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

A bird’s eye view of genomic projects in the ANSES laboratory for food safety

Dr. Michel-Yves MISTOU,
Head of the Foodborne pathogens department

Nanyang Technological University Food Technology Centre (NAFTEC)
Food Safety Risk Assessment, 5th November 2018

NANYANG
TECHNOLOGICAL
UNIVERSITY
SINGAPORE

Laboratory for food safety

Head, Laurent Laloux

3 departments

Sea products

Chemistry

Foodborne Pathogens

- Staff 140 people
- European Community Reference Laboratory (CRL)
- **Accredited EN ISO 17025** for most of its activities. In particular regulatory analyses performed under National (13) and European (2) References Mandates

Foodborne Pathogens Department (FBP)

Enteric viruses

Noroviruses, HAV, HEV

Salmonella & Listeria

Toxins producing bacteria

Staphylococcus, B. cereus, C. perfringens

High-Throughput PCR platform

E. Coli STEC

Official Laboratory FBO investigations

Genomics & Modelisation

- 3 units focused on pathogens
- 2 transversal teams
- 1 technological platform
- 1 official laboratory

Staff of about 80 people

- *4 NRLs mandates*

Listeria, Staphylococcus, Salmonella and Enteric viruses

- *2 EURL mandates*

Listeria, Staphylococcus

Collections of FB Pathogens:

- *Salmonella* (15,000 isolates)
- *Listeria* (5000)
- SCP (2000)
- *B. cereus* (500)
- *C. perfringens* (200)

Typology of activities in the FBP Dpt

Detection, **typing** and **characterization** of FB pathogens

✓ **Surveillance** (official control plans and coordination of lab networks (unofficial))

✓ **FBO investigations**

✓ **Scientific & technical support** to Anses Risk Assessment and regulated products divisions, National and European public health authorities

✓ **Coordination** of laboratories networks through **Technical support** and organization of **Proficiency trials** and **Training courses**

✓ **Research projects** : Innovative methods for pathogens detection, counting, typing, characterization, quantitative modelisation of risk assessment (QMRA), Source attribution, FBO investigations …

> **Implementation of Whole Genome Sequencing Approaches**

Finding our way to WGS

Conventional methods

PFGE

MLST

MLVA

PCR

Conventional Serotyping

qPCR

Antibiograms

Unique technical platform

> Many challenges in term of expertise and staff training / financial resources / IT infrastructure / regulatory issues …

Our strategic line

- **Sequencing – The choice of *Externalisation***

Public tender: Sequences are produced on a public platform.
60 € per genome – 10 days.

- **Data Analysis - Build bioinformatics *expertise***

Hire competent people / Build IT infrastructure / develop *in-house pipelines* / Dissemination of the competence among microbiologists

- **Learn while doing - *Genomics Research projects***

> 20 ongoing financed research projects / Valorizing Anses bacterial *collections* / Building collaborations /

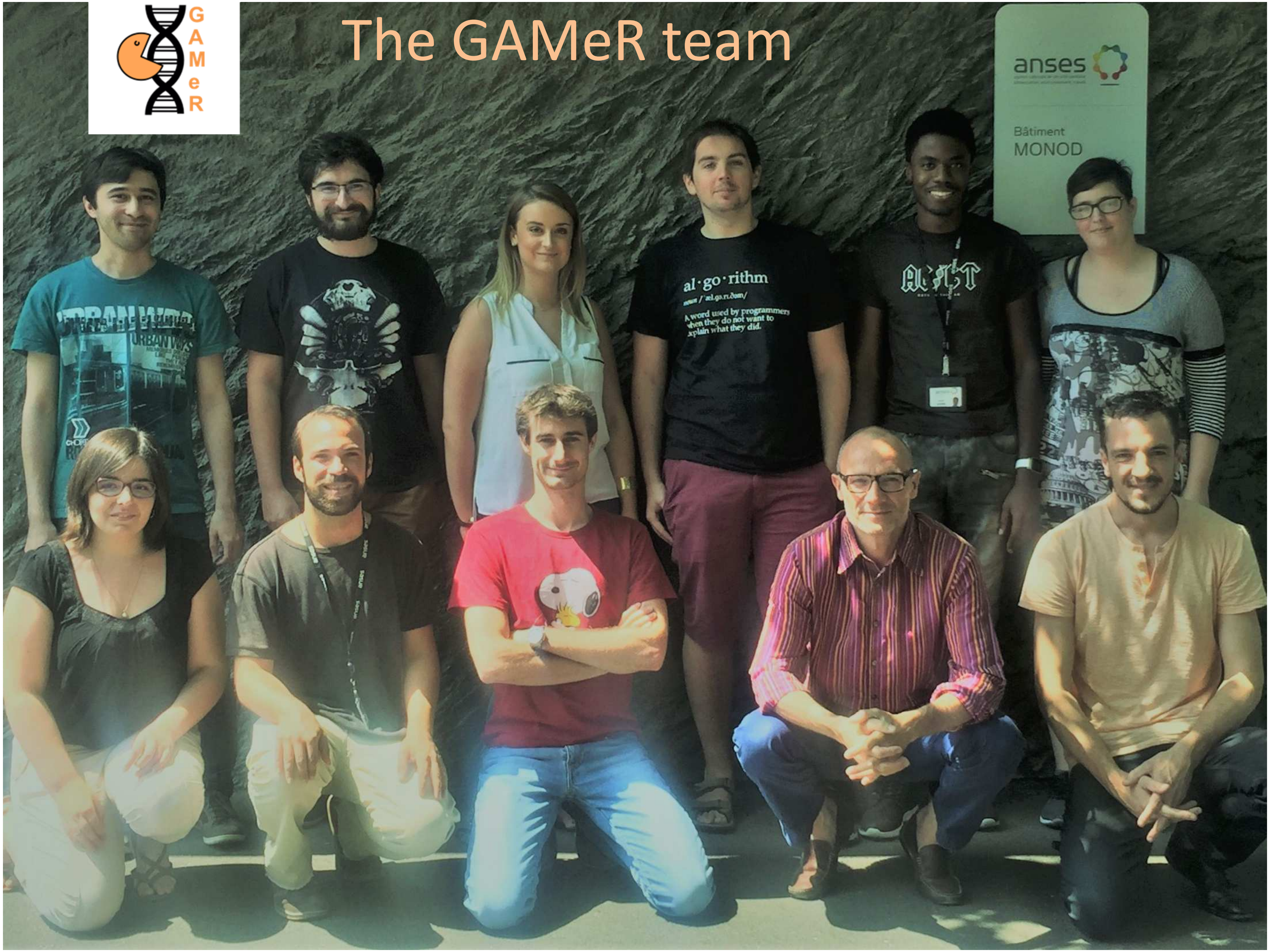
Creation of the Genome Analysis Modelisation and Risk Team (GAMeR)

