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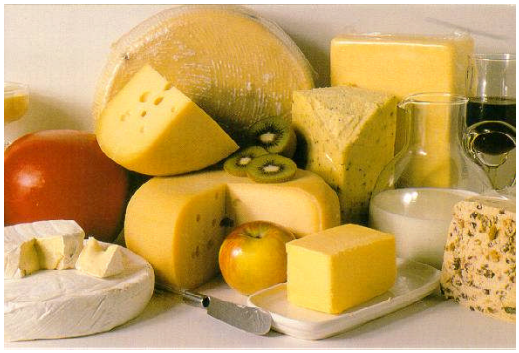
Improved bio-traceability of unintended micro-organisms and their substances in food and feed chains



Key external stakeholders:
EU – the funding agency
Researchers
Irish Farmhouse Cheesemakers Association
Food Safety Authority of Ireland

Practical implications for stakeholders:
This research has had an impact as follows:

- *Listeria monocytogenes* occurrence on farms was about 19%, indicating that cross contamination could occur on farms
- Using predictive modelling for determination of growth of *L. monocytogenes* in food is not always accurate
- Similar strains of *L. monocytogenes* can be isolated from multiple food chains
- Of 109 raw milk samples tested, 6% contained *L. monocytogenes*
- The results suggest that the farm environment external to the processing environment may in some cases be the source of processing environment contamination with *L. monocytogenes.*
- The data obtained contributes to a better understanding of the potential risk that *L. monocytogenes* presents to cheese producers (growth on the product, if it is contaminated) and constitutes a very useful set of data for further modelling studies in food.
- Persistent strains of *L. monocytogenes,* that are more difficult to control, were identified in some processing environments

Main results:

- Sixteen cheesemaking facilities were sampled during the production season at monthly intervals over a one-year period. Thirteen facilities were found to have samples positive for *L. monocytogenes* on at least one occasion
- 19% of samples at farm level were positive for *L. monocytogenes*
- This study demonstrates the prevalence of *L. monocytogenes* in the dairy farm and processing environments and the need for good hygiene practices to prevent its entry into the food chain
- Predictive modeling is not always applicable to food

Opportunity / Benefit:

- Contamination of food processing facilities (not food) was shown. There is an opportunity to use this pre-emptive knowledge to improve hygiene at processing facilities and prevent future issues with food contamination
- Predictive modeling is not always applicable to food – challenge studies are necessary
- A database of pulsed field gel electrophoresis (PFGE) profiles of *L. monocytogenes* isolates from Ireland was generated

Collaborating Institutions:

Principally the Danish Technical University, Copenhagen and the University of Veterinary Medicine, Vienna. There were 45 other participants in the project.

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Teagasc project team: Kieran Jordan, Edward Fox, Sol Schvartzman
External collaborators: Danish Technical University, Copenhagen and the University of Veterinary Medicine, Vienna.
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1. Project background:

L. monocytogenes is a foodborne bacteria responsible for the disease listeriosis. Although listeriosis is uncommon, the mortality associated with it is 20-30%. *L. monocytogenes* occurs widely in the environment so elimination of it is an unrealistic aim. Awareness of its occurrence and routes of transmission are essential in its control, particularly with respect to strains that persist in processing environments. PFGE is a valuable tool in tracing routes of contamination and in comparing isolates obtained from different sources. Regulations in the EU allow up to 100 cfu/ml (g) in foods that cannot support growth of the organism. Therefore, knowledge on the ability of foods to support growth of *L. monocytogenes* and the factors affecting such growth is essential.

2. Questions addressed by the project:

- What is the occurrence of *L. monocytogenes* in cheese processing facilities and on farms?
- What are the sources and putative transfer routes of *L. monocytogenes*?
- Is there a difference in growth of *L. monocytogenes* in raw and pasteurised milk?
- Can models of growth that are generated in laboratory media be used to describe growth in food?
- Are there similar strains of *L. monocytogenes* isolated from different sources?

