VIRAL INFECTION

Neurons fight back

Viral infection induces an innate immune response in neurons, a group led by Monique Lafon at the Pasteur Institute in Paris, France, has found. It was previously thought that the nervous system relied solely on glial cells to detect infection.

Lafon and colleagues used *in vitro* cultures of a human postmitotic neuron-derived cell line, NT2-N, which displays characteristics of fully mature human neurons upon transplantation into the brain, and infected them with rabies virus (RABV) or herpes simplex type 1 virus (HSV-1).

Searching for neuronal genes of which transcription is upregulated during infection through microarray analysis (using cut-off of a twofold increase to identify upregulated genes), they found that RABV infection increased the transcription of 228 genes, and HSV-1 infection increased the transcription of 263 genes. RABV upregulated 56 genes of the immunity cluster of genes (24% of all genes upregulated by RABV), whereas HSV-1 upregulated only 13 genes in this cluster (4.9% of all genes upregulated by HSV-1). Of all the genes upregulated by RABV, the 25 genes for which expression was most strongly stimulated were all involved in the innate immune response, including the interferon-β (IFN-β) gene, IFN-β primary and secondary response genes, as well as genes for chemokines, inflammatory cytokines and molecules with antiviral activities. Infection with HSV-1, on the other hand, did not upregulate IFN-β gene transcripts. Microarray results were confirmed by real-time PCR, immunocytochemistry and ELISA.

Microarray analysis also revealed that NT2-N cells express genes that code for molecules which can sense double-stranded RNA (dsRNA, a molecular signature of RNA viruses), including Toll-like receptor 3 (TLR3). This was confirmed by immunocytochemistry using antibody directed against the human TLR3. Treatment of NT2-N cells with dsRNA led to the upregulation of some genes that are involved in innate immunity. Similar treatment with lipopolysaccharide, found on the outer leaflet of the outer membrane of Gram-negative bacteria, had limited effect. In addition, human postmitotic neurons were responsive to a treatment with IFN-β, suggesting that they also express receptors to IFN-α/β (IFN-Rα/β).

These results establish that human neurons are capable of sensing and responding to viral infection, and that dsRNA could be the main viral element that is sensed by human neurons as a trigger of an innate immune response.

Annie Tremp

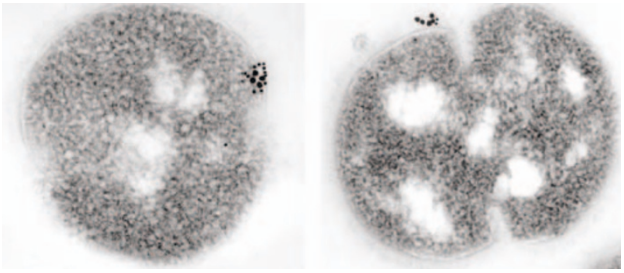
References and links

ORIGINAL RESEARCH PAPER Préhaud, C., Mégret, F., Lafage, M. & Lafon, M. Virus infection switches TLR-3-positive human neurons to become strong producers of beta interferon. *J. Virol.* **79**, 12893–12904 (2005)

BACTERIAL SECRETION

Specialized ExPort

Gram-positive microorganisms face a unique challenge when it comes to protein folding and secretion. Although all microorganisms lack the endoplasmic reticulum (ER) found in eukaryotes, Gram-negatives have a periplasmic space where nascent polypeptides can be folded before secretion. This space is lacking in Gram-positives, and the mechanisms these organisms use to ensure that their vast quantities of secreted proteins are folded correctly have been largely unknown until recently.



Immunogold electron micrographs showing the co-localization of HtrA tagged at the C terminus (18-nm beads) and SecA (12-nm beads). Reproduced with permission from *Molecular Microbiology* © (2005) Blackwell Publishing.

In the streptococci, it was known that, although only the general secretory (Sec) pathway is present, secretion is a complex process that involves chaperones and accessory factors. In addition, the existence of a putative secretion microdomain in *Streptococcus pyogenes* was first revealed by Jason Rosch and Michael Caparon in *Science* last year. Then, immunogold electron microscopy showed that SpeB — a cysteine protease, and one of the most abundant *S. pyogenes* secreted proteins — was localized to a discrete location at the cell hemisphere. Given that they also detected a high concentration of Sec translocons in this area, Rosch and Caparon proposed that this location was a specialized microdomain for protein export, which they termed the ExPortal.

