



Science writer **Meriel Jones** takes a look at some recent papers in SGM journals which highlight new and exciting developments in microbiological research.

## Rotavirus versus coronavirus in newborn calves

**Aich, P., Wilson, H.L., Kaushik, R.S., Potter, A.A., Babiuk, L.A. & Griebel, P. (2007).** Comparative analysis of innate immune responses following infection of newborn calves with bovine rotavirus and bovine coronavirus. *J Gen Virol* **88**, 2749–2761.

Diarrhoea, especially in developing countries, is a serious cause of illness and death in children. It also affects young farm animals and results in greater financial loss to cattle producers than any other infectious illness. Many species of bacteria and viruses produce the symptoms, including bovine rotavirus (BRV) and bovine coronavirus (BCV). BRV infections clear up more rapidly than those of BCV, which can persist and re-occur in adult cattle. Apart from this, both viruses cause similar clinical and physiological consequences in calves, despite many physical differences. They both infect the lining of the digestive tract, seriously damaging its surface and causing diarrhoea.

The body has protective immune responses to infections, some of which are specific to the digestive tract. Researchers in Canada wondered whether these responses differed between BRV and BCV. One way to check was to look at which genes were switched up or down during the infection. The researchers did this using commercial DNA microarrays that

allow thousands of genes to be screened simultaneously, as well as methods to monitor individual genes. They were particularly interested in what happened to genes known to be important in cell growth and the immune system.

Both viruses affected the amount of product from some genes involved in cell proliferation, reducing some and increasing others. They frequently had the same effect, but sometimes had opposite effects. The overall result was to increase the products from genes that promoted cell proliferation in the infected tissues. Interpreting effects on the immune system was more difficult because less is known about how this operates in the intestine during viral infections. The researchers focused on genes that are known to be activated following viral infection and realized that each virus induced a distinct immune response. There were some very important differences that affected the proinflammatory response and also the production of the important antiviral interferons.

The researchers gained an overall impression that intestinal cell division and proliferation were more active after BRV infection than following BCV infection, which fits with the fact that the intestine recovers more rapidly from BRV infection. The two viruses have developed different strategies to fox the natural antiviral defences. Although they may both use the same strategies to evade the immune response, they activate different aspects of the innate immune response. Successful new treatments for diarrhoeal disease will need a good understanding of the ways that different viruses cause the same symptoms, as illustrated by this study.

◀ Histology of jejunal mucosa in control and BRV- and BCV-infected loops. Tissues were collected 18 h post-infection, fixed and stained with haematoxylin and eosin. (a) Control loop and (b) BRV-infected loop were collected from the same animal. (c) Control loop and (d) BCV-infected loop were collected from the same animal. *Palok Aich, VIDO, University of Saskatchewan, Saskatoon, Canada*



## A sticky problem

**Corrigan, R.M., Rigby, D., Handley, P. & Foster, T.J. (2007).** The role of *Staphylococcus aureus* surface protein SasG in adherence and biofilm formation. *Microbiology* **153**, 2435–2446.

*Staphylococcus aureus* is a bacterium that lives inside the noses of up to 80 % of people. Most of the time it is a harmless inhabitant, but it can cause disease once inside cuts or operation wounds. To do this, *S. aureus* has several proteins that each interact with specific proteins on the surface of the human cells. Once the bacterial cells are attached, they grow into multiple layers embedded in a matrix material that results in the bacteria becoming resistant to both antibiotics and the immune system.

Researchers in Dublin, in collaboration with colleagues in Manchester, have reported their latest work on a gene that encodes one of the proteins known to be involved in these processes, SasG. One end of the protein contains a region called the A domain that is exposed on the surface of the bacterial cell, while the other end anchors the protein to the cell wall. In between there are from 2 to 10 identical structural protein blocks, called B repeats. SasG forms fibrils that coat *S. aureus* cells and these can prevent other *S. aureus* proteins attaching to their targets on human cells. However, it was discovered that SasG also has the property of sticking *S. aureus* to nasal epithelial cells.

The number of B repeats affected the role of SasG. SasG with five or more repeats could stick to human cells and form biofilms, but physically blocked binding of other *S. aureus* proteins to human cell surface proteins. The fact that clinical isolates have both more and less than five B repeats implies that SasG may interfere with biofilm formation in some strains, but be part of the process in others. The researchers speculate that these contradictory properties might become important during an infection if the *S. aureus* cells gained an advantage by detaching from the human cells to spread further around the body.

## No answer to blocked catheters

**Macleod, S.M. & Stickler, D.J. (2007).** Species interactions in mixed-community crystalline biofilms on urinary catheters. *J Med Microbiol* **56**, 1549–1557.

Caring for patients with long-term bladder catheters is often complicated by the catheters becoming blocked by crystalline deposits. Bacteria form biofilms on the surface of the catheter and generate ammonia from the urea in the urine. This creates conditions in which salts in the urine can crystallize, blocking the catheter. This can result in kidney infections that can be very difficult to treat effectively.

*Proteus mirabilis* is well known for causing this problem. Researchers at Cardiff have recorded the species of bacteria they have isolated from catheters. Out of 106 patients, only 30 had biofilms caused by *P. mirabilis* alone. All the others had been colonized by two or more species. Almost half the catheters colonized by *Providencia stuartii* were also colonized by *P. mirabilis*. In contrast, only one of 14 catheters colonized by *Morganella morganii*, and no *Enterobacter cloacae*-containing biofilms, was infected by *P. mirabilis*. This obvious difference immediately suggested that *M. morganii* and *E. cloacae* might be in some way antagonistic to *P. mirabilis*.

