

RESEARCH HIGHLIGHT

EPIFOOD MOLECULAR EPIDEMIOLOGY OF ZOONOTIC BACTERIA IN THE FOOD CHAIN IN LUXEMBOURG

Too small to be seen with the naked eye, the planet's most abundant life form could nearly pass unnoticed, if not for its manifold effects on everyday life. Bacteria, tiny unicellular organisms, arose several billion years ago and have since then been a key factor for all other life on Earth. Indeed, bacteria run the most fundamental processes in our ecosystems: without them, there would be no carbon or phosphorous cycle as we know it and the nitrogen cycle would not even exist. Besides, if not for bacterial photosynthesis, scientists believe, the atmosphere would never have accumulated enough oxygen to allow the beginning of all other life forms.

But not all bacteria are benign – they can cause a variety of diseases, one example is the very prevalent bacterial gastroenteritis, also known as food poisoning. Four of the main culprits, and consequently the main subjects of the recently completed EPIFOOD study (Molecular Epidemiology of Zoonotic Bacteria in the Food Chain in Luxembourg), are the food-borne bacteria Salmonella, Escherichia coli, Campylobacter and Listeria. "Even now, we do not know exactly, how many people in Luxembourg are affected every year with bacterial gastroenteritis. Infections are, more often than not, not reported. But we do know it is a very common problem, especially in summer, when bacteria thrive in the warm temperatures", says Dr Joël Mossong, the project coordinator from the National Health Laboratory (Laboratoire National de la Santé - LNS). "In addition to this lack of human medical data we used to find

a big discrepancy between reported human infections and animal infections. For Salmonella, for example, a yearly average of 300-400 human cases was matched with only 10-20 bacteria isolated from meat in the food chain." The bacteria in question are all zoonotic meaning that they are transmitted from animals to humans. Pork, beef and chicken are common carriers; humans infect themselves by ingesting contaminated food, either by eating undercooked animal products themselves (for example raw meat or eggs) or through cross-contaminated food (for example by not sufficiently cleaning kitchen knives or cutting boards).

Out of the need for a better surveillance system arose the FNR-financed EPIFOOD project. Its aim was to locate and genetically fingerprint the bacterial strains present both in the food chain and in the human population in order to find source reservoirs and track common transmission pathways. Dr Catherine Ragimbeau, a molecular biologist specifically employed for this project and still with the LNS today, helped to set up the molecular methods applied for that purpose. "The techniques we use are comparable to those used in forensic science", says Mossong. "Except that our "criminals" are bacteria." The PFGE (Pulsed Field Gel Electrophoresis) method used in the LNS laboratory pinpoints the exact variant of a specific serovar of a specific bacteria. In contrast, the more commonly used method of real time PCR would only deliver a line-up of suspects, so to say. But sometimes even PFGE fails: some bacterial strains are too similar and PFGE cannot deliver conclusive evidence. In such cases, the EPIFOOD team dug deeper and used MLVA (Multi locus variable number tandem repeat analysis). More than 600 different strains were thus genotyped over the course of the project.











According to Mossong, the implementation of these sophisticated genotyping and detection techniques will be of huge benefit, if there ever is another epidemic such as the one in 2006. A good example illustrating the usefulness of such specific identification was the 2008 *Salmonella* Typhimurium outbreak in Denmark. The Danes made an international alert, following which the team at the LNS checked its MLVA bacterial profile library. Strangely enough, they stumbled upon a single case of a Luxembourg resident that had travelled to Denmark prior to becoming ill, had consumed a single food item at the airport and travelled back to Luxembourg the same day. Although the Danish authorities have not yet been able to trace the exact source of their epidemic, the Luxembourgish data has nonetheless helped to narrow the search down substantially.

"Food does not stop at borders. The collaboration on a European level is very important", says Mossong. The same principle applies on a national level. As such, another one of the project's most notable achievements is certainly the creation of a functional collaboration between the Health Inspection Unit of the Health Directorate Luxembourg, the Veterinary Services Administration, the Food Control Unit of the LNS and the Technical Services for Agriculture Administration, all working together to guarantee a better surveillance programme of zoonotic bacteria.

"The Salmonella outbreak in 2006 showed the past short-comings of the collaboration between the different institutions. This was certainly not due to a lack of motivation, however", claims Mossong, "but rather to a limitation in manpower and funding." The contribution of the *Fonds National de la Recherche* helped redress this problem, at least temporarily. "At the closing of the project, the partners have taken as much as they could on board and OSQCA (*Organisme pour la sécurité et la qualité de la chaîne alimentaire*), a new Health and Food Safety section responsible for the coordination of bacteria surveillance in the food chain, was founded within the Luxembourgish government. " says Mossong. Meanwhile, the EPIFOOD team will embark on a new project: HYPOCAMP aims to investigate the role of environmental sources in human campylobacteriosis.