Now, in a paper available online in *Molecular Microbiology*, Rosch and Caparon have undertaken a more detailed analysis of the ExPortal, providing evidence to support their suggestion that it provides a specialized environment in which newly formed proteins can interact with chaperones and accessory proteins. Their recent experimental work has focused on HtrA (DegP), an extracellular serine protease also known to function as a chaperone for several *S. pyogenes* secreted proteins, including SpeB. Using immunoelectron microscopy, it was found that the distribution of HtrA on the streptococcal surface was similar to the distribution of the ExPortal — clustered at a single discrete location distal from both cell poles. Further work showed that HtrA co-localizes with SpeB, providing evidence that the ExPortal provides an environment in which chaperones and substrates can interact.

Previous work had shown that both the proteolytic and chaperone functions of HtrA can be involved in protein folding and maturation. Here, Rosch and Caparon found that the serine-protease activity of HtrA was crucial for SpeB maturation, although they did not show directly

ANTIFUNGALS

A helping hand

Hsp90 is well known for its role as a molecular chaperone — a protein that assists the folding of other proteins ('clients'). Now, research published in *Science* reveals that Hsp90 might also influence the evolution of new traits by potentiating the phenotypic effect of genetic variation. Studies of the evolution of drug resistance in several strains of pathogenic fungi demonstrate an essential role for Hsp90 and its client protein calcineurin, and implicate Hsp90 as a novel antifungal target.

As a chaperone for many signal transducers, Hsp90 is able to 'buffer' the effects of genetic variation by enabling the cell to tolerate mutations. Although Hsp90 is highly inducible following environmental stress, the demands of stress-induced protein misfolding can outpace its induction, enabling previously silent mutations to act combinatorially and generate new phenotypes. It now seems that Hsp90 has yet another role in the emergence of new traits: by allowing a mutation to have immediate effects rather than buffering against it, HSP90 might actually potentiate the appearance of new phenotypes.

Leah Cowen and Susan Lindquist examined the role of Hsp90 in the evolution of resistance to antifungal drugs. Using rapid selection of three strains of *Saccharomyces cerevisiae* with varying levels of Hsp90, they demonstrated that the development of resistance depended on high-level expression of Hsp90. Moreover, Hsp90 was required to maintain resistance rather than just to cope with the initial selection stress.

The Hsp90-dependent effect was specific to mutants generated by rapid selection, which favours mutations that prevent the accumulation of toxic metabolites, rather than gradual selection which involves upregulation of a multidrug transporter. However, in 11 previously identified *S. cerevisiae* drug-resistant deletion strains, all were found to be Hsp90-dependent, showing that Hsp90 can influence the resistance caused by a variety of different genetic lesions.

But how does Hsp90 achieve this? One possibility is that a common regulator exists to mediate Hsp90-dependent effects on different mutations. An obvious candidate was calcineurin, an Hsp90 client known to regulate the cell's response to azoles. Accordingly, inhibition of calcineurin strongly reduced fluconazole resistance in all Hsp90-dependent resistant strains.

These findings reveal an attractive therapeutic strategy against fungal infection, as similar results were seen with several fungal pathogens. It is particularly significant that Hsp90 was crucial for the evolution of antifungal resistance in clinical isolates of *Candida albicans* collected from an HIV-infected individual. With continued exposure to fluconazole, the clinical isolates evolved towards Hsp90-independent resistance, prompting speculation that Hsp90 initially allows the phenotype to be expressed but that environmental stress drives the cell towards stabilizing the resistant phenotype. Inhibiting Hsp90 early in infection could therefore render resistant fungal pathogens sensitive to conventional treatment or could prevent the initial development of antifungal-drug resistance. Moreover, Hsp90 inhibitors are already being evaluated in clinical trials for cancer, and seem to be well tolerated at levels that achieve significant inhibition.

Joanna Owens, Associate Editor,
Nature Reviews Drug Discovery

References and links

ORIGINAL RESEARCH PAPER Cowen, L. & Lindquist, S. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. *Science* **309**, 2185–2189 (2005)

that HtrA acts on SpeB. It was also found that HtrA must be anchored in the membrane at the ExPortal to function in SpeB maturation.

