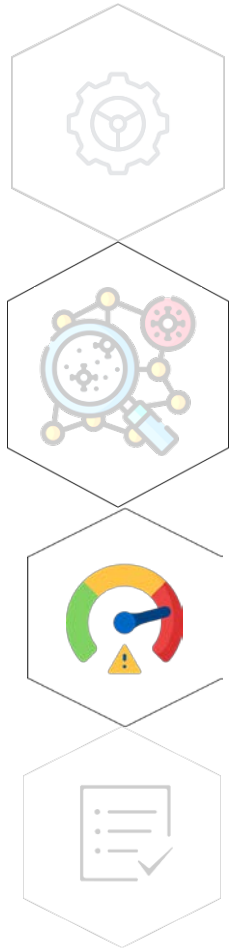
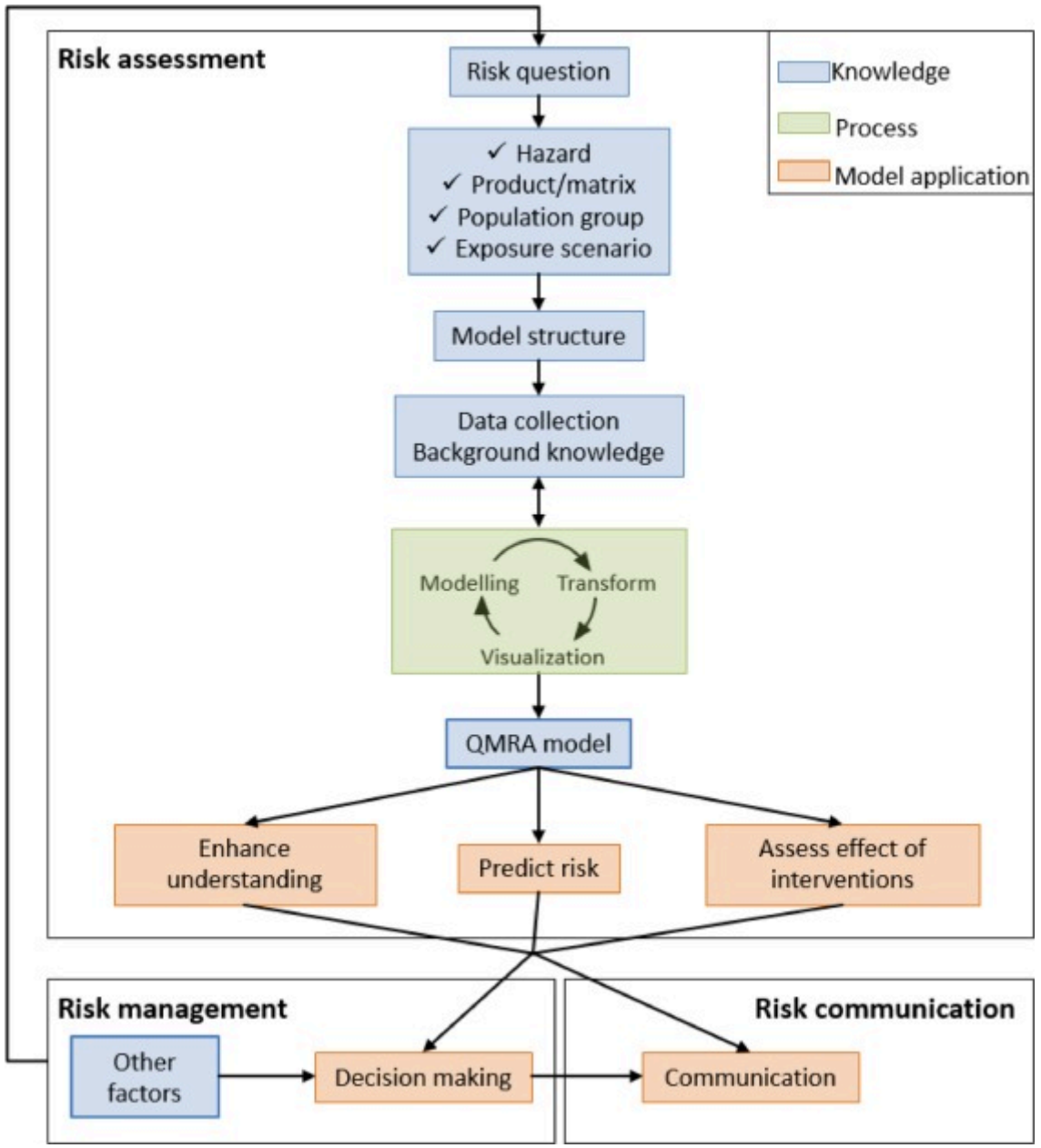
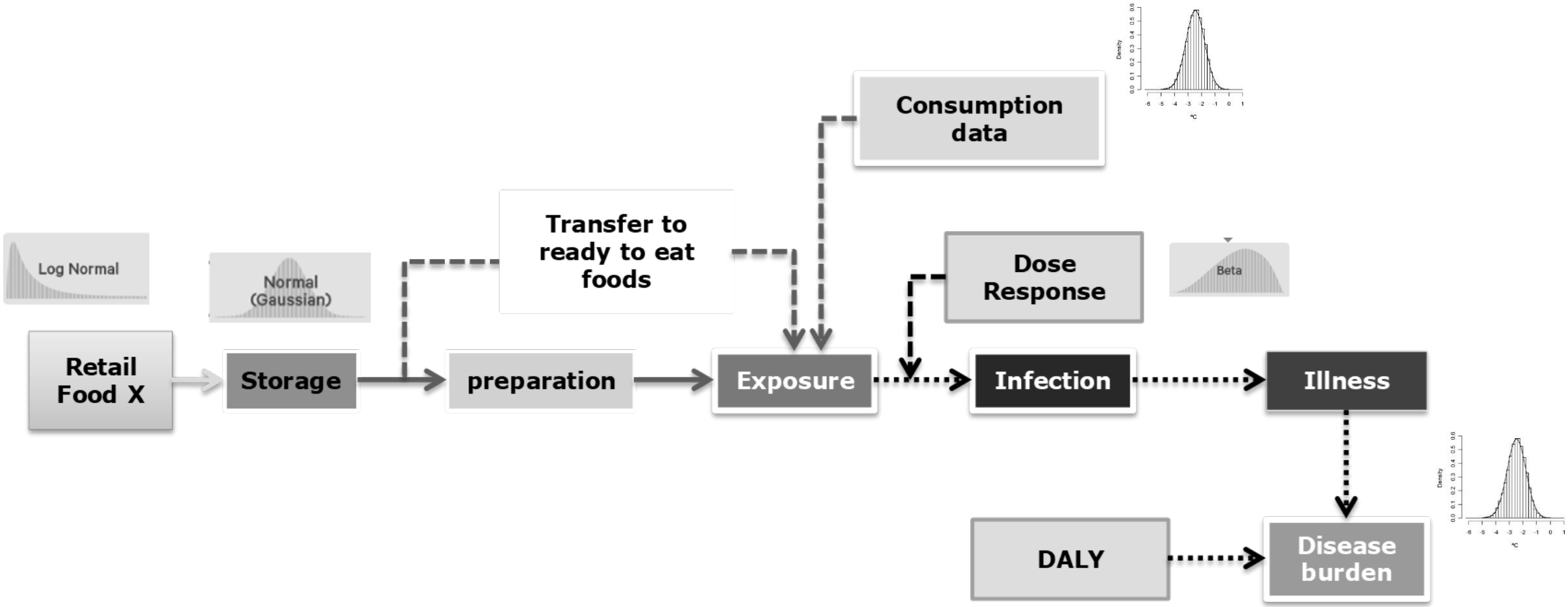