3. The experimental studies:

- Farms and cheese processing facilities were sampled for *L. monocytogenes*
- Mathematical models to describe the growth of *L. monocytogenes* on the surface of cheese was used
- The difference between growth of *L. monocytogenes* in pasteurised and unpasteurised milk cheese was studied
- PFGE profiles from *L. monocytogenes* isolates from different sources in Ireland were determined establishing a national database of *L. monocytogenes* profiles.

4. Main results:

L. monocytogenes in the farm and processing environments

This study aimed to determine the occurrence of *L. monocytogenes* in 1) the Irish dairy farm environment and 2) the cheese processing environment.

1) Two hundred ninety-eight environmental samples were collected from 16 farms in the southern region of Ireland. A number of farms within the group supply raw milk to the unpasteurized milk cheese industry. The samples taken included cow faeces, milk, silage, soil, water, etc. Samples were enriched in *Listeria* enrichment broth and incubated for 48 h, followed by plating on chromogenic agar Listeria Ottavani & Agosti and further incubation of the plates for 24 to 48 h. Presumptive *L. monocytogenes* isolates were purified and confirmed by PCR targeting the *hly* gene. And 51 isolates were compared using PFGE. Overall, 19% of the samples (57 of 298) were positive for *L. monocytogenes.* These were serotyped using conventional and PCR methods; serotypes 1/2a, 1/2b, and 4b made up 78% of the typeable isolates. A correlation was found between the level of hygiene standards on the farm and the occurrence of *L. monocytogenes.* There was little difference in the occurrence of *L. monocytogenes* between farms supplying milk to the unpasteurized milk cheese industry and those supplying milk for processing. From the 51 isolates examined by PFGE, there were 40 individual PFGE types. Four of the PFGE types were common to multiple farms, and five farms had isolates with indistinguishable PFGE types in multiple locations on the farm. Indistinguishable PFGE types were common to multiple farms in different geographical locations up to 200km apart. The results indicate multiple niches for the organism in the dairy farm environment. The presence of *L. monocytogenes* in samples related to animals other than cattle indicates that there are multiple possible vectors of contamination. The farm environment harbors a diverse collection of *L. monocytogenes* isolates that must be considered as possible agents of food contamination.

2) Sixteen cheesemaking facilities were sampled during the production season at monthly intervals over a one year period. Samples were divided into 4 categories; cheese, raw milk, processing environment and external to the processing environment (samples from the farm such as silage, bedding, and pooled water).

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In order to attempt to identify the source, persistence and putative transfer routes of contamination with the *L. monocytogenes* isolates, they were differentiated using PFGE and serotyping. Thirteen facilities were found to have samples positive for *L. monocytogenes*. Of the 250 isolates, there were 52 different pulsotypes. No pulsotype was found at more than one facility. Two facilities had persistent pulsotypes that were isolated on sampling occasions at least 6 months apart. Of the samples tested, 6.3% of milk, 13.1% of processing environment and 12.3% of samples external to the processing environment, respectively, were positive for *L. monocytogenes*. Pulsotypes found in raw milk were also found in the processing environment and on only one occasion one of the pulsotypes from raw milk was also found in cheese. One of the pulsotypes isolated from the environment external to the processing facility was found on the surface of cheese, however, a number of them were found in the processing environment. The results suggest that the farm environment external to the processing environment may in some cases be the source of processing environment contamination with *L. monocytogenes*.

This study demonstrates the prevalence of *L. monocytogenes* in the dairy farm and processing environments and the need for good hygiene practices to prevent its entry into the food chain.

Strain comparisons

A retrospective analysis of isolates of 222 *L. monocytogenes* strains from human and non-human sources in Ireland was undertaken by PFGE. Human clinical isolates from other countries were also examined. Eight small clusters of human and non-human isolates (mostly serotype 4b) that were indistinguishable from one another were detected, suggesting potential sources for human infection. For non-human isolates, some PFGE types appeared to be exclusively associated with a single source, whereas other PFGE-types appeared to be more widely disseminated. Indistinguishable, or highly related clusters of isolates of Irish and non-Irish origin suggest that some PFGE patterns may be globally distributed.