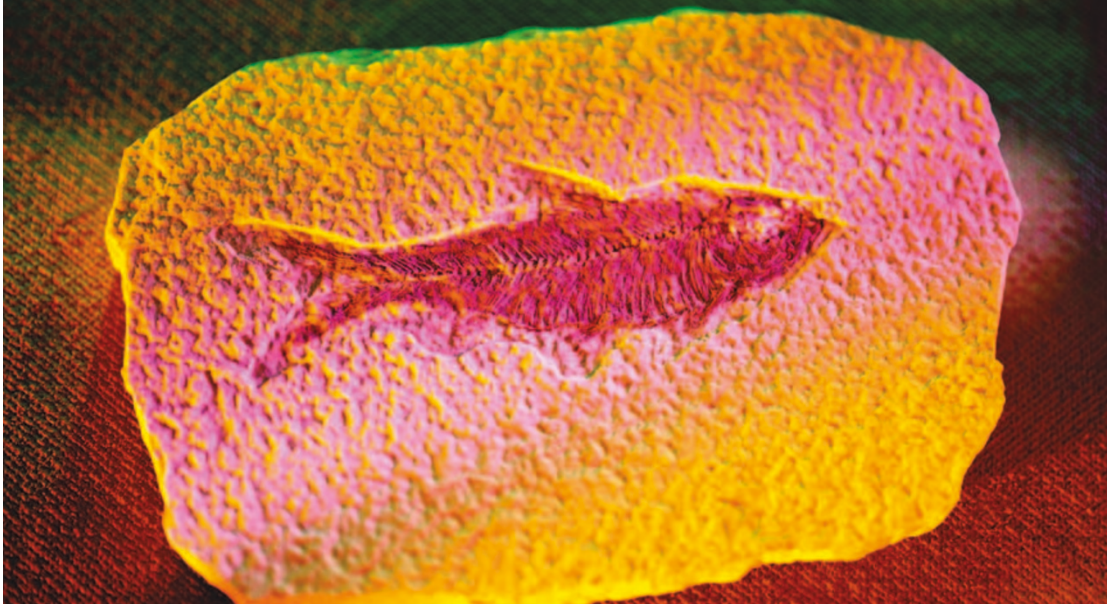
Unlike Gram-negatives, which can contain up to five different secretion systems, the streptococci encode only the Sec system, and the discovery that the Sec apparatus was concentrated into a single location — the ExPortal — rather than being evenly distributed on the cell surface was perhaps contrary to expectations. However, Rosch and Caparon have now shown that the ExPortal might provide the streptococci with the functional equivalent of the periplasm or ER, allowing a subset of nascent polypeptides to mature and fold correctly before secretion.

Sheilagh Molloy

References and links

ORIGINAL RESEARCH PAPER Rosch, J. W. & Caparon, M. G. The ExPortal: an organelle dedicated to the biogenesis of secreted proteins in *Streptococcus pyogenes*. *Mol. Microbiol.* Sep 2005 (doi:10.1111/j.1365-2958.2005.04887.x)

FURTHER READING Rosch, J. & Caparon, M. A microdomain for protein secretion in Gram-positive bacteria. *Science* **304**, 1513–1515 (2004) | Campo, N. *et al*. Subcellular sites for bacterial protein export. *Mol. Microbiol*. **53**, 1583–1599 (2004)



VIROLOGY

Resurrecting the past

In the 1918–1919 influenza pandemic, one-third of the world's population is believed to have been infected with the virus, and up to 50 million people died. Now, almost 90 years later, the genome sequence of the causative virus has been completed, not only facilitating comparative genomic and phylogenetic analysis, but also allowing researchers to reconstruct this deadly pathogen in the laboratory.

The 1918 genome-sequencing project started in 1995, when Jeffrey Taubenberger's group began analysing archived autopsy samples. Using these samples in conjunction with lung tissue from an influenza victim buried in a gravesite in Alaska that was covered with permafrost, Taubenberger and his collaborators have now been able to determine the complete genome sequence of the 1918 virus. The genomes of influenza A viruses comprise eight separate genome segments. The sequences of five of these segments have been published previously, and it is the sequences of the trimeric polymerase complex, comprising the PA, PB1 and PB2 proteins, that are now reported by Taubenberger *et al*. in *Nature*.