Risk analysis



Microbial risk assessment



Genomics and risk assessment

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 23 October 2019

doi: 10.2903/j.efsa.2019.5898

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

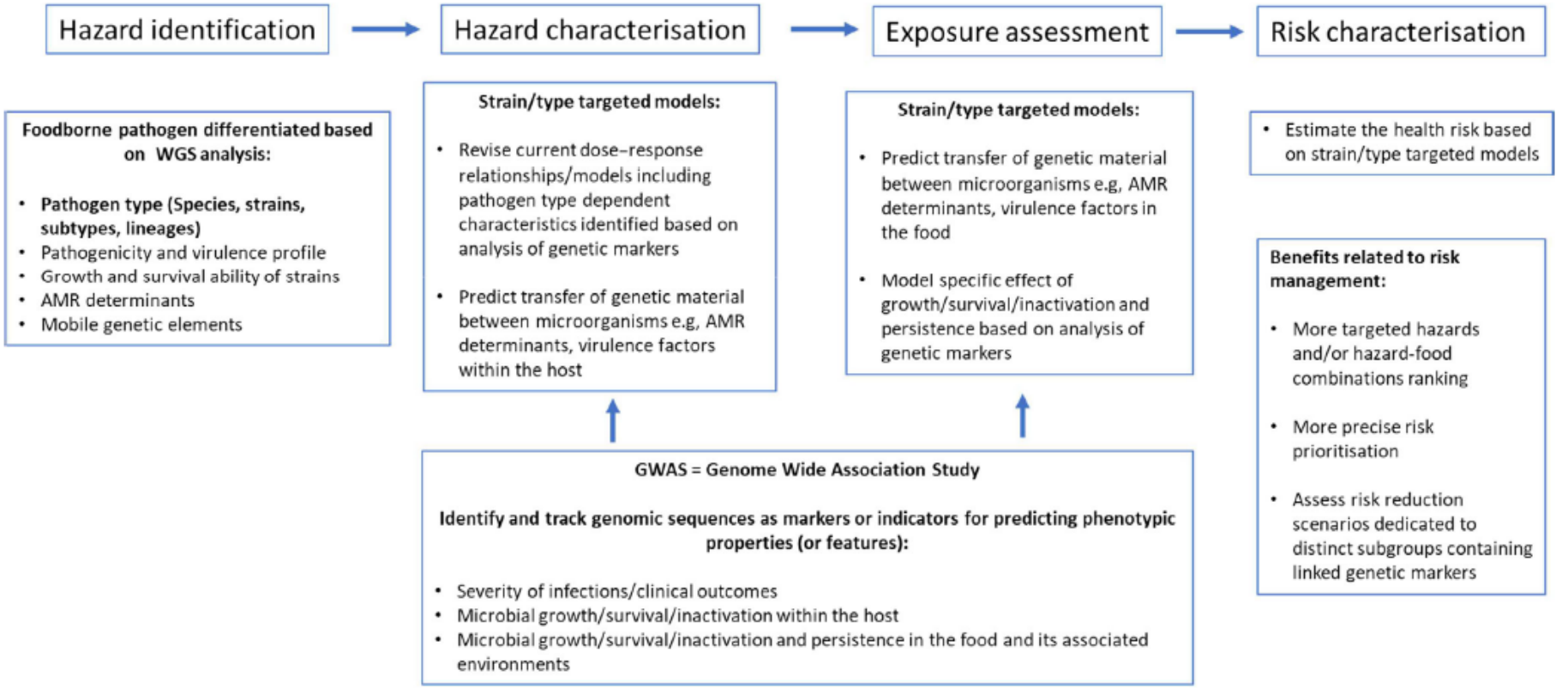
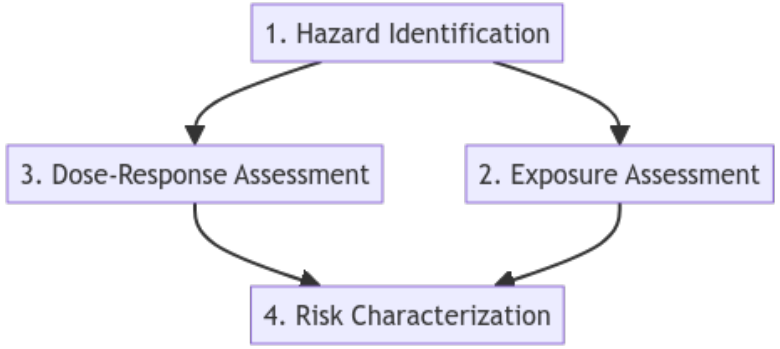


