anses
French agency for food, environmental
and occupational health & safety

*Investigate, evaluate, protect*

# Sequencing Technology and its application to risk assessment
## National Perspectives

**C. Grastilleur / C. Cordevant – Strategy and Programs Directorate**

*66th MEETING OF THE EFSA ADVISORY FORUM* - Tuesday 5 December 2017

# Conventional typing methods> High Throughput Sequencing

A multitude of pathogen-dependent methods

Phage-T

PFGE

MLST

AFLP

MLVA

RFLP

Serotyping

SLST

RAPD

AMR

CRISP-R based

Rep-PCR

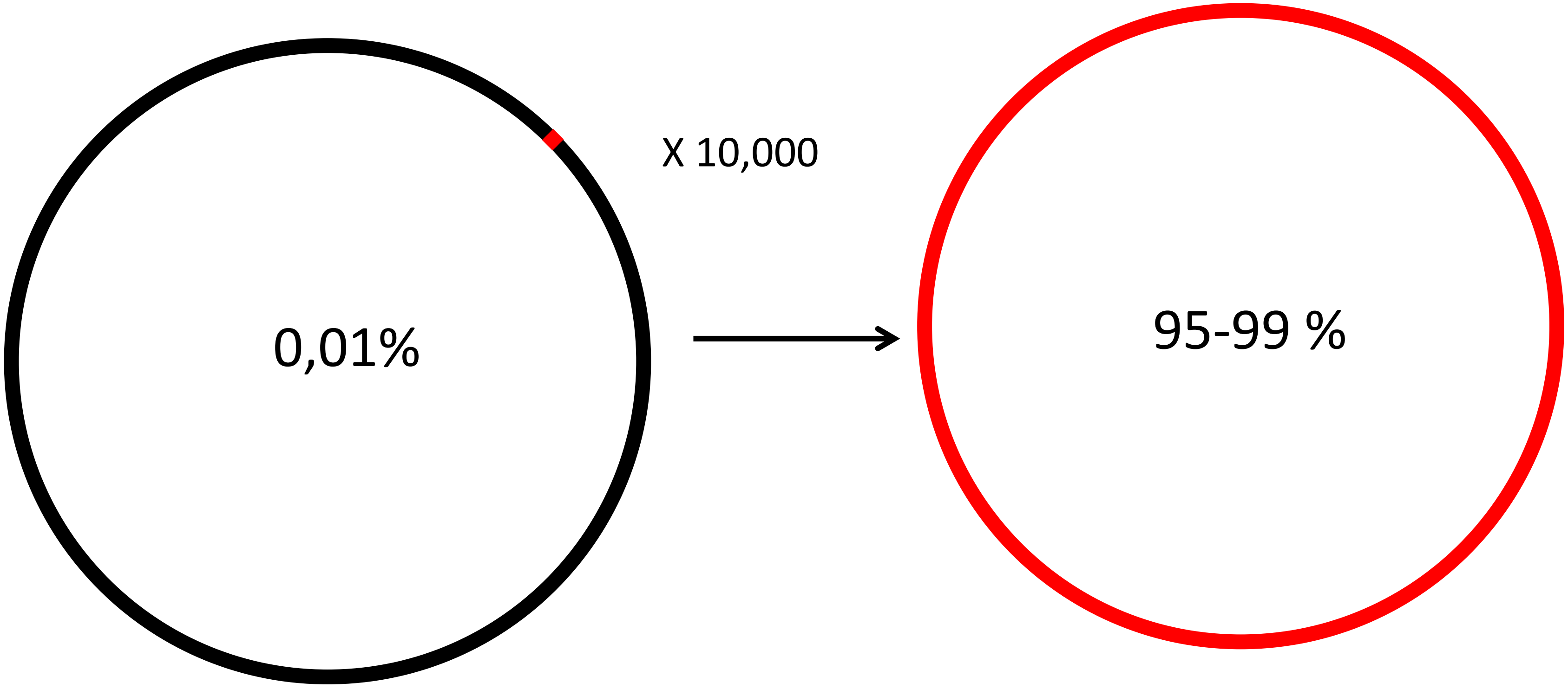
A unique technological platform

High Throughput Sequencing
(WGS/NGS)

Surveillance / Biological Properties / Epidemiological Investigation

# MSLT *vs* WGS

**Amount of information**



X 10,000

0,01%

95-99 %

**MLST**

**WGS**

# The challenge for National organisations

**Financial / Organizational / Technical / Scientific / Policy**

- Access to sequencing technology (volumes, costs, deadlines)
- New skills and expertise
- IT infrastructure: Storage / calculations / network / cloud access
- Validation of methods – Standardization
- Data analysis, portability and inter-laboratory harmonization
- Database governance: sharing, sustainability, accessibility of data