Analysis of the polymerase-complex genes confirms the conclusion drawn about the origin of the 1918 virus from analysis of the other genome segments. In contrast to the 1957 and 1968 pandemic viruses, which are thought to be reassortants between a Eurasian waterfowl strain and a human-adapted strain, the 1918 strain is not a reassortant but is an entirely avian virus that adapted to humans. The precise source of the virus remains unknown, however, as the sequence differs from that of all avian sequences available for analysis and so might have come from an evolutionarily isolated source.

Interestingly, several of the amino-acid changes from the avian consensus sequence seen in the polymerase-complex genes have been observed in the highly pathogenic H5N1 viruses currently in circulation.

In a separate study published in *Science*, Terrence Tumpey *et al*. used reverse genetics to generate a virus with the same coding sequence as the 1918 virus. The reconstructed virus proved highly virulent in mice, with a virus titre 4 days after inoculation that was 39,000 times greater than after infection with a modern influenza strain. In addition, mice infected with the reconstructed virus lost up to 13% of their body weight 2 days after infection, and the virus could be lethal in as little as 3 days. As well as recreating the 1918 strain, Tumpey *et al*. also created viruses containing different combinations of 1918 genes, and determined that the polymerase complex and haemagglutinin are required for maximal replication in human cells.

Recreating the 1918 virus has raised safety concerns in some quarters, but all work with the virus was carried out under strict Biosafety Level 3 enhanced conditions, and the reconstructed virus was handled by a single individual. As fears of a new influenza pandemic continue to grow, such work is vital to understand the highly pathogenic influenza viruses.

Sheilagh Molloy

References and links

ORIGINAL RESEARCH PAPERS Taubenberger, J. T. *et al*. Characterization of the 1918 influenza virus polymerase genes. *Nature* **437**, 889–893 (2005) | Tumpey, T. M. *et al*. Characterization of the reconstructed 1918 Spanish influenza pandemic virus. *Science* **310**, 77–80 (2005)

FURTHER READING Reid, A. H., Taubenberger, J. K. & Fanning, T. G. Evidence of an absence: the genetic origins of the 1918 pandemic influenza virus. *Nature Rev. Microbiol*. **2**, 909–914 (2004)

BACTERIAL PATHOGENESIS

Defeating the cost of GAS

The pili of Gram-negative pathogens have important roles in virulence and protection, and their biology has been well characterized. Little is known, however, about the extended surface organelles of Gram-positive pathogens. Mora and colleagues, reporting in *Proc. Natl Acad. Sci. USA*, have now shown that the Gram-positive group A *Streptococcus* (GAS) has surface pili, and that each of the four variants corresponds to specific Lancefield T antigens and confers protection against lethal GAS challenge in a mouse model of infection.

GAS produces two major classes of protein antigens: trypsin-sensitive M antigens and trypsin-resistant T antigens. The authors initially searched the five available GAS genomes, and found that each of the four variant fibronectin-binding, collagen-binding T-antigen (FCT) regions that are in all genomes codes for pilus-like structures. The T6 antigen, recognized in the original Lancefield serotyping system, forms the backbone of one pilus; the major pilus component of each of the other three also represents specific Lancefield T antigens.

Further analyses revealed that GAS pili are composed of members of a family of extracellular matrix-binding proteins and that, structurally, they resemble the pili described for the Gram-positive *Corynebacterium diphtheria* and *Streptococcus agalactiae* (a group B *Streptococcus*; GBS). Furthermore, although the exact function of the pili remains elusive, the authors showed that, as in GBS, the components are effective protective antigens.

GAS infections can range from mild skin infections to severe, life-threatening conditions such as necrotizing fasciitis. Mora *et al.* suggest that the pilus structures they have identified could be crucial virulence factors and, as such, represent potential vaccine targets against the wide variety of GAS-induced diseases.