Figure 2: Future perspectives for WGS to add value to microbial risk assessment

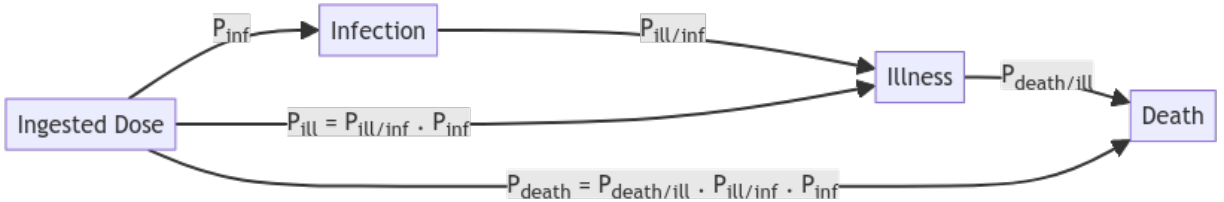
Dose-response: definition and use

Part of QMRA

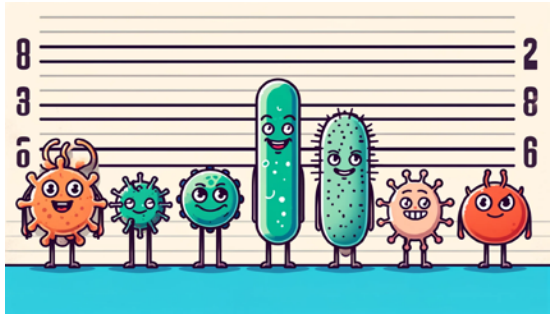


Definition: DR modeling uses mathematical relationships to describe the **probability** of adverse health effects due to exposure to a specific dose.

Health effects : infection, illness…



Measurement of "dose": Dose levels are measured in number of microorganisms (could be oocysts, CFUs, PFUs, or number of genome copies)

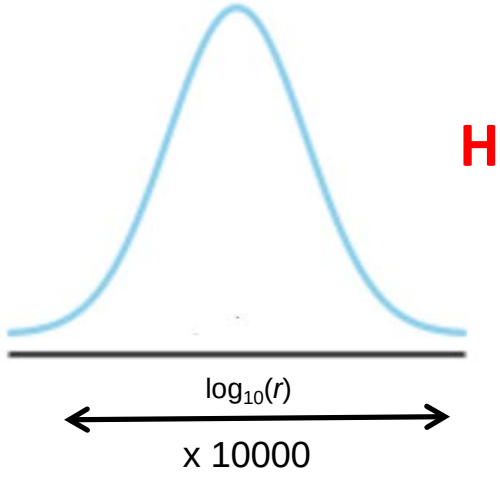


Dose-response: data and limits

Large uncertainty

- Parameter uncertainty
- Data uncertainty: Are the (outbreak) data relevant to describe my population and strain variabilities?