1st jan 2016

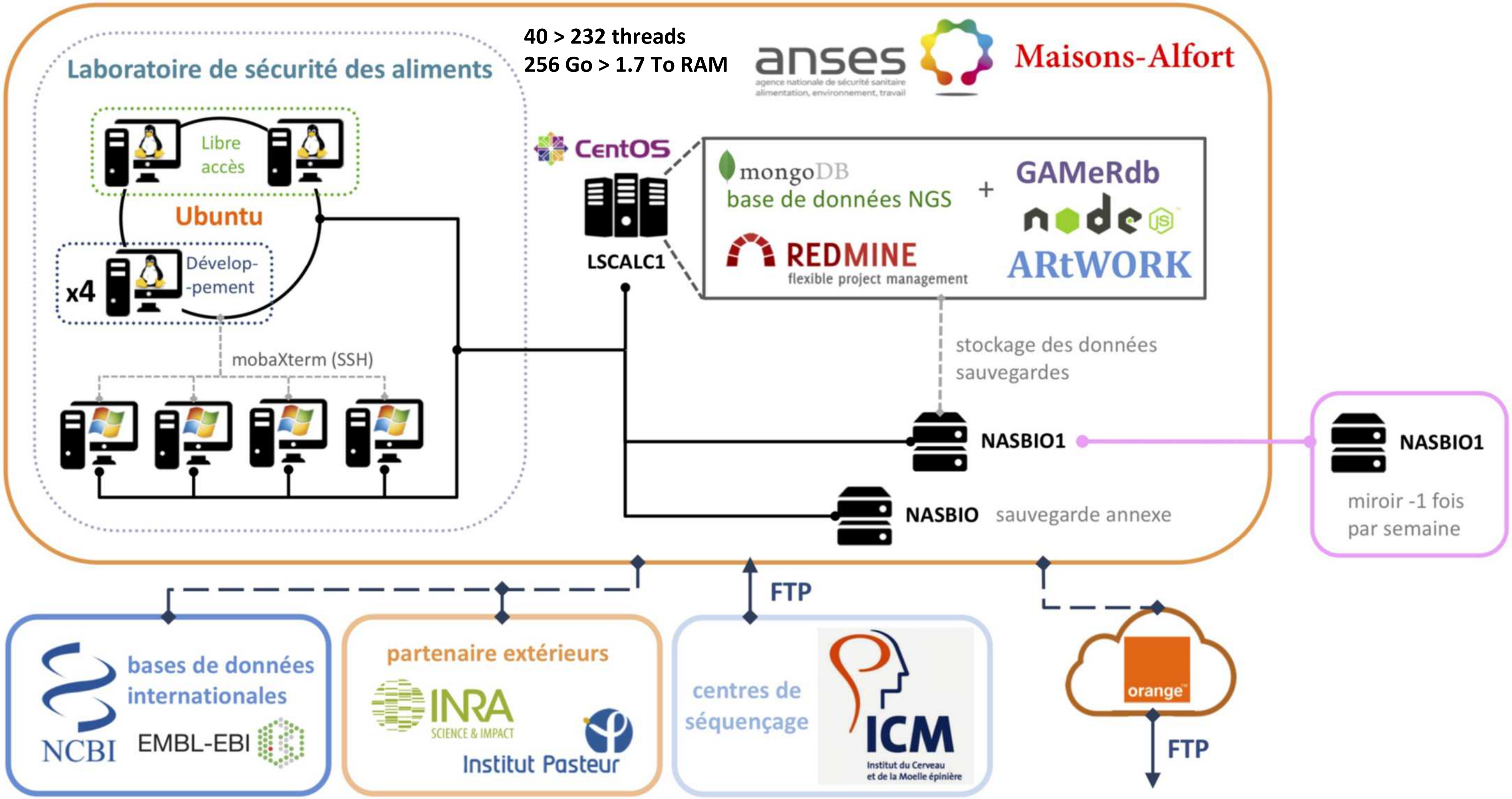
Objectives of the GAMeR Team:

