LISTADAPT

Adaptive traits of *Listeria monocytogenes* to its diverse ecological niches

One HEALTH EJP

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WHAT CAUSES LISTERIOSIS AND WHY IS IT A ONE HEALTH ISSUE?

Zoonoses are diseases that can be transmitted between animals and human, directly or indirectly. Foodborne zoonotic disease are caused by the consumption of food products contaminated with micro-organisms, such as bacteria, viruses and parasites, capable of causing infections (European Food Safety Authority EFSA Factsheet foodborne zoonosis, EFSA topics foodborne zoonotic diseases 2022).

In the European Union, over 350,000 human cases of foodborne zoonotic diseases are reported each year, the most common causes being the bacteria *Campylobacter spp.*, *Salmonella spp.*, *Yersinia spp.*, pathogenic *Escherichia coli* and *Listeria monocytogenes* (EFSA topics foodborne zoonotic diseases 2022). *Listeria monocytogenes* (*Lm*) cause listeriosis, a disease that is often severe with high hospitalisation and mortality rates (EFSA Topics – Listeria). *Lm* tolerate salt and can multiply at low temperature, making it able to contaminate many foods, even in food processing environment. High risk foods include ready-to-eat meat and fish products, unpasteurised dairy products and raw vegetables (Listeriosis - WHO Factsheet, EFSA Topics – Listeria).

Lm can be found in various ecological niches, such as soil, water, vegetation and animals' faeces, from both the natural and farm environments (Listeriosis - WHO Factsheet). Soil, in particular, is a primary ecological niche for *Lm* and may thus be important in its transmission from natural/farm environment to food and food-processing environment. Farm animals are also an additional important reservoir for *Lm* and contribute to contamination of the farm environment through fecal shedding. The genetic background of *Lm* is very diverse. Classification of *Lm* (using Multilocus sequence typing (MLST)) into clonal complexes (CCs) and sequence types (STs), is used to describe the *Lm* population structure. Certain clonal complexes (CCs) are overrepresented in food production and represent a challenge to food safety. Many studies compared the genomic background of the *Lm* strains between food, food processing environments and sporadic human cases but little is known on the genetic make up to strains isolated from animals and environment.

The scientific community working on listeriosis would benefit of an investigation of the genetic background of Lm strains found in animals and in the environment and comparison to the food strains' one, to provide a better understanding of the *Lm* transmission routes from the farm/natural environment to processing environments.

The One Health EJP uses its unique position to facilitate a collaborative approach between institutes to deliver important multisectoral research, and attain optimal health and wellbeing outcomes for humans, animals, and the environment. We bring together 43 acclaimed European scientific institutes and the Med-Vet-Net Association working together on 47 research projects to address potential and existing risks that originate at the animal-human -environment interface.
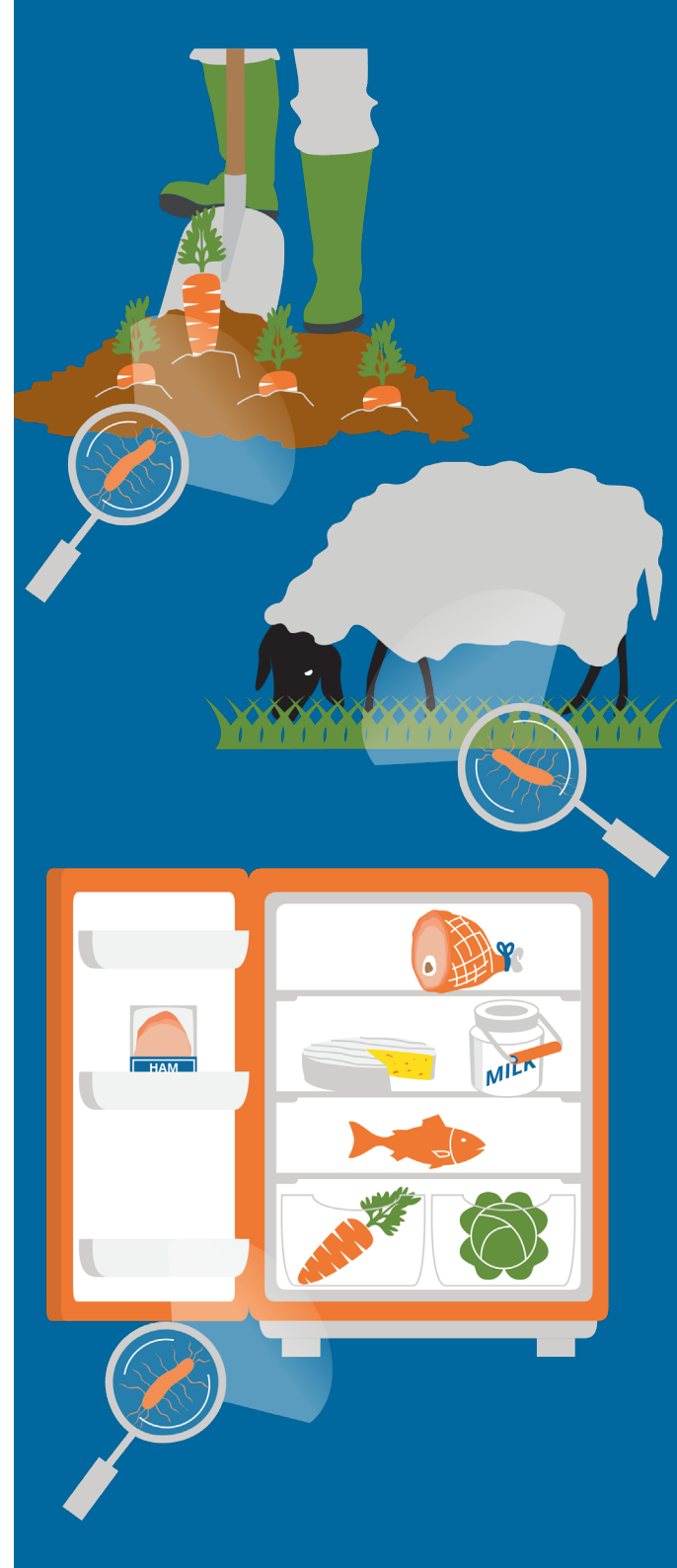
WHAT IS THE LISTADAPT PROJECT?