Strain variability

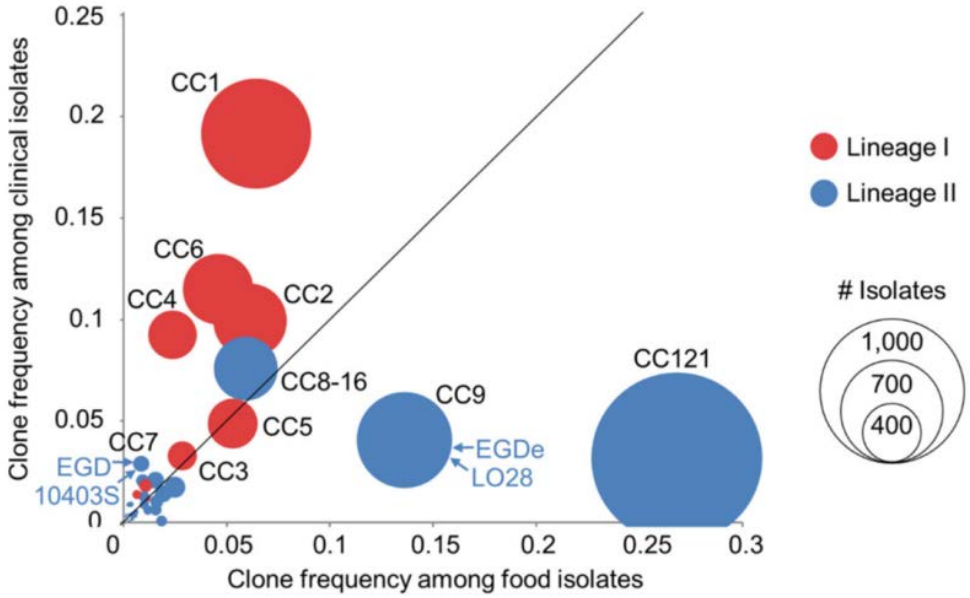


How WGS can help to improve DR?

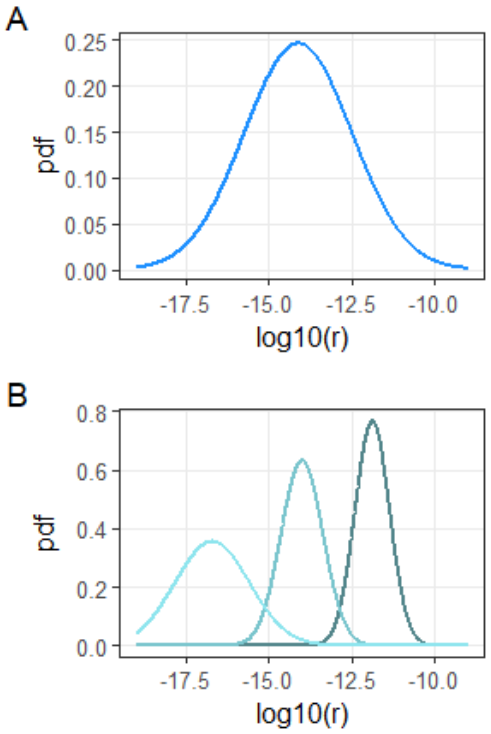
Tipping point for WGS in risk assessment

Is already there for dose response… driven by epidemiological data

L. monocytogenes



Maury et al. 2016



Fritsch et al. 2018

JEMRA
Joint FAO/WHO Expert Meetings on Microbiological Risk Assessment

To be published in 2024

New *L. monocytogenes* dose response (Pouillot et al. 2024)

Article

Updated Parameters for *Listeria monocytogenes* Dose–Response Model Considering Pathogen Virulence and Age and Sex of Consumer

Régis Pouillot [1,*], Andreas Kiermeier [2], Laurent Guillier [3], Vasco Cadavez [4,5] and Moez Sanaa [6,*]

World Health Organization

Step 1: Classify the strains according to : virulence

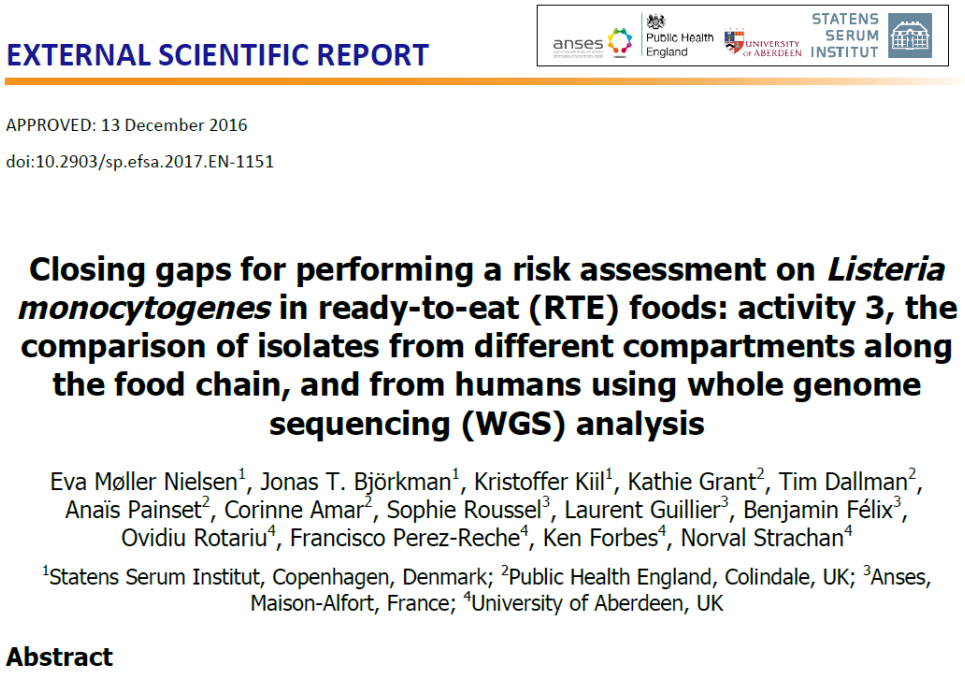
- **More virulent**: CC1, CC101, CC2, CC220, CC224, CC4, CC451, CC54, CC6, CC7, CC87)
- **Virulent**: CC14, CC155, CC177, CC18, CC20, CC21, CC26, CC3, CC37, CC379, C388, CC398, CC5, CC59, CC8, CC403 and all others
- **Less Virulent**: CC121, CC204, CC31, CC9, CC193, CC19, ST214