- Develop the IT infrastructure
- Develop in-house data analysis pipelines
- Organize training sessions for the scientific staff
- Provide full Assistance to genomics projects in the FBP Dpt
- Scientific seminars / thematic days
- Research projects

The GAMeR team



Setup of a GNU/Linux based-environment



Laboratoire de sécurité des aliments

Libre accès

Ubuntu

x4

Dévelop-
-pement

mobaXterm (SSH)

40 > 232 threads
256 Go > 1.7 To RAM

anses
agence nationale de sécurité sanitaire
alimentation, environnement, travail

Maisons-Alfort

CentOS

LSCALC1

mongoDB
base de données NGS

+

GAMeRdb

node JS

REDMINE
flexible project management

ARtWORK

stockage des données sauvegardes

NASBIO1

NASBIO1
miroir -1 fois par semaine

NASBIO sauvegarde annexe

FTP

bases de données internationales

NCBI

EMBL-EBI

partenaire extérieurs

INRA
SCIENCE & IMPACT

Institut Pasteur

centres de séquençage

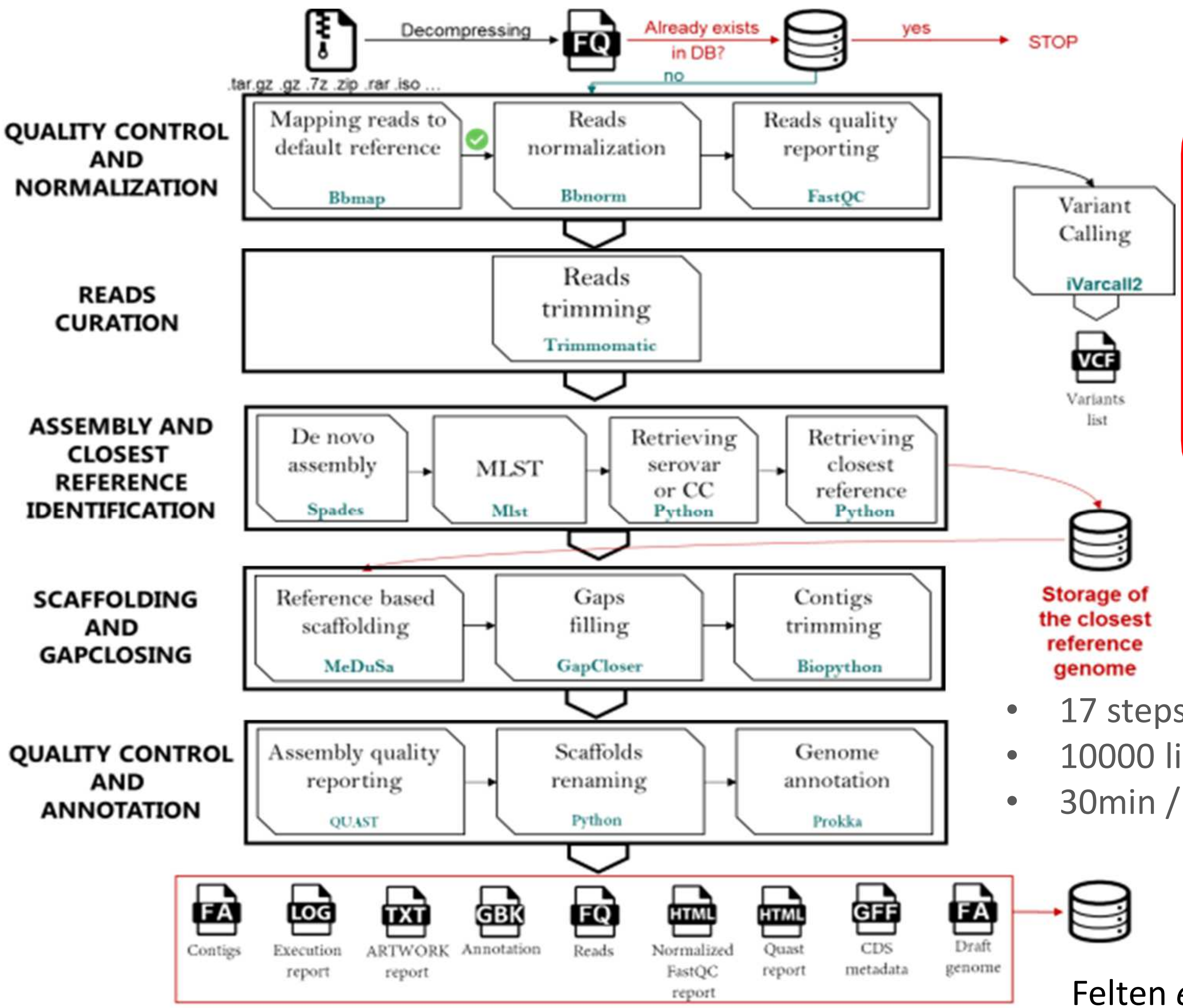
ICM
Institut du Cerveau et de la Moelle épinière

orange

FTP

- Training microbiologist and molecular biologists staff to command lines
- Easy access to calculation and storage resources

ARTwork pipeline: the entry door of WGS data



- **Data Quality control**
- **Data curation**
- **Variant calling**
- **Assembly**

(*de novo*, scaffolding, gap closing)