The OHEJP LISTADAPT project involves 7 partners across Europe: the French Agency for Food, Environmental and Occupational Health & Safety (ANSES) and the French National Research Institute for Agriculture, Food and Environment (INRAe), the Austrian Agency for Health and Food Safety (AGES), the Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZ AM) in Italy, the National Food Institute (DTU) in Denmark, the National Food Agency (NFA) in Sweden and the Norwegian Veterinary Institute (NVI) in Norway.

With these partnerships between human, animal and plant health, food safety, and environmental protection institutes, the LISTADAPT project was designed to compare the genetic background of *Lm* strains found in animals and in the environment with the food strains' one, to gain new understanding of *Lm* adaptation mechanisms in food and provide a better understanding of the genetic population structure of *Lm* strains.

At the start of the project, several important knowledge gaps were identified, including:
- The poor understanding of the ecology of *Lm*. Some strains pose a serious challenge to the food industry as they are overrepresented in all food sectors and can persist in different food processing plants for years. Interestingly, the clonal complexes that are the most commonly found in food and food-processing environments, posing a serious challenge in food industry, are rarely reported in animals or natural/farm environments.
- The limited number of *Lm* strains available from natural environment and wildlife and the little knowledge on the genetic make-up of these strains.
- The sparse knowledge on (i) how strains become part the food chain, (ii) the routes of transmission of strains from outdoor environments and animals to food.

The LISTADAPT project aimed to address these knowledge gaps by:
- Focusing on a large and diverse collection of *Lm* strains isolated from farming/wild animals and farming/wild environment in different European countries.
- Looking for molecular markers of interest such as mobile genetic elements or genes underlying survival in soil and/or adaptation in food processing environment.
- Combining phenotypic and genotypic data to investigate the behaviour and evolution of *Lm* strains.

The LISTADAPT project enhanced links with National and European veterinary and environmental laboratories groups, by collecting many strains. LISTADAPT made possible to promote inter sectorial research and develop new collaborative studies. By helping to assess the true importance of the environmental and animal strains as sources of foodborne infections for public health, the LISTADAPT project will make it possible to assess the relevance of monitoring plans, in agricultural soils, in farming animals and in wildlife.

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LISADAPT PROJECT OUTCOMES

The key aims of the OHEJP LISTADAPT project were to collate a large dataset of *Lm* genomes from diverse ecological niches and phenotypic data from a panel of Lm strains, to improve understanding of the *Lm* population structure and the *Lm* evolutionary history, facilitate the detection of the emerging *Lm* clones and identify genetic traits related to the adaptation of *Lm* to particular ecological niches.

To achieve the LISTADAPT first goal, 22 partners, including food, environment, veterinary and public health laboratories, collated a dataset of 1,575 high-quality draft genomes originating from *Lm* strains collected in 20 European countries. The constructed dataset covers a wide genetic diversity of *Lm* since it includes about 80 different clonal complexes (CCs) including the most prevalent CCs in Europe and worldwide. These CCs were collected from natural environment (wild animals and natural environment), primary production (farm environment and farm animals with or without listeriosis symptoms) until Food Processing Environment and food products.