New *L. monocytogenes* dose response (Pouillot et al. 2024)

Step 2. Get the Proportion of each virulent group in food and human cases

RTE	More Virulent	Virulent	Less Virulent	Unknown	N
RTE Seafood	12%	35%	51%	1%	290
RTE Meats	20%	20%	59%	1%	176
RTE cheese and dairy	33%	47%	12%	8%	89
Human Sporadic Cases	60%	29%	8%	3%	262

Moller-Nielsen et al, 2017 Data for Seafood, Meat, cheese and sporadic cases



EXTERNAL SCIENTIFIC REPORT

APPROVED: 13 December 2016

doi:10.2903/sp.efsa.2017.EN-1151

Closing gaps for performing a risk assessment on *Listeria monocytogenes* in ready-to-eat (RTE) foods: activity 3, the comparison of isolates from different compartments along the food chain, and from humans using whole genome sequencing (WGS) analysis

Eva Møller Nielsen[1], Jonas T. Björkman[1], Kristoffer Kiil[1], Kathie Grant[2], Tim Dallman[2], Anaïs Painset[2], Corinne Amar[2], Sophie Roussel[3], Laurent Guillier[3], Benjamin Félix[3], Ovidiu Rotariu[4], Francisco Perez-Reche[4], Ken Forbes[4], Norval Strachan[4]

[1]Statens Serum Institut, Copenhagen, Denmark; [2]Public Health England, Colindale, UK; [3]Anses, Maison-Alfort, France; [4]University of Aberdeen, UK

Abstract

New *L. monocytogenes* dose response (Pouillot et al. 2024)

Step 3. Get an exposure model

From EFSA:

- Exposure of the EU consumers to *L. monocytogenes* from Seafood, Meats and Cheese
- Empirical Distribution of the Contaminated servings of Lm per sub-populations

SCIENTIFIC OPINION

EFSA Journal

ADOPTED: 6 December 2017

doi: 10.2903/j.efsa.2018.5134

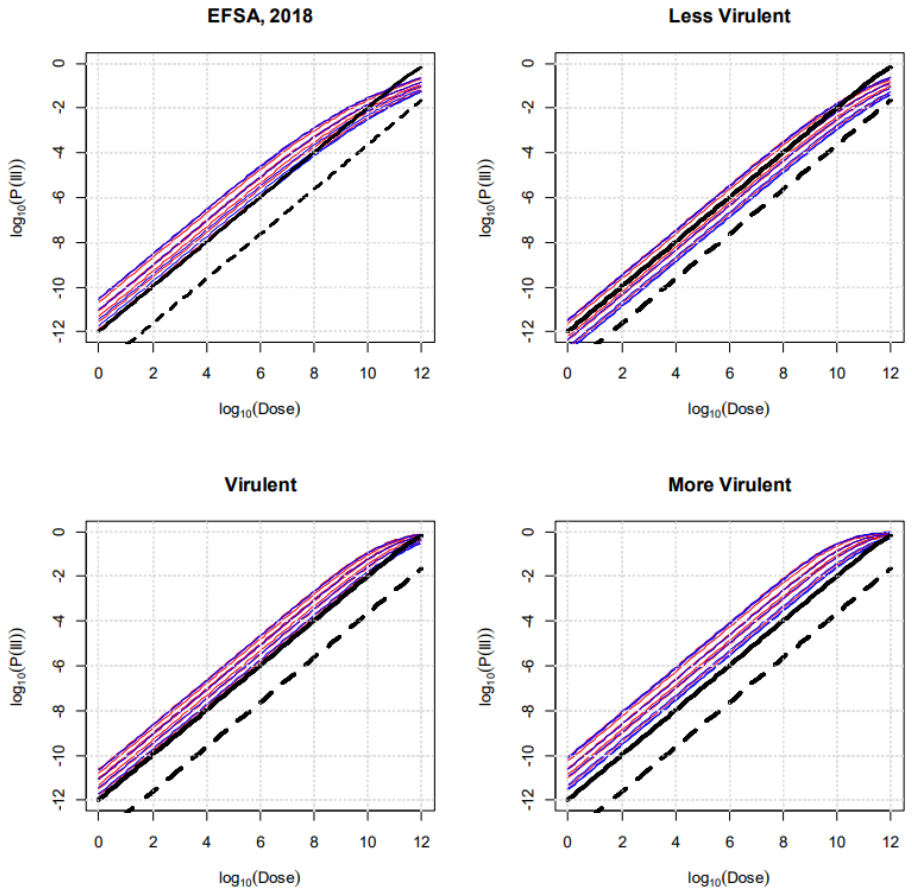
***Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU**

EFSA Panel on Biological Hazards (BIOHAZ),
Antonia Ricci, Ana Allende, Declan Bolton, Marianne Chemaly, Robert Davies,
Pablo Salvador Fernández Escámez, Rosina Girones, Lieve Herman,
Konstantinos Koutsoumanis, Birgit Nørrung, Lucy Robertson, Giuseppe Ru, Moez Sanaa,
Marion Simmons, Panagiotis Skandamis, Emma Snary, Niko Speybroeck, Benno Ter Kuile,
John Threlfall, Helene Wahlström, Johanna Takkinen, Martin Wagner, Davide Arcella,
Maria Teresa Da Silva Felicio, Marios Georgiadis, Winy Messens and Roland Lindqvist

Abstract

New *L. monocytogenes* dose response (Pouillot et al. 2024)

Step 4. Infer DR parameters



README

FoodsDR

This repository contains the R code and data necessary to derive the manuscript "**Updated parameters for the dose-response model for *Listeria monocytogenes* considering pathogen virulence and age and sex of consumer**". from Régis Pouillot, Andreas Kiermeier, Laurent Guillier, Vasco Cadavez, and Moez Sanaa. *Foods* **2024**, *13*(5), 751 (https://doi.org/10.3390/foods13050751), as well as the link to install the `doseresponsemodels` package.