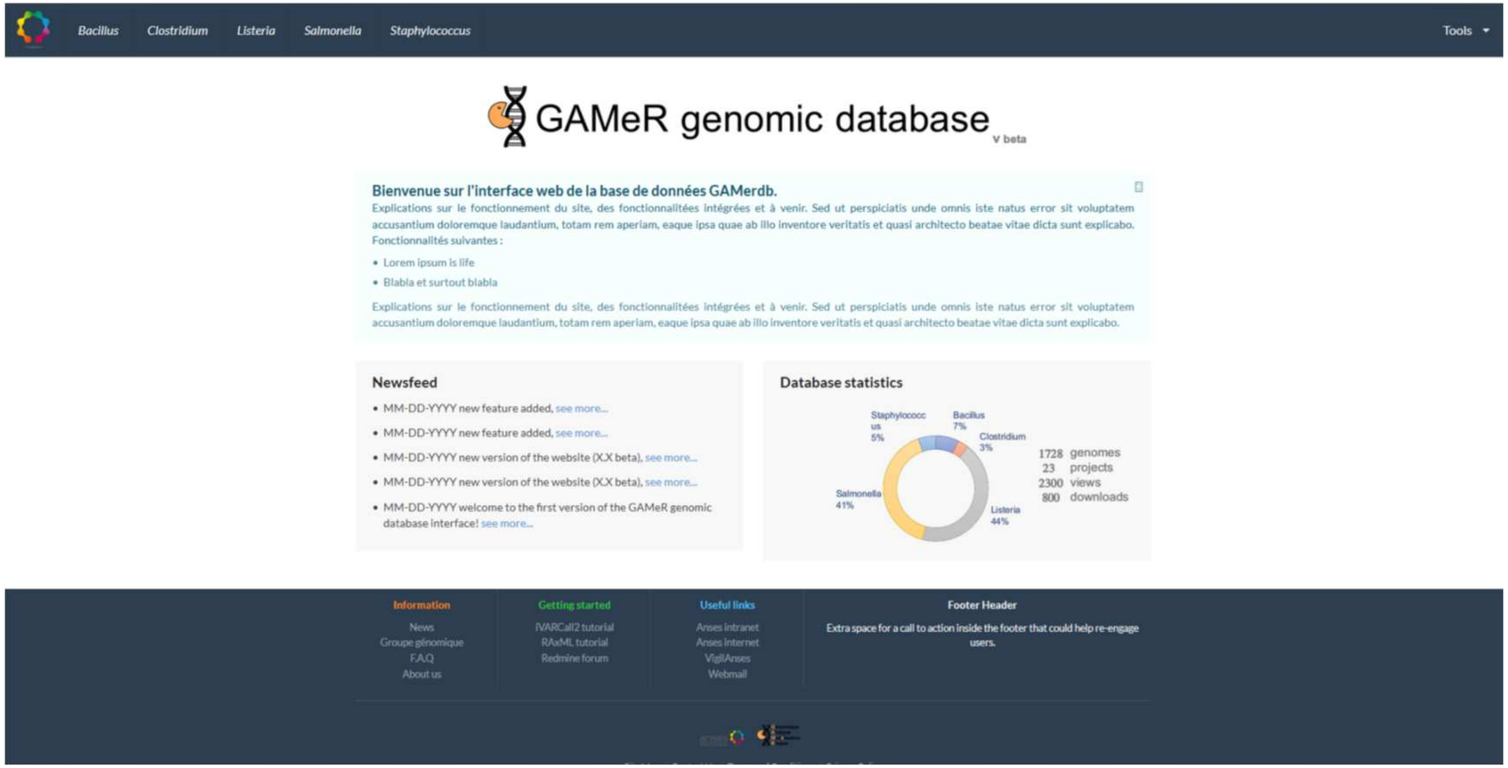
- **Typing (MLST)**
- **Annotation**

- 17 steps
- 10000 lines
- 30min / strain

Felten *et al, BMC microbiol., 2017*
Henri *et al., Frontiers microbiol., 2017*

Development of a local DB for easy and secure access to genomic data and analysis

- Storage / traceability
- Web interface for easy querying of genomic data and Artwrk analysis



Bienvenue sur l'interface web de la base de données GAMerdb.

Explications sur le fonctionnement du site, des fonctionnalitées intégrées et à venir. Sed ut perspiciatis unde omnis iste natus error sit voluptatem accusantium doloremque laudantium, totam rem aperiam, eaque ipsa quae ab illo inventore veritatis et quasi architecto beatae vitae dicta sunt explicabo.
Fonctionnalités suivantes :

- Lorem ipsum is life
- Blabla et surtout blabla

Explications sur le fonctionnement du site, des fonctionnalitées intégrées et à venir. Sed ut perspiciatis unde omnis iste natus error sit voluptatem accusantium doloremque laudantium, totam rem aperiam, eaque ipsa quae ab illo inventore veritatis et quasi architecto beatae vitae dicta sunt explicabo.

Newsfeed

- MM-DD-YYYY new feature added, see more...
- MM-DD-YYYY new feature added, see more...
- MM-DD-YYYY new version of the website (X.X beta), see more...
- MM-DD-YYYY new version of the website (X.X beta), see more...
- MM-DD-YYYY welcome to the first version of the GAMeR genomic database interface! see more...