Modelling growth of *L. monocytogenes*

The dynamics of the physicochemical characteristics of foods help to determine if growth of pathogens will occur in the food. The aim of this work was to determine if growth initiation varied between a cheesemaking, milk and tryptic soy broth (TSB). Growth of a two-strain mix of *L. monocytogenes* at combinations of four initial pH values and five water activity (a_w) values was determined in cheese, milk and TSB. Each condition was repeated six times, and growth initiation probability was modelled with logistic regression models. Growth initiation boundaries were obtained for each matrix type. The results showed that the growth limits were matrix dependent. In the three matrix types, a_w was the most important factor affecting the probability of growth initiation. The growth interface wideness and position in cheese, milk and TSB were dissimilar, indicating that the use of models evaluated in TSB or milk could not be used to predict the behaviour of *L. monocytogenes* under cheesemaking conditions. Predictive models generated in liquid media are not necessarily adaptable to solid food, and the generation of real food models is necessary.

No growth of *L. monocytogenes* occurred during raw milk cheesemaking, whereas growth did occur in pasteurised milk. During ripening, growth occurred in raw milk cheese, but inactivation occurred in pasteurised milk cheese. The behaviour observed for *L. monocytogenes* was modelled using a logistic primary model coupled with a secondary cardinal model, taking into account the effect of physicochemical conditions (temperature, pH, water activity and lactate). This complex model had an acceptable quality of fit on the experimental data. The estimated optimum growth rates can be used to predict the fate of *L. monocytogenes* during cheese manufacture in raw or pasteurized milk in different physicochemical conditions. The data obtained contributes to a better understanding of the potential risk that *L. monocytogenes* presents to cheese producers.

5. Opportunity/Benefit:

- Contamination of food processing facilities (not food) was shown. There is an opportunity to use this pre-emptive knowledge to improve hygiene at processing facilities and prevent future issues with food contamination

6. Dissemination:

Technology transfer to various audiences included:

- Scientific community: the project resulted in 9 papers in peer reviewed journals, 7 book chapters, 2 PhD theses, 14 conference presentations and 1 article in T-Research.
- Conference: a Conference on Listeria for science and industry was held 2009, 2010, 2011 and 2012.

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The data generated from this project was disseminated at these conferences.

Main publications:

1. Edward M. Fox, Niall deLappe, Patricia Garvey, Paul McKeown, Martin Cormican, Nola Leonard, and **Kieran Jordan.** 2012. Pulsed-field gel electrophoresis (PFGE) analysis of *Listeria monocytogenes* isolates of clinical, animal, food, and environmental origin from Ireland. Journal of Medical Microbiology, in Press.
2. Edward M. Fox, Nola Leonard and **Kieran Jordan.** 2011. Physiological and transcriptional characterisation of persistent and non-persistent *Listeria monocytogenes* isolates. Applied and Environmental Microbiology, 77, 6559-6569.
3. M.S. Schvartzman, A. Maffre, F. Tenenhaus-Aziza, M. Sanaa, F. Butler, **K. Jordan**. 2011. Modelling the fate of *Listeria monocytogenes* during manufacture and ripening of smeared cheese made with pasteurised or raw milk. International Journal of Food Microbiology, 145, S31–S38.

Popular publications:

1. Troubleshooting the environmental source of contamination in a small food manufacturing plant. **Kieran Jordan**, Karen Hunt and Edward Fox. 2012. Case studies in food safety and authenticity. J. Hoorfar. Published by Woodhead Publishing.
2. *Listeria monocytogenes* in milk, cheese and the dairy environment. Anthony D. Hitchins, **Kieran Jordan**, Martin Wagner and Moez Sanaa. 2011. In: Rapid detection, characterization and enumeration of food-borne pathogens, Chapter 18, pages 257-284. Editor: J. Hoorfar, ASM Press.
3. **Jordan**, K.N. and Burgess, K. (2011). How safe is our food? In: Oral Presentation at What's for Lunch? Conference, Brussels, 20-Sep-2011.

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