R package to use the Dose Response models

Package installation

```
install.packages("devtools")
devtools::install_github("rpouillot/doseresponsemodels")
```

Usage

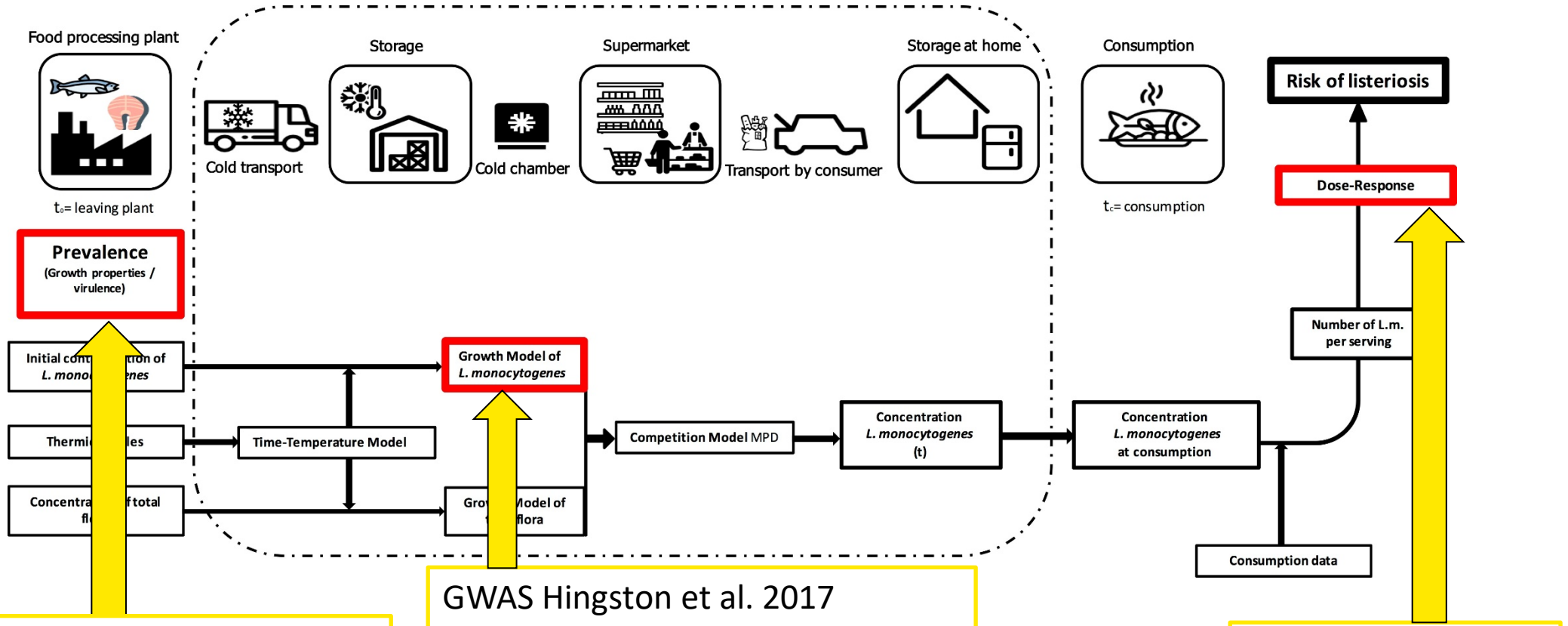
The `doseresponsemodels::DRQuick()` function provides a "quick" version of the function to derive the marginal probability of invasive listeriosis in a given population for a given dose in CFU (actual dose if the argument `Poisson = FALSE` or average dose if the argument `Poisson = TRUE`) using the "JEMRA" 2004, the "Pouillot" *et al.*, 2015, the "Fritsch" *et al.* 2018, the "EFSA", 2018 dose-response models or the model developed within this project ("EFSAMV" for more virulent strains, "EFSAV" for virulent strains, or "EFSALV" for the less virulent strains).

```
library("doseresponsemodels")
help('DRQuick')
DRQuick(1:10,  model="JEMRA", population = 1:2)
DRQuick(1:10,  model="Pouillot", population = 1:11)
DRQuick(1:10,  model="EFSA", population = 1:14)
DRQuick(1:10,  model="EFSAMV", population = 1:14)
```

How to do in practical terms?