Database statistics

1728	genomes
23	projects
2300	views
800	downloads

Information
News
Groupe génomique
F.A.Q
About us

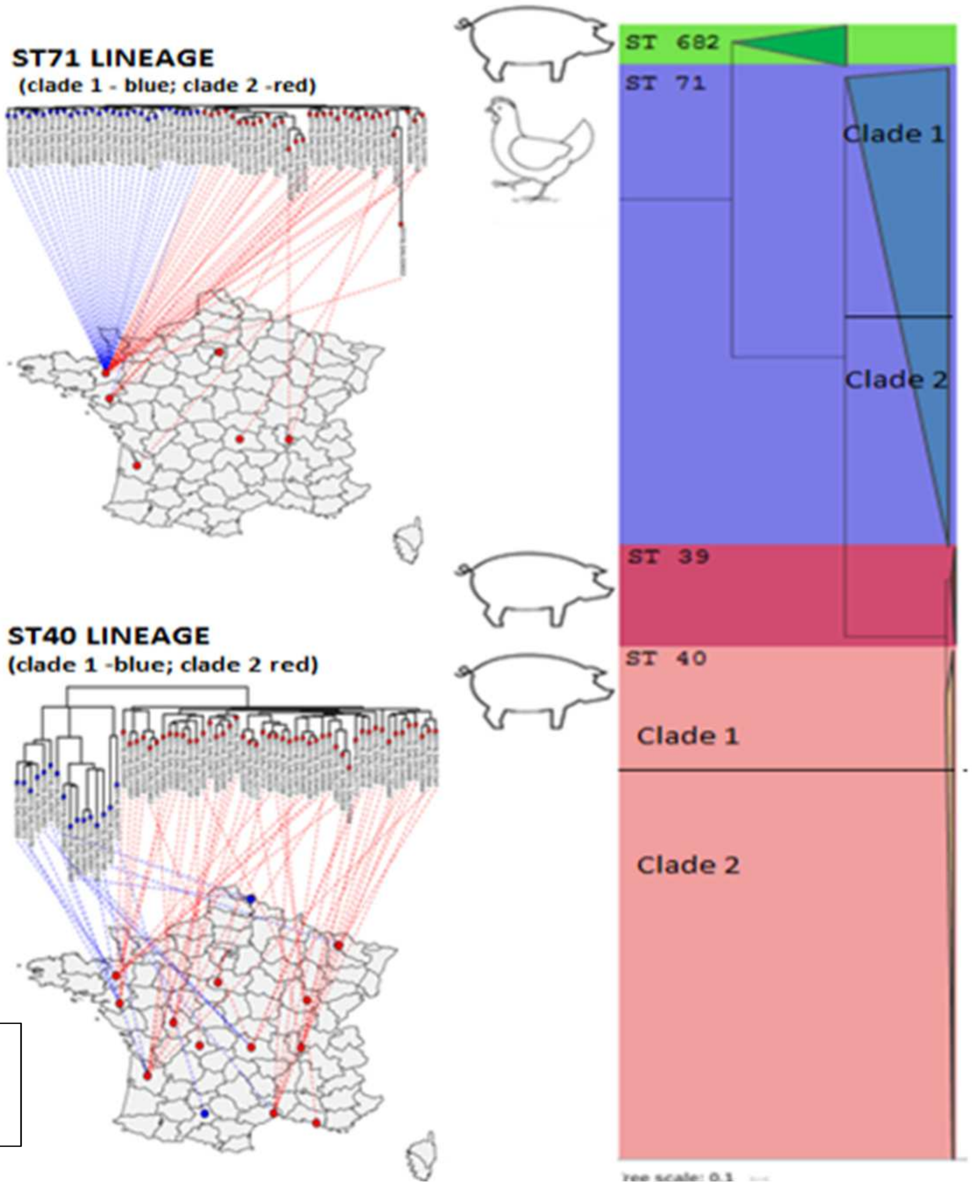
Getting started
iVARCall2 tutorial
RAxML tutorial
Redmine forum

Useful links
Anses intranet
Anses internet
VigilAnses
Webmail

Footer Header
Extra space for a call to action inside the footer that could help re-engage users.

Phylogenetic analysis of *Salmonella* Derby isolates

- Derby is among the Top 5 clinical serovar in France
- *S.* Derby 1st serovar isolated in pork meat in Europe
- Low discriminatory power (ID*: 0,789) of the PFGE



✓ Sequencing and analysis of 140 animal and food strains representative of the production sectors and geographical origins (France 2014-2015)