Sharon Ahmad

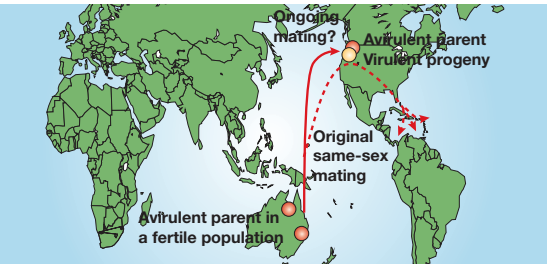
References and links

ORIGINAL RESEARCH PAPER Mora *et al*. Group A *Streptococcus* produce pilus-like structures containing protective antigens and Lancefield T antigens. *Proc. Natl Acad. Sci. USA* **102**, 15641–15646 (2005)

FUNGAL BIOLOGY

Same-sex mating and host-range expansion

Microbiologists from Duke University have unraveled the story behind the emergence of a virulent fungus strain that was responsible for an 1999 outbreak of meningoencephalitis on Vancouver Island, Canada. Two clonal lineages of the same 'sex' — one local strain found in the Pacific Northwest, and another that originated in Australia — combined through sexual reproduction to yield the hypervirulent recombinant genotype that was primarily responsible for the outbreak.



The origin of a *Cryptococcus gattii* outbreak. An original α–α mating event involving an avirulent α parent that likely originated in Australia (orange circle) yielded a virulent recombinant (yellow circle), and ongoing α–α mating could enable robust infectious spore production and outbreak expansion. Image adapted from Fraser *et al.* © (2005) Macmillan Magazines Ltd.

Cryptococcus gattii is a yeast normally restricted to tropical and subtropical regions of the world. Identification of this pathogen as the causative agent of the Vancouver Island outbreak indicated a new and expanded geographical range for this microorganism, prompting questions about its origin and evolution. To address these questions, Joseph Heitman, James Fraser and colleagues undertook a large-scale genealogical analysis of *C. gattii* isolates that were linked to the outbreak and compared them with each other and with the global *C. gattii* population. This analysis revealed that the outbreak isolates comprised two distinct genotypes — a major genotype (95% of isolates) that is hypervirulent and a minor genotype (5% of isolates) that is less virulent and has an identical genotype with fertile isolates from an Australian recombining population. This analysis also revealed evidence of sexual reproduction between the outbreak genotypes; however, instead of the classical fungal **a**–α sexual cycle, Fraser *et al.* found evidence that the major-outbreak hypervirulent genotype descends from a same-sex mating event involving two α mating-type parents.

Further studies will be required to ascertain the prevalence of same-sex *C. gattii* mating in nature and whether this process facilitated the production of infectious fungal spores. However, these findings do establish the importance of same-sex reproduction in fungi by allowing the expansion of a pathogen into a new geographical region, ultimately resulting in an infectious-disease outbreak in humans. The earlier demonstration of same-sex mating in *Cryptococcus neoformans*, a sibling species of *C. gattii*, and the authors' assertion that other parasites, including *Trypanosoma cruzi*, *Leishmania* species and *Plasmodium falciparum*, could harbour a same-sex cycle that produces progeny with altered characteristics, has fascinating implications for our understanding of parasite pathogenicity and host range.

David O'Connell

References and links

ORIGINAL RESEARCH PAPER Fraser, J. A. *et al.* Same-sex mating and the origin of the Vancouver Island *Cryptococcus gatti* outbreak. *Nature* **437**, 1360–1364 (2005)

FURTHER READING Idnurm, A. *et al.* Deciphering the model pathogenic fungus *Cryptococcus neoformans*. *Nature Rev. Microbiol.* **3**, 753–764 (2005) | Lin *et al.* Sexual reproduction between partners of the same mating type in *Cryptococcus neoformans*. *Nature* **434**, 1017–1021 (2005) | Kidd *et al.* Comparative gene genealogies indicate that two clonal lineages of *Cryptococcus gattii* in British Columbia resemble strains from other geographical areas. *Eukaryot. Cell* **4**,1629–1638 (2005)

BACTERIAL PHYSIOLOGY

Signal interference

Bacteria use chemical signals called autoinducers to communicate with one another — a phenomenon known as quorum sensing. By producing and importing autoinducers, bacterial cells establish the density of their own and other species and synchronize the expression of fundamental genes. Now, a new report published in *Nature* shows that certain bacteria can interfere with autoinducer-mediated signals, disrupting quorum-sensing behaviour in competing microorganisms.

Previous work by Karina Xavier and Bonnie Bassler had characterized the quorum-sensing mechanisms of *Escherichia coli* and *Vibrio harveyi*: both species communicate using the autoinducer AI-2. A positive feedback loop operates in *E. coli* — consumption of AI-2 induces the expression of the *lsr* operon, which encodes the transporter that imports AI-2. In *V. harveyi*, detection of AI-2 by extracytoplasmic receptors modulates the expression of genes that are involved in bioluminescence and type three secretion.