Fritsch et al. 2018, MRA

Implementation for cold smoked salmon-related listeriosis



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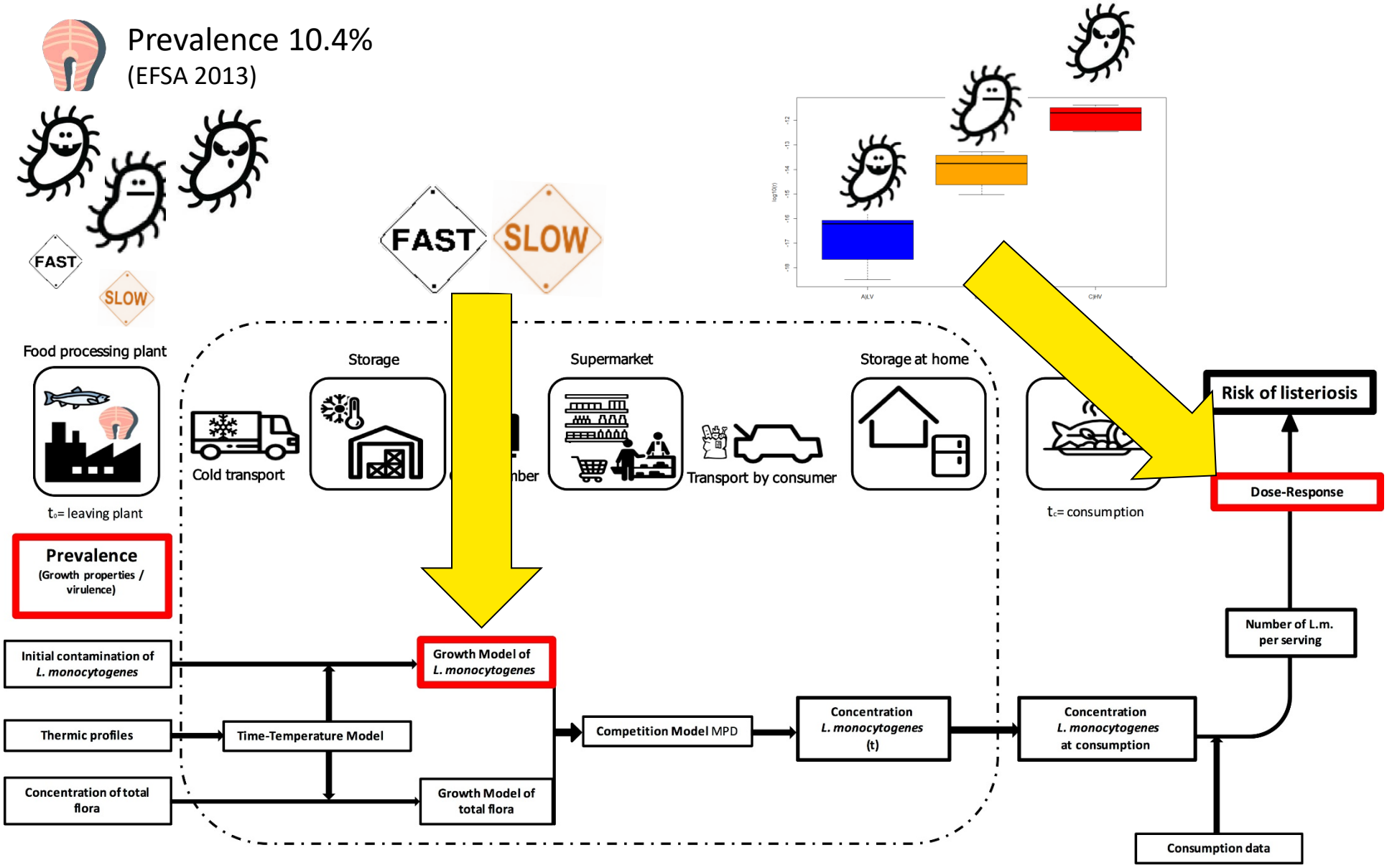
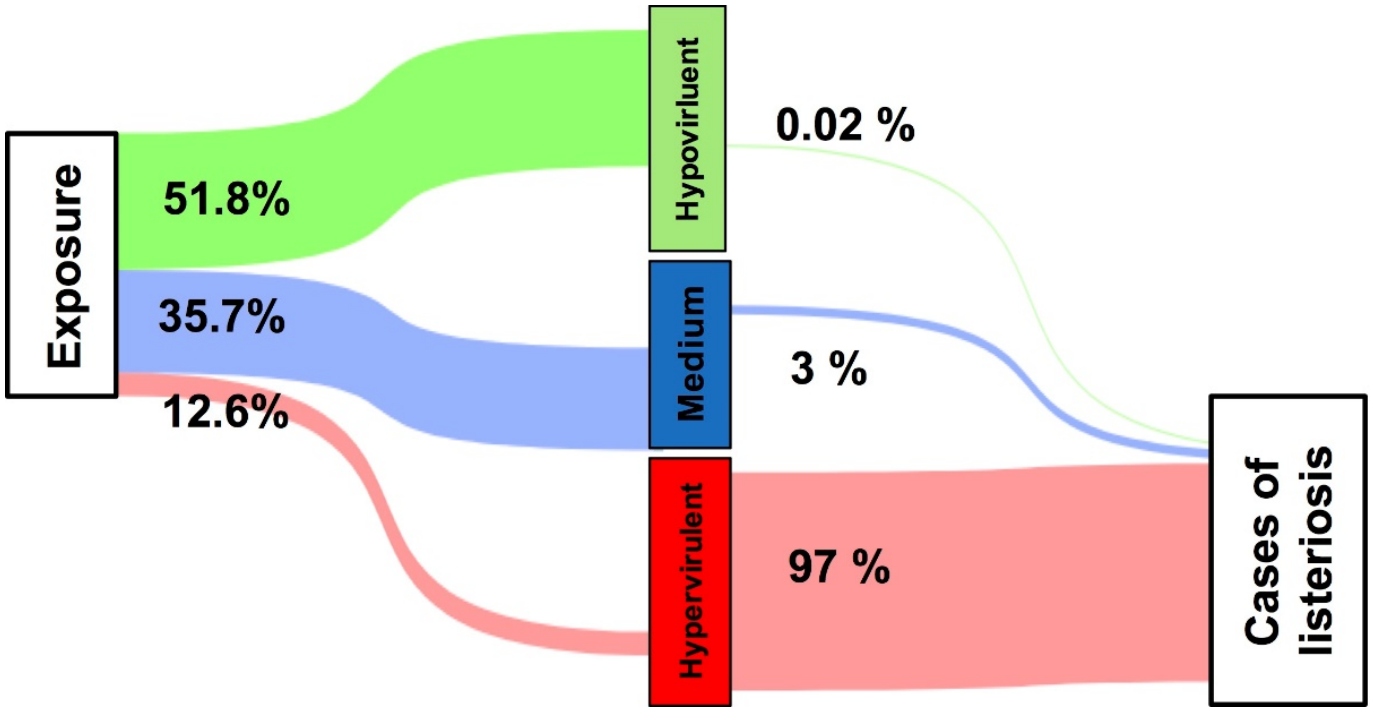