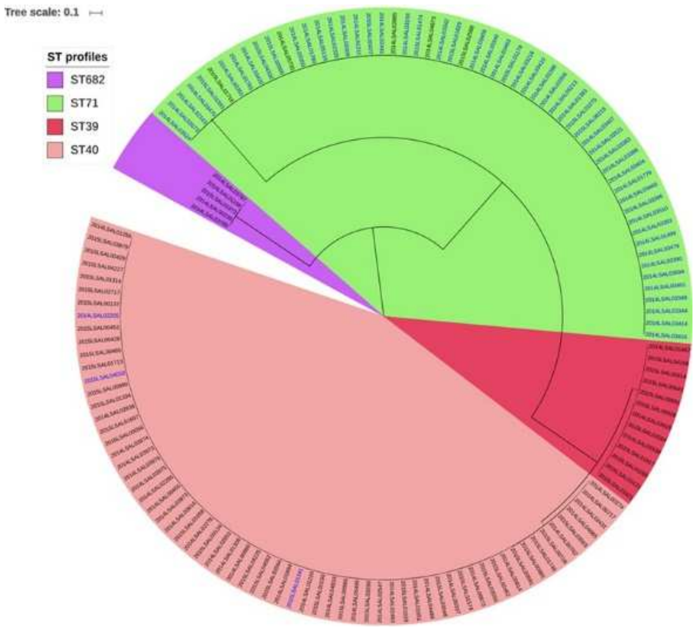
✓ **One serovar : 6 genetic clades !**

✓ **Host-clade specific associations**

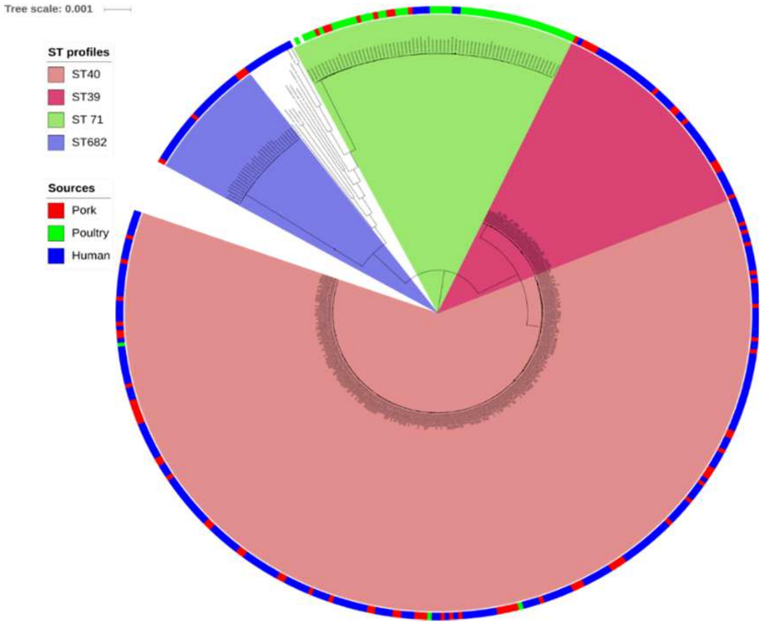
Sevellec et al., Frontiers microbiol., 2017
Sevellec et al., Genome announc., 2017

Clarification of the *Salmonella* derby epidemiological situation

Adding **302 human clinical** isolates into the picture (collab. NRC Salmonella Institut Pasteur)



Food/animal



Food/animal + human **(blue)**

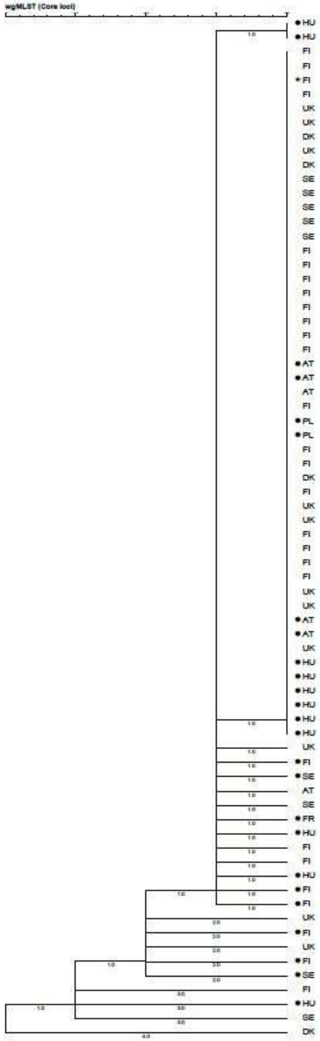
✓ **98% of the human isolates** are linked to the genetic clades associated to the **pork sector** : Actionable information !

Sevellec et al., submitted

FBO investigation at the european level

July 2018 - Multi-country outbreak of *L. monocytogenes* ST6
47 human cases, 9 deaths

- The EURL interviewed its NRL network, selected, received, sequenced and analyzed 29 isolates from 6 member states.
- Strong microbiological links between human and non-human isolates (0-7 allelic differences).
- Epidemiological and WGS analysis point to a common source related to frozen corn contaminated in a hungarian freezing plant whose activities have been halted.



ecdc
EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL

efsa
European Food Safety Authority

anses
agence nationale de sécurité sanitaire alimentation, environnement, travail
Connaître, évaluer, protéger
EURL *Listeria monocytogenes*