# Contribution of HTS technologies to ANSES' activities

- Ultimate discrimination of isolates:
  - Robust phylogenies studies
  - Specific and accurate epidemiological investigations
- Phenotypic prediction (virulence, adaptation, AMR!?)
- Rapid methods development (targeted PCR) for hazards characterization
- Integration into Animal Health models - Vehicles and sources better apprehended
- Microevolution > retrospective analysis of emergence > predictive models of emergence
- Exploration of strains collections

Better hazards knowledges > Evolution of National & EU Regulation -
with focus point / targeted Regulation

# A multitude of initiatives to prepare the HTS transition

## In the field of food safety

- *Congress 2015-2016 (FAO, GMI, SFM, ASM, EFSA, CDC ...)*
- *Expert groups (GMI, eCDC, WHO, FAO, EFSA ... CDC, FDA)> reports*
- *ISO TC 34 / SC 9 / WG 25 food / microbiology / NGS*
- *CDC-FDA ($ 30M 2014 GenomTrackr Program)*
- *PHE (Salmonella Surveillance)*
- *European Programs & Food Security*

  *- COMPARE H2020 (2015-2020) - Global - All foodborne pathogens*

  *- BioContam EFSA Call (2015-2017) - Listeria biodiversity*

  *- EFFORT H2020 (2013-2018) - AMR epidemiology & metagenomics*

  *- ENGAGE EFSA Call (2016-2018) - HTS E. coli & Salmonella*

# Strains collections investigation with WGS

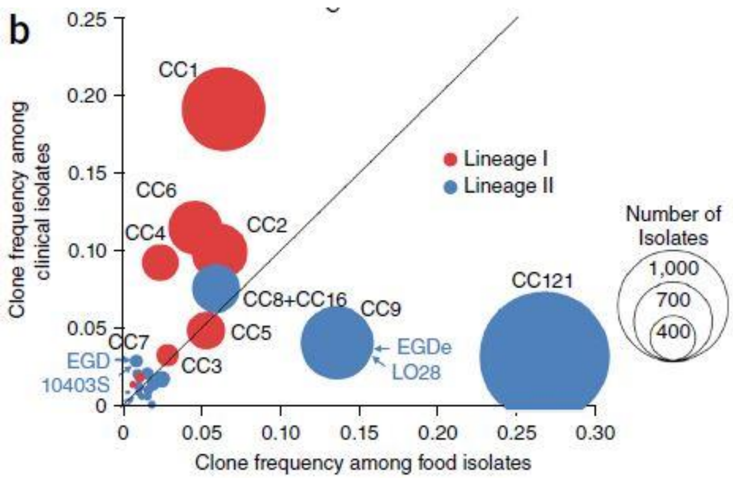
## Uncovering *Listeria monocytogenes* hypervirulence by harnessing its biodiversity (Nature genetics, 2016)

- Investigation of *Listeria* strains collections from clinical and food origins (NRL & NRC)

- Identification of clones associated with clinical cases (Food listeriosis or with human central nervous system (CNS) or maternal-neonatal (MN) listeriosis)

**Comparative genomics**

> Identification of new virulence factors - Experimental validation

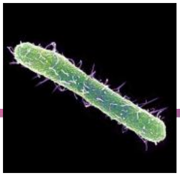


Clinical Isolates

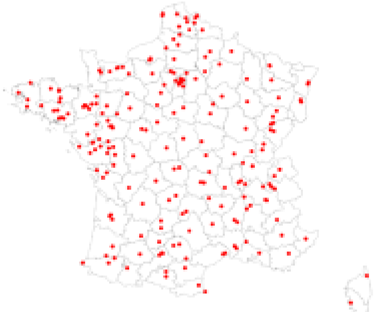
Food Isolates

**> Impact on Regulation?**

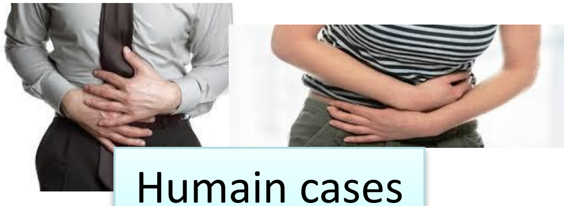
# Salmonella foodborne outbreak – WGS investigation 1/2



