Of Badgers, Bacteria, and Bed Sheets

The menu at a recent Society for Applied Microbiology meeting ranged from highly sophisticated molecular genetics to classical simplicity

Bernard Dixon

Amid continuing controversy over the culling of Britain’s badgers to prevent them transmitting tubercle bacilli to cattle, there is new evidence that badgers carry the agent of another human disease. Presented during the Society for Applied Microbiology’s recent conference in Brighton, data from Lauren Perrin with other researchers at the University of Salford and elsewhere indicated that the animals are a competent reservoir host for *Borrelia burgdorferi*, the agent of Lyme borreliosis. As this condition is currently emerging rapidly in the United Kingdom, the finding is both important and disquieting. It comes from a two-year study to determine the prevalence and diversity of *B. burgdorferi* infecting badgers, ticks feeding on them, and questing ticks. Results from a survey in Woodchester Park, Gloucestershire, included a 6% prevalence of *B. afzelii* in ear biopsies from badgers and a 5% prevalence of infection in questing *Ixodes ricinus* nymphs.

Other data reported in Brighton extended our knowledge of Lyme disease elsewhere in Europe. Cees van den Wijngaard with colleagues at the Centre for Infectious Disease Control in Bilthoven, the Netherlands, presented the first estimates of the incidence and public health significance of the disease to include disseminated and persisting Lyme-related symptoms. From a global perspective, this was also the first study of the impact of Lyme borreliosis in disability-adjusted life years. The work in the Netherlands revealed that the infection causes a substantial burden of disease, the greater part being attributable to Lyme-related persisting symptoms.

A second Dutch group, including investigators from the Bilthoven laboratory with collaborators in Moscow, Russia, described their efforts to gain more insight into public health risks posed by *Borrelia miyamotoi* in the Netherlands. Using a newly developed serological assay, they confirmed that infections with this organism do occur in tick-exposed individuals—and that the condition is currently underdiagnosed. The findings were coupled with a warning, for both the Netherlands and other countries where *Ixodes ricinus* ticks are endemic, that doctors should consider *B. miyamotoi* in patients reporting tick bites and a febrile illness of unknown etiology.

Europe has been experiencing the predations of another emerging pathogen recently, with an evolutionary pathway no doubt still incomplete. Verotoxin-producing enteroaggregative *Escherichia coli* came to light as a pathotype causing a large outbreak of hemolytic uremic syndrome in Germany. An O104:H4 strain that had acquired a VT-encoding phage, its recognition prefaced the detection of other strains with similar features. Valeria Michelacci and coworkers at the Istituto Superiore di Sanita in Rome, Italy, argued that these bacteria have been imported into Europe from low-income countries with which many of the reported cases had known or suspected epidemiological links.

Adriana Cabal with a team at SaBio-ERIC in Ciudad Real, Spain, described a new real-time assay for the detection of nine *E. coli* virulence genes and four serotype-related genes to determine whether cattle can serve as reservoirs for intestinal pathotypes typically associated with humans, such as the O104:H4 strain in the German outbreak. Although not yet fully validated, the assay appears to be a valuable addition to our armory for characterizing disease-causing versions of *E. coli*.

Advances in whole-genome sequencing are, of course, throwing increasing light on the ways in which bacteria evolve, and on their impact on their hosts. As part of this research avenue, thousands of bacterial genomes are being sequenced in laboratories throughout the world. Nevertheless, as Sam Sheppard of the University of Swansea, United Kingdom, pointed out at the SfAM
meeting, microbiologists face a continuing challenge in developing methods of analyzing these data. One approach, pioneered in human genetics, is genome-wide association mapping, in which variations in DNA sequences across the genome are related to specific phenotypes. Researchers have found it difficult to apply this method to bacteria because of their strong population structure based on clonal reproduction.

Sheppard described an innovative technique which he and his Swansea colleagues have employed to study genetic variation in the zoonotic food-poisoning organism *Campylobacter* as it moves through the food chain. Contaminated poultry meat is a major source of human infection—the strains that infect humans being a subset of those that colonize chickens on the farm.

“To understand better the genes and alleles associated with survival at different stages, we sequenced over 500 genomes from chickens on the farm, abattoirs, retail meat outlets and patients,” Sheppard said. “We divided the genomes into overlapping 30-bp words, allowing simultaneous analysis of homologous and nonhomologous sequence variation, and highlighted words that were significantly overrepresented in particular stages, compared with our expectations founded on population structure.”

Sheppard and his colleagues identified words that showed significant association across different clonal complexes, and mapped them onto an annotated reference genome to assess their functionality. “This provided a list of candidate genes that could be important in the passage of *Campylobacter* from farm to fork.” The Swansea work highlights the value of genome-wide studies for our comprehension of the genetic basis of phenotypic variation in bacteria. There are also implications here for the identification of virulence, transmission, and survival factors in pathogens, as well as for broader investigations into the ecological genomics of nonpathogens.

Laura Piddock at the University of Birmingham, United Kingdom, has led an unusual, long-term study to identify a hitherto unknown mechanism of antibiotic resistance in *Salmonella enterica*. She and her collaborators have investigated fluoroquinolone and multidrug resistance in a series of isolates of *S. enterica* serovar Typhimurium from a patient who failed to respond to antibiotic treatment during the 1990s. They originally “struggled” to identify the precise nature of the mutations conferring resistance to numerous antibiotics, as well as the relationship between particular mutations and phenotype.

“Before whole-genome sequencing, with such complex phenotypes we could only investigate the roles of genes already implicated in, or known to confer, drug resistance,” Piddock said. “More recently, genomics has transformed our capacity to understand antibiotic resistance phenotypes. It has also allowed us to identify putative mechanisms of resistance, from which we have been able to design experiments to demonstrate the phenotypes caused by specific mutations.” In this way, the Birmingham team discovered a new mode of resistance mediated by the efflux pump AcrB, the transporter component of the AcrAB-TolC multidrug efflux system.

Alongside frontier science of this sort, I am always attracted by advances reported in present times but achieved by techniques which the pioneers of microbiology might have used aeons ago. One previous SfAM meeting included a poster on the leaching of organisms from cow pats into the underlying soil. Louis Pasteur could have done this work—which, despite its simplicity, was important in relation to our understanding of the dissemination of enteropathogenic *E. coli*.

A corresponding item on the Brighton agenda was a report by Joanna Tarrant and others at De Montfort University, Leicester, United Kingdom, on their discovery that inadequately decontaminated bed sheets could be contributing to *Clostridium difficile* infections in UK hospitals. They have found that its spores can survive the National Health Service’s washing standard of 71°C for three minutes, and while they used ribotyping to verify that the spores were the same before and after washing, the crucial evidence came from a far more elementary test of thermotolerance.

If, in my fantasy world, I were ever to return to the lab, there might still be things for me to do.