efsa
European Food Safety Authority
ecdc

JOINT ECDC–EFSA RAPID OUTBREAK ASSESSMENT

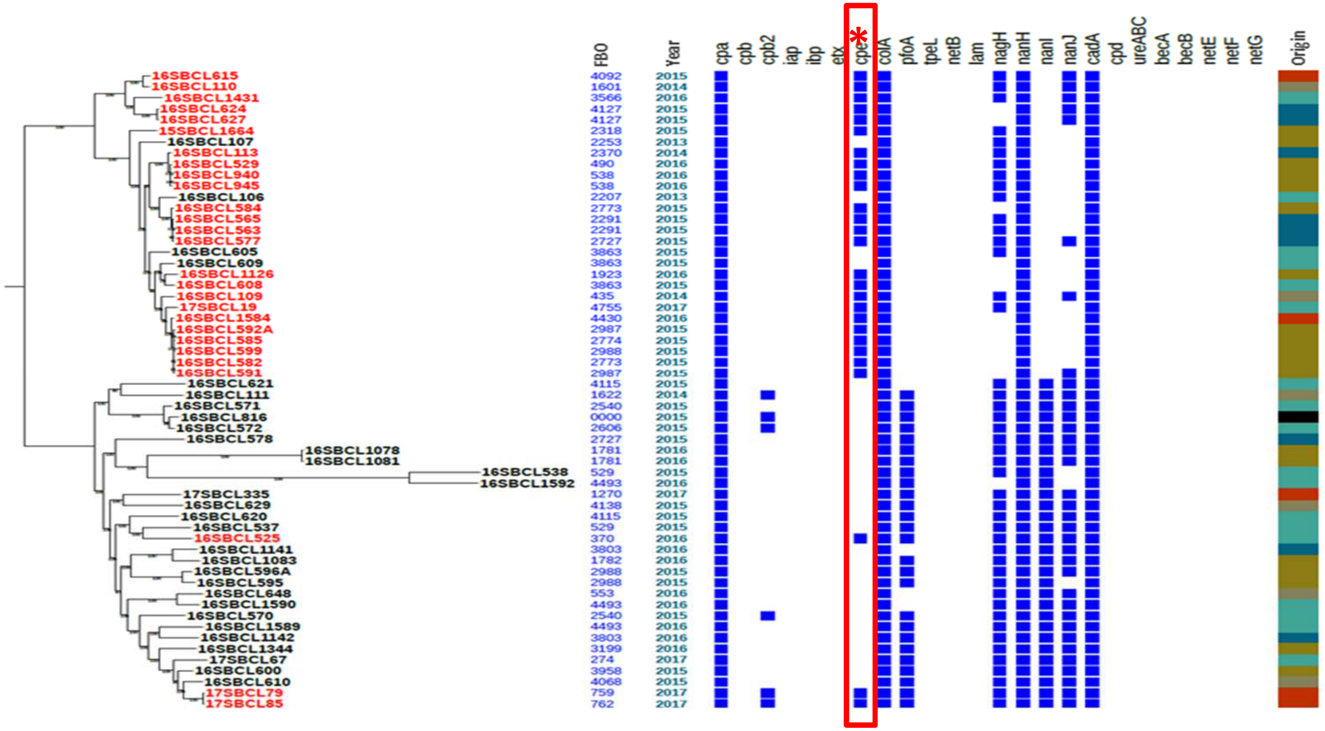
Multi-country outbreak of *Listeria monocytogenes* serogroup IVb, multi-locus sequence type 6, infections linked to frozen corn and possibly to other frozen vegetables – first update

3 July 2018

Large-scale Genomic Analyses and Toxinotyping of *Clostridium perfringens* Implicated in Foodborne Outbreaks in France

- 15,000 humans cases in EU (EFSA,2016)
- Ranks 4th in toxin producing bacteria associated with FPO
- Genetic diversity of *C. perfringens* is poorly characterized
- No typing methods relevant for FBO investigation is available

✓ **Genome analysis of 141 isolates involved in 42 French FBOs**



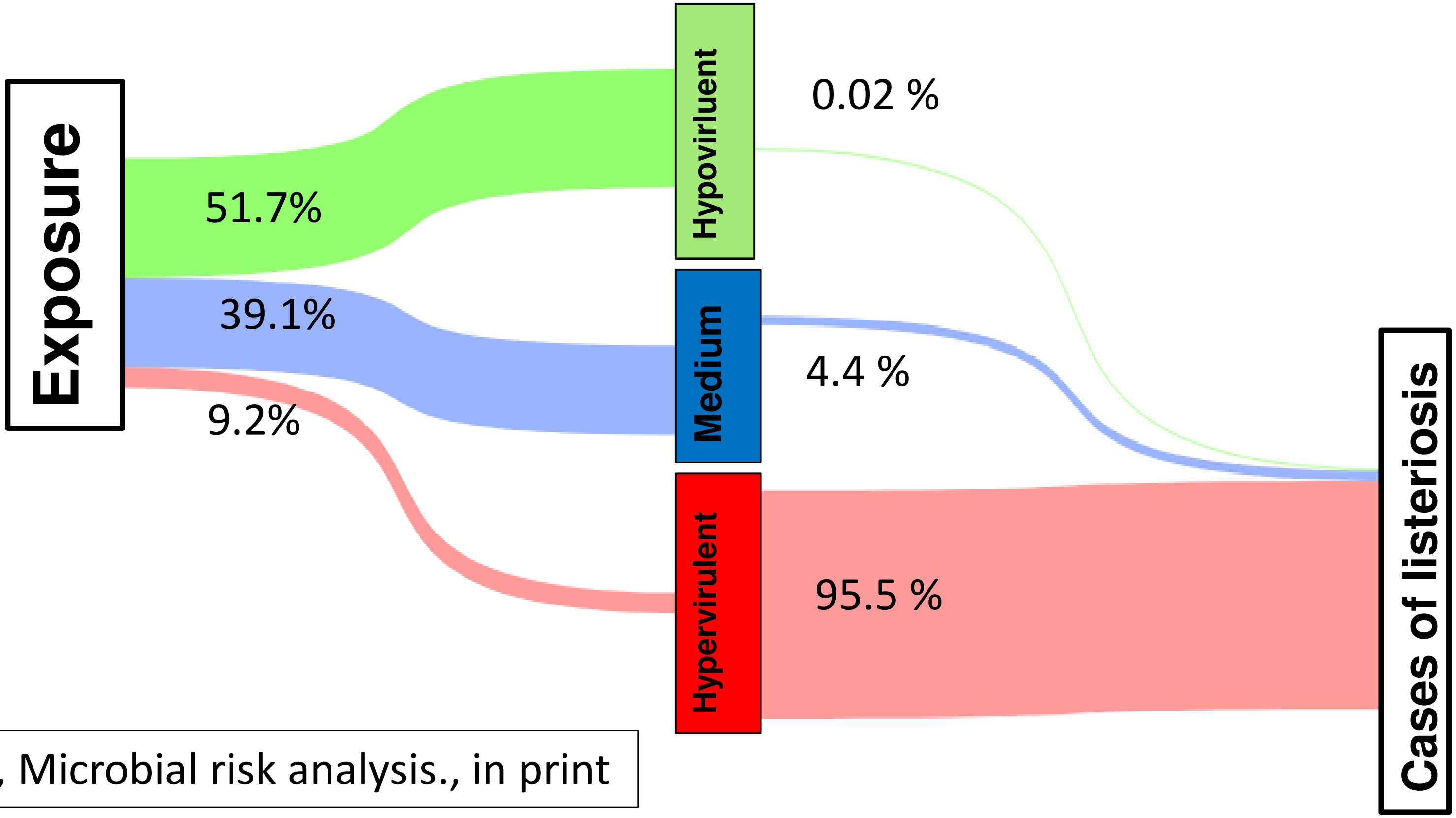
Genomic analysis revealed new features for *C. perfringens* food poisoning