To test this, a temperature-controlled glass chamber was used as a model bladder, with a catheter as its outlet through which artificial urine could drain. The authors inoculated it with bacteria and waited until the catheter blocked up. These model infections took around 18.7 hours to block the catheter if *P. mirabilis* was used on its own, compared with about 50 % longer when *P. mirabilis* was mixed with *E. cloacae*. On its own, *E. cloacae* never managed to block the catheter during the experiments. The results indicated that *P. mirabilis* out-competed the other organism so that the catheter blocked, although more slowly than from a *P. mirabilis* infection alone.

The researchers wondered what would happen if the other bacteria were given a head start, so they carried out another series of experiments where they let the other bacteria grow in the model system for 72 hours and then added *P. mirabilis*. All the other bacteria managed to form a biofilm on the surface of the catheter within the 72 hours, although their numbers decreased once the *P. mirabilis* cells arrived. Again, the catheters only became encrusted once *P. mirabilis* was added.

Unfortunately, these experiments showed that any antagonism between *P. mirabilis* and other bacteria commonly found in clinical catheter infections has only a minor and temporary effect. The implication is that infection with *P. mirabilis* is always likely to lead to a blocked catheter.

## Classifying phytoplasmas

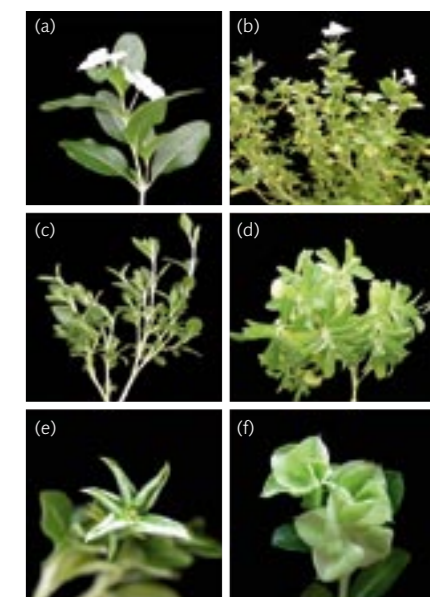
**Wei, W., Davis, R.E., Lee, I.-M. & Zhao, Y. (2007).** Computer-simulated RFLP analysis of 16S rRNA genes: identification of ten new phytoplasma groups. *Int J Syst Evol Microbiol* **57**, 1855–1867.

**Martini, M., Lee, I.-M., Bottner, K.D., Zhao, Y., Botti, S., Bertaccini, A., Harrison, N.A., Carraro, L., Marcone, C., Khan, A.J. & Osler, R. (2007).** Ribosomal protein gene-based phylogeny for finer differentiation and classification of phytoplasmas. *Int J Syst Evol Microbiol* **57**, 2037–2051.

Phytoplasmas are small, wall-less bacteria that live within plant cells; they are associated with over 600 plant diseases and are transmitted by insects. They cannot grow outside their hosts, which makes identifying them quite a challenge. Most conventional methods for identification of bacteria cannot be applied to the phytoplasmas, and therefore criteria for formal naming of species cannot be fulfilled. However, the advent of molecular biological methods has significantly improved understanding of diversity and genetic inter-relationships among phytoplasmas. The currently accepted method to distinguish each species involves analysing the essential 16S rRNA gene. The standard way to do this involves digesting the DNA with a series of enzymes to generate a characteristic RFLP pattern. The current phytoplasma classification scheme, designed in the early 1990s by researchers at USDA, is based on distinct RFLP pattern types. Now this research group has published new developments in phytoplasma classification.

The researchers found more than 800 sequences of phytoplasma 16S rRNA genes in computer databases, and analysed these sequences to create virtual RFLP patterns. They found that the virtual patterns matched experimental gel patterns. Moreover, they identified several new patterns characterizing previously unrecognized groups of phytoplasmas. These results support the value of RFLP patterns in phytoplasma identification and provide a new practical identification tool that will be available through the internet ([www.ba.ars.usda.gov/data/mppl/virtualgel.html](http://www.ba.ars.usda.gov/data/mppl/virtualgel.html)). However, some phytoplasmas with distinctive biological or ecological properties are not distinguishable on the basis of 16S rRNA analysis. In collaboration with colleagues in Italy, Oman and Florida, the USDA team tested whether other genes could provide fine-scale differentiation. The genes for two proteins, RplV and RpsC, gave the results they were looking for and allowed discrimination amongst biologically distinct strains that could not be distinguished by 16S rRNA.

These new developments advance identification and taxonomy of phytoplasmas, and contribute to deepening knowledge of these intriguing microbes in the 21st century.



▲ Phytoplasma infection in *Catharanthus roseus*. Compared to a healthy plant (a), symptoms include yellowing (b), shoot proliferation (c), witches'-broom growth (d), phyllody (e, leaf-like structures in place of flowers) and virescence (f, green colour in place of normal flower colour). *Yan Zhao, Molecular Plant Pathology Lab, Beltsville, USA*