



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

The 'eyes' have it

Niesalla, H., McNeilly, T.N., Ross, M., Rhind, S.M. & Harkiss, G.D. (2008). Experimental infection of sheep with visna/maedi virus via the conjunctival space. *J Gen Virol* **89**, 1329–1337.

Sheep and goats can suffer a long-term and debilitating inflammatory disease, affecting their lungs, joints, udders and brains, that is eventually fatal. The condition is caused by one of several small ruminant lentiviruses, including visna/maedi virus (VMV). The virus targets lymphoid tissue and the circulating macrophages of the immune system. The EU has been supporting evaluation of a vaccination strategy against this condition in sheep. As part of this, a team in Edinburgh have been checking the transmission routes of VMV infection. The main ones are thought to be through milk for young animals, but via aerosols for adults. The infected animals exhale and cough a mixture of infected cells and free viral particles into the air. This fits with the fact that the lung and upper respiratory tract are efficient sites for contracting the infection. However, VMV infection has also been found in eyes. Even though the eye has several natural defences, including enzymes like lysozyme, many viral infections can

start there. The researchers therefore set out to discover whether this disease could be caught from virus landing in the animals' eyes.

After infecting sheep with VMV, the authors checked for any disease symptoms or signs of the virus over 13 months. Virus was detected circulating in the blood in some of the animals within 2 weeks of infection, and in all of them within a month. The presence of antibodies, termed seroconversion, meant that the virus was not only present but active, and the sheep's immune system was attempting to counter any pathological effects. All of the sheep infected through the windpipe seroconverted, but only one of the four infected via the eyes did so, and then only 8 months after infection. A long delay between infection and evidence of virus activity was not unexpected because this is a well-known feature of infections with VMV and related viruses. Post-mortem examinations of the sheep showed that almost all the animals had inflammation in their lungs, typical of the early stages of the disease.

For a successful vaccination programme it is important to understand how the disease is transmitted and the full range of symptoms. These tests showed, for the first time, that VMV can infect through the eyes as well as through inhalation, and from quite low numbers of viral particles.

Novel bacterium from the whale-carcass ecosystem

Miyazaki, M., Nogi, Y., Fujiwara, Y., Kawato, M., Kubokawa, K. & Horikoshi, K. (2008). *Neptunomonas japonica* sp. nov., an *Osedax japonicus* symbiont-like bacterium isolated from sediment adjacent to sperm whale carcasses off Kagoshima, Japan. *Int J Syst Evol Microbiol* **58**, 866–871.

Sperm whale carcasses on the deep-sea floor form unique biological communities. A novel genus of marine worm, *Osedax*, has recently been discovered in such environments. These worms consist of a crown, trunk and root structure, and symbiotic bacteria exist in the root systems. Researchers from the Japan Agency for Marine-Earth Science and Technology have discovered a novel species of symbiotic bacteria in these worms during an investigation of a sperm whale carcass ecosystem off Kagoshima in Japan.

After running a battery of identification tests on the bacterium, it became obvious that it was closely related to other species of symbiotic marine bacteria, but different enough to warrant description as a novel species of the genus *Neptunomonas*, for which the name *N. japonica* was proposed. The only other known member of this genus had been found in creosote-contaminated sediment on the other side of the Pacific Ocean.

The genus *Osedax* is closely related to tubeworms, and it is thought that the method of acquisition of their symbiotic bacteria may be the same, i.e. horizontal transmission from the environment. *Osedax* worms are also known as zombie worms because of their ability to devour bones. The symbiotic bacteria are thought to help them digest the oils and fats in the bones.



Two pathogens with one drug

Zeidner, N.S., Massung, R.F., Dolan, M.C., Dadey, E., Gabitzsch, E., Dietrich, G. & Levin, M.L. (2008). A sustained-release formulation of doxycycline hyclate (Atridox) prevents simultaneous infection of *Anaplasma phagocytophilum* and *Borrelia burgdorferi* transmitted by tick bite. *J Med Microbiol* **57**, 463–468.

Ticks can transmit several diseases to wild and domestic animals, and people. The hard tick *Ixodes scapularis* carries *Borrelia burgdorferi* from its animal reservoir in the wild American white-footed mouse to people. This bacterium causes Lyme borreliosis (Lyme disease). There are 500–2,000 new cases of Lyme borreliosis in the UK each year, but it is the most common vector-borne disease in the USA with about 20,000 new cases annually. The effects on people vary considerably, from mild to very severe. The symptoms are numerous, ranging from feeling unwell with flu-like symptoms through tiredness with joint or muscle pains to a rash, digestive upsets, headaches and effects on the central nervous system. The best treatment is antibiotics over several months to kill the bacteria, starting as soon as possible after receiving an infected tick bite. Short courses of antibiotics have also been used to successfully treat Lyme disease.