- Heterogeneous contamination (multiple isolates) of food *C. perfringens* is frequent 25%
- Food poisoning is not always associated with the presence of the *cpe* gene
- An unexpected diversity of *cpe* loci – Impact on toxin production ?
- Development of a multiplex PCR-method targeting 19 toxin genes for rapid typing of isolates

Abdelrahim et al., Frontiers microbiol., in print

Feeding QMRA with genomics data

- Risk assessments neglects within-species heterogeneity in microbial virulence
- Genomics offer the discriminatory power to distinguish pathotypes



Fritsch et al., Microbial risk analysis., in print

Others on going WGS projects

H2O2O, COMPARE project (DTU Food coordination), 20 M€

- Phylogenetic-independent approach to FBO investigation
- Microevolution of *Salmonella* monophasic variants (APHA, DTU)

H2O2O, EJP OneHealth project, 90 M€ (Anses coordination)

- **RADAR** project 12,000 Plasmids curated DB construction (RIVM coord.)
- **Listadapt** Comparative genomic analysis of 1000 Lmo isolates from environmental sources

- ***Bacillus thuringiensis*** **hazard** in **organic food** products

(Under coordination of the ANSES regulated products division)

- **Genomic-based design of genetic markers for *Salmonella* serotyping –patent pending**

Arnaud FELTEN, Nicolas RADOMSKI, Sabrina CADEL-SIX, Patrick FACH

People involved

- **Multi-country outbreak Listeria monocytogenes – Salmonella & Listeria Unit**

Benjamin FELIX (EURL *Listeria monocytogenes*), Jean-Charles LEBLANC, David ALBERT

- **DerbyClone project - Salmonella & Listeria Unit**

Yann Sevellec (PhD), Sabrina CADEL-SIX, Nicolas Radmoski, Arnaud FELTEN
Financed by the ministry of agriculture

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DE L'AGROALIMENTAIRE
ET DE LA FORÊT

- **Clostridium *perfringens* genomic characterization – Toxin producing bacteria Unit**

Abdelrahim ABABAKAR (PhD), O. FIRMESSE, N. RADOMSKI, JA Hennekinne
Financed by the ministry of agriculture

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- **MQRA *L. monocytogenes – Feeding MQRA models with genomics data***

Lena Fritsch (PhD), Laurent Guillier, Jean-Christophe AUGUSTIN

Aknowledgments

- French Ministry of Agriculture
- DG Santé EURL SCP and *Listeria* Mandates
- EU commission Horizon 2020 program COMPARE
- EURLs Laboratoty Networks
- French Ministry of Research ANR
- Pasteur Institute Salmonella CNR, Pasteur Institute Collection
- DTU
- Technical institutes supporting livestock sectors

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Institut Pasteur

INRA
SCIENCE & IMPACT

AGENCE NATIONALE DE LA RECHERCHE
ANR

One HEALTH EJP

European Commission
Directorate-General for Health and Food Safety (DG SANTE)

European Commission
Horizon 2020
European Union funding
for Research & Innovation

compare

DTU
Technical University
of Denmark

efsa
European Food Safety Authority

A last message

"Impact of whole genome sequencing of foodborne pathogens on public health protection"

Joint scientific conference organized by

The French Agency for food, environmental and occupational health & safety (ANSES) ; The Federal risk assessment institute (BfR), Germany ; The Danish Technical University National Food institute (DTUFood) ; The National Institute of food and drug safety evaluation (NIFDS), Korea.

Paris, 26-28 March 2019

https://www.anses.fr/en/content/joint-scientific-conference-ansesbfrdtufoodnifds

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Investigate, evaluate, protect

DTU Technical University of Denmark

BfR
Bundesinstitut für Risikobewertung

MINISTRY OF FOOD AND DRUG SAFETY
National Institute of Food and Drug Safety Evaluation

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