How to do in practical terms?

Implementation for cold smoked salmon-related listeriosis

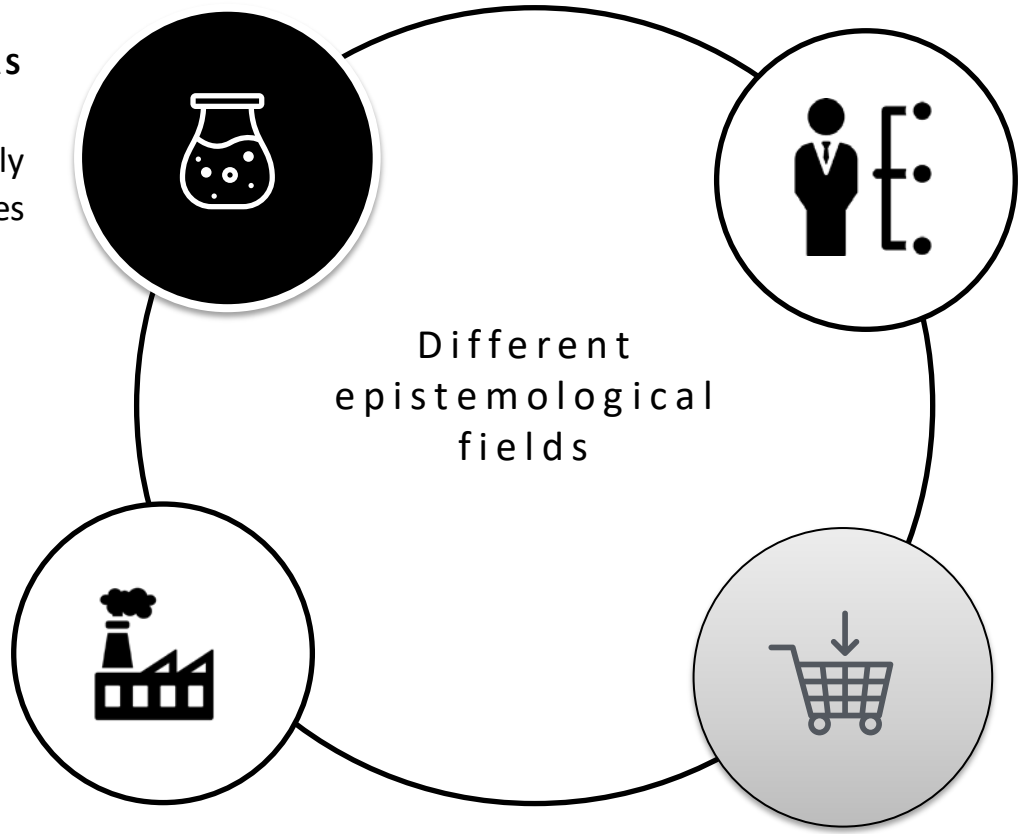


These results raise questions about the management measures associated with the different strains

Are we ready to change ?

RISK ASSESSORS

Risk could be managed differently according to virulence types



RISK MANAGERS

Do we have zero risk for the low virulence class?

INDUSTRY

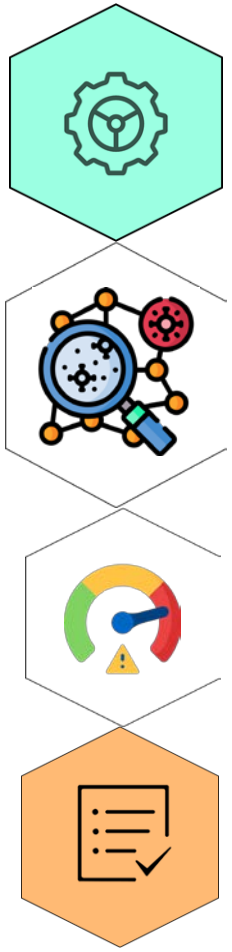
Do we have a method to rapidly characterize virulence ?

CONSUMERS

Is it a “safe” or “not safe” food?

Outline

1. Context
2. Epidemiological investigations and source attribution
3. Risk assessment
4. Conclusion



Conclusions: what has (will) WGS brought us?

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