- ✓ Grouped cases of salmonellosis - December 2016 => catered dishes suspected



**Epidemiological survey**



Humain cases

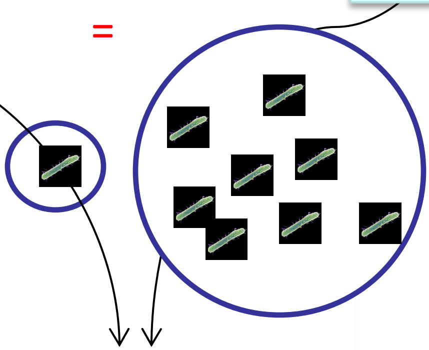
ANSES *Salmonella Network*
*(n=130* including official laboratories)
Anses USEL MA

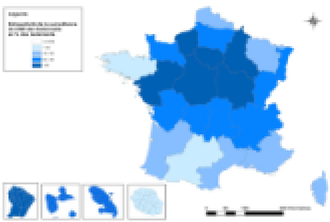
13 human strains
**CNR**

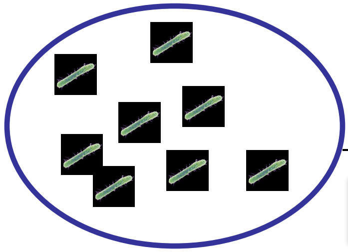
Control data set
17 strains of the network
Last 2 months
same serovar
food / animals

1 food strain
Anses LCSV

?
=







**National Reference Lab**

Statistical analysis of
genomic variations = SNP analysis

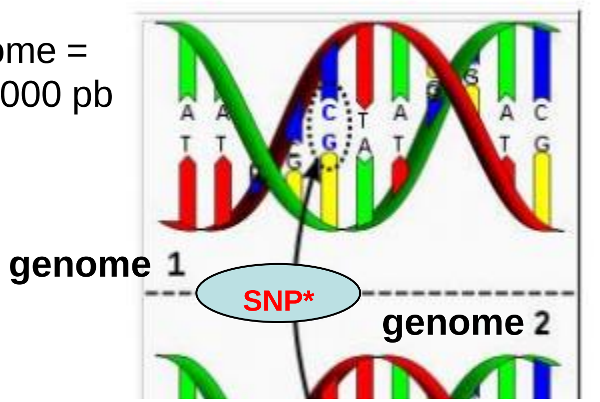
~1400 LABM

# Salmonella foodborne outbreak – WGS investigation 2/2

## Genomic variations (SNPs) & statistical analysis of links

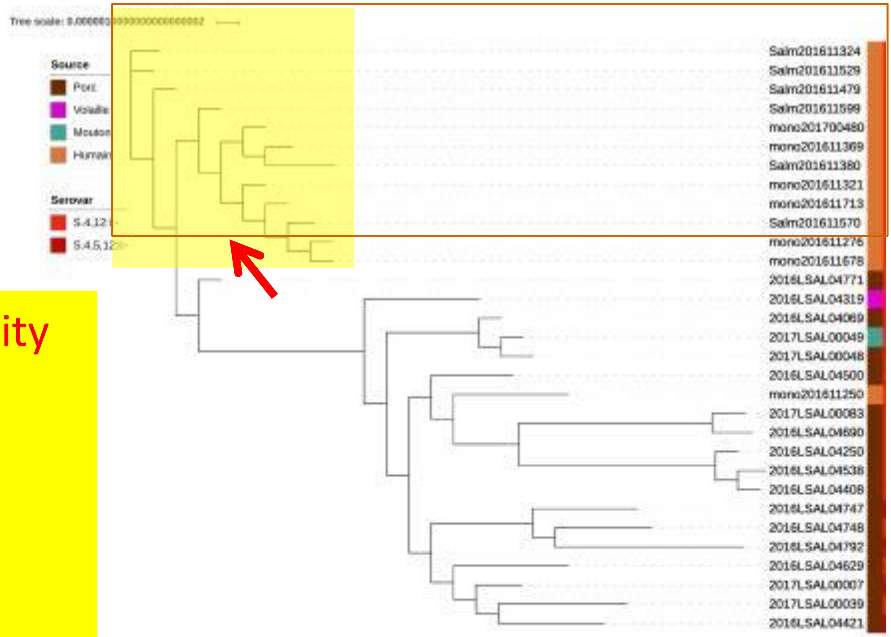
- ✓ Molecular characterization method
  - *a. R&D*
  - *b. Validation*
  - *c. Application to surveillance*

1 genome =
5, 000, 000 pb



- ✓ Highly efficient tool for isolate discrimination tool
- ✓ Genetic link between isolates (= phylogenetic tree)
- ✓ Statistical analysis

➔ 12 human strains linked to the catering ham strain !



⇨ Statistical definition of the genomic proximity threshold between linked and unrelated strains

Applicable specifically to each health alert investigation

# *Thank you for your attention*