In this study, the authors first confirmed that *E. coli* and *V. harveyi* communicated using AI-2. Co-culture experiments showed that AI-2 production by either *E. coli* or *V. harveyi* induced the expression of the *E. coli lsr* operon. Similarly, in *V. harveyi*, AI-2 from either species activated the bioluminescence genes and repressed type-three-secretion genes. This cross-species communication is especially interesting as *E. coli* and *V. harveyi* detect AI-2 signals with different chemical structures, and chemical interconversion presumably takes place in the surrounding media.

In mixed consortia, the induction of quorum-sensing genes by AI-2 is not equivalent in different species. In model *V. harveyi–E. coli* cultures, the consumption of AI-2 by *E. coli* interfered with the expression of the *V. harveyi* quorum-sensing regulon, reducing light production and de-repressing a type-three-secretion locus.

Furthermore, *E. coli* AI-2 consumption interfered with the quorum-sensing behaviour of *Vibrio cholerae*, extending these observations to communities that could co-colonize the human enteric system.

The authors conclude with an intriguing proposal — perhaps eukaryotes have developed specific associations with microorganisms that communicate using AI-2. The manipulation of AI-2 signals by these bacteria would maintain normal eukaryotic microflora and protect the host against pathogenic bacteria.

Shannon Amoils

References and links

ORIGINAL RESEARCH PAPER Xavier, K. B. & Bassler, B. L. Interference with AI-2 mediated bacterial cell–cell communication. *Nature* **437**, 750–753 (2005)

FURTHER READING Veneville, A. *et al.* Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. *Nature Rev. Microbiol.* **5**, 361–446 (2005)

IN BRIEF

TECHNIQUES AND APPLICATIONS

Defining genes in the genome of the hyperthermophilic archaeon *Pyrococcus furiosus*: implications for all microbial genomes

Poole, F. L. II *et al. J. Bacteriol.* **187**, 7325–7332 (2005)

Does automated genome annotation produce reliable and reproducible results? Not necessarily, according to Farris L. Poole and co-workers. A comparison of the genome annotations of the archaeon *Pyrococcus furiosus* from three different databases revealed significant discrepancies. In particular, the number and size of the open reading frames differed among the different databases. Transcriptional analyses and recombinant protein production confirmed the presence of at least 17 genes in *P. furiosus* that had not been identified in the original annotation. It is therefore imperative that all microbial genome annotations are carefully scrutinized and, ideally, backed up by experimental analyses.

ENVIRONMENTAL MICROBIOLOGY

Fungal contamination of bedding

Woodcock, A. A. *et al. Allergy* 20 Oct 2005 (doi:10.1111/j.1398-9995.2005.00941.x)

Woodcock and colleagues identify a fungal 'field of dreams'. The authors show that regularly used pillows in the UK harbour up to 16 different species of fungus, most commonly *Aspergillus fumigatus*. Synthetic and feather pillows might therefore represent a previously unidentified source of fungal infection, with implications for susceptible patients with respiratory disease.

SYMBIOSIS

A secondary symbiosis in progress?

Okamoto, N. & Inouye, I. *Science* **310**, 287 (2005)

This study describes the formative stages of a secondary endosymbiosis between a protist, here referred to as Hatena ('enigmatic' in Japanese), and its symbiont — a green algae of the genus *Nephroselmis*. The study illustrates the profound morphological changes in both the host and the symbiont that accompany endosymbiosis. Ultrastructural data and observations of natural populations are used to outline the life cycle of Hatena.

EPIDEMIOLOGY

Comparative phylogenomics of the food-borne pathogen *Campylobacter jejuni* reveals genetic markers predictive of infection source

Champion, O. L. *et al. Proc. Natl Acad. Sci. USA* **102**, 16043–16048 (2005)

Although poultry has traditionally been regarded as the main source of human *Campylobacter jejuni* infection, this is currently unproven, owing to the limitations of traditional typing methods. In this study, Champion *et al.* combine DNA-microarray-based genomotyping with Bayesian-based algorithms to determine the phylogeny of *C. jejuni*. The population structure of *C. jejuni* comprises a livestock-associated and a non-livestock-associated clade. Surprisingly, most human isolates were found in the non-livestock sources, suggesting an environmental reservoir of the pathogen and highlighting the utility of this approach.