However, *I. scapularis* can harbour other pathogenic bacteria. Human granulocytic anaplasmosis, caused by *Anaplasma phagocytophilum*, was first described in 1990 from the mid-western USA, but is now found increasingly along the north-eastern seaboard and the upper mid-western states. It is therefore possible to become infected simultaneously with *A. phagocytophilum* and *B. burgdorferi*. Doxycycline is the best antibiotic for treating acute infections of *A. phagocytophilum* and may also be effective against *B. burgdorferi*, although amoxicillin is usually the first choice.

Researchers at the CDC in the USA, in collaboration with QLT Laboratories, wanted to see whether doxycycline could also be used to prevent both infections. They compared the effectiveness of a single oral dose of doxycycline with one injection of a slow-release version of the same antibiotic. For the test, the researchers allowed bacteria-infected ticks to bite mice and then treated them with the two antibiotic formulations. The health of the mice was monitored for 3 weeks. The slow-release injection protected all the mice from both species of pathogenic bacteria. In comparison, over 70% of the mice succumbed to infection when their therapy was a single oral dose of antibiotic, exactly the same result as in untreated mice. After this decisive result, the researchers wondered if this could be developed into a strategy to block or eliminate the bacterial infections in the wild animals and ticks, and as a basis to develop novel platforms to deliver doxycycline to people to prevent infection. As always, prevention of infections is preferable to devising cures. They are now evaluating field trials of novel doxycycline formulations to see whether this is possible.

Just say NO!

Mills, P.C., Rowley, G., Spiro, S., Hinton, J.C.D. & Richardson, D.J. (2008). A combination of cytochrome *c* nitrite reductase (NrfA) and flavorubredoxin (NorV) protects *Salmonella enterica* serovar Typhimurium against killing by NO in anoxic environments. *Microbiology* **154**, 1218–1228.

Nitric oxide (NO) is a highly reactive gas encountered by microbes in many environments. Some bacteria encounter NO within animal tissues, where macrophage cells use it as a toxic molecule to repel the invaders. For every natural toxin, some bacteria have evolved methods to counter the effects. NO is no exception. It is known that bacteria can have several defences against NO, but it has not been clear if they are all important. *Salmonella* bacteria are able to survive and even grow and divide within macrophages. They have at least three enzymes that can metabolize NO. Scientists from Norwich and Dallas, have now clarified how each enzyme protects the bacteria. They focused on *Salmonella* Typhimurium, which causes severe food poisoning. One enzyme, HmpA, was already known to detoxify NO in aerobic conditions. Indeed, some studies have indicated that it contributes to the virulence of *Salmonella* towards mice. There are two more enzymes that can handle NO: NorV is found in the bacterial cytoplasm; NrfA lies between the membranes and cell-wall polymers that surround and protect the cell. The advantages of this position are that the enzyme can detoxify NO before it enters the cell properly, and it can also channel some of the energy from this reaction into the energy-conserving system of the cell. NrfA therefore gives the cell the potential to use this toxin as fuel.

The researchers carried out a careful series of tests, measuring the growth of *S. Typhimurium* in aerobic and anaerobic conditions with different levels of NO gas dissolved in the growth media. They compared a series of bacterial strains that lacked the genes for one, two or all three of the enzymes. This, for the first time, showed that under anaerobic conditions NrfA protected the cells efficiently from NO, with NorV mopping up any NO that got into the cytoplasm. The cells could even boost the level of NrfA activity in response to NO in the growth medium. In addition, the importance of HmpA to counter NO under normal aerobic conditions was confirmed. By analysing the growth characteristics of an *S. Typhimurium* strain that lacked all three proteins, the researchers got a strong hint that there was at least one more NO-detoxifying system waiting to be discovered.

The advantage to the bacterium of these multiple methods to counteract one toxic molecule is that they give flexibility in the many environments in which the bacteria may find themselves exposed to this gas.

► Atomic force microscopy image of *Salmonella* Typhimurium. Roy Bongaerts & Patrick Gunning, Imaging Partnership, Institute of Food Research, Norwich (www.ifr.ac.uk)

◄ *Osedax* worms on a whale carcass. Masayuki Miyazaki, Japan Agency for Marine-Earth Science and Technology, Yokosuka, Japan

