New and Lethal