All the produced genomes in LISTADAPT are available to the scientific community (umbrella *Bioproject* submitted to European Nucleotide Archive (ENA)), they are also reported in deliverable D-JRP7-1.4: Report on strain collection and strategy for selection of strains for sequencing and in the following research article: Félix, B., Sevellec, Y., Palma, F. *et al*. (2022), A European-wide dataset to uncover adaptive traits of *Listeria monocytogenes* to diverse ecological niches. Sci Data 9, 190 https://doi.org/10.1038/s41597-022-01278-6. The dataset will contribute to improve to a better understanding of the ecology of *Lm* by identifying new markers conferring the potential to colonize the different ecological niches and provides a basis for the discovery of the genetic traits underlying *Lm* transmission routes from the farm/natural environment to food and food processing environment. It may also help to assess the importance of animal and food strains for human infection.

Such genetic traits could be of use for EFSA and ECDC to control the risks related to *Lm*, to design control strategies and then to prevent the recurrence of diseases which are both social and economic burden to the European society. The food, environment, veterinary and public health laboratories can use the 1,575 genomes to predict phenotypic traits of strains, by using bioinformatics methods and/or to uncover new genes or new mechanisms related to virulence of clinical and animal strains.

The LISTADAPT project made it possible to strengthen the collaboration between the consortium and a team of DTU (Danish Technical University) that will use the 1,575 *Lm* genomes and metadata to develop a tool predicting the resistance of *Lm* strains to biocides used in food industry. The genomes will also be used in an important research project between INRAE and Anses, that aims to develop tools for the detection and quantification of persistent intracellular *Lm*. This new project aims to progress the knowledge of this phenomenon's biology, on the mechanisms leading to asymptomatic carriage in hosts, and to propose diagnostic, therapeutic and preventive solutions for at-risk human populations.

Researchers from the LISTADPT project also collected phenotypic data, such as resistance to antibiotics, effects of biocides, bacterial adhesion and biofilm formation, survival and persistence in different ecological niches on a smaller panel of 200 *Lm* strains. The 200 strains panel has been selected to be balanced between reservoirs, sub-reservoirs, sampling area and CCs with high and low degree of genetic relatedness.

The obtained phenotypic data are centralised in a database that will soon be made accessible to the scientific community in the coming months and were reported in deliverables D-JRP7-3.1, 3.2, 3. 3, 3. 4, 3.5, 3.6-Phenotypic data of 200 strains isolated from food, animals and environment. The phenotypic data will be compiled with all the genomic data for a mixomics approach. This data would bring new information on the behaviour and evolution of *Lm* strains outside the human host and its the ability of to colonise different environments and has the potential to help anticipate the emergence of new genetic types. This phenotypical data will be valuable for EURL activities such as selection of strains for challenge testing of low pH foods, and for risk assessments of foods with preservatives. Further, these results can be used to assess the validity of predictive models that have been developed with few strains for strains with other genetic characteristics.

In addition, ADEPALE, a professional federation of the French food industry, is very interested in the results; to improve their understanding of the adaptation mechanisms of the strains in the plant chain from the soil to the finished plant products and wish to set up a research collaborative project with ANSES on this subject.

SCIENTIFIC PUBLICATIONS

The LISTADAPT project worked towards understanding of the Lm population structure, identifying genetic traits related to the adaptation of Lm to particular ecological niches and facilitating the detection of the emerging *Lm* clones. The research conducted in this project led to the publication of research articles, that can benefit to the whole scientific community.

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Palma F, Radomski N, Guérin A, Sévellec Y, Félix B, Bridier A, Soumet C, Roussel S, Guillier L. 2022. Genomics elements located in the accessory repertoire drive the adaptation to biocides in *Listeria monocytogenes* strains from different ecological niches. *Food Microbiology* 2021-02 DOI: 10.1016/j.fm.2021.103757

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MEET THE TEAM

Each of the One Health EJP projects creates a unique Europewide network of institutes with wide-ranging expertise to achieve objectives using a cohesive One Health approach. We bring together scientific communities with expertise in medical, veterinary, and environmental health, and use integrated approaches to solve complex global issues.

The LISTADAPT project worked collaboratively to achieve its aims and to produce impactful scientific outcomes which may benefit scientists and policy-makers worldwide.

The One Health EJP members of this project were from the following institutes:
- French Agency for Food, Environmental and Occupational Health & Safety (ANSES)
- French National Research Institute for Agriculture, Food and Environment (INRAe)
- Austrian Agency for Health and Food Safety (AGES)
- Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale" (IZ AM), Italy
- National Food Institute (DTU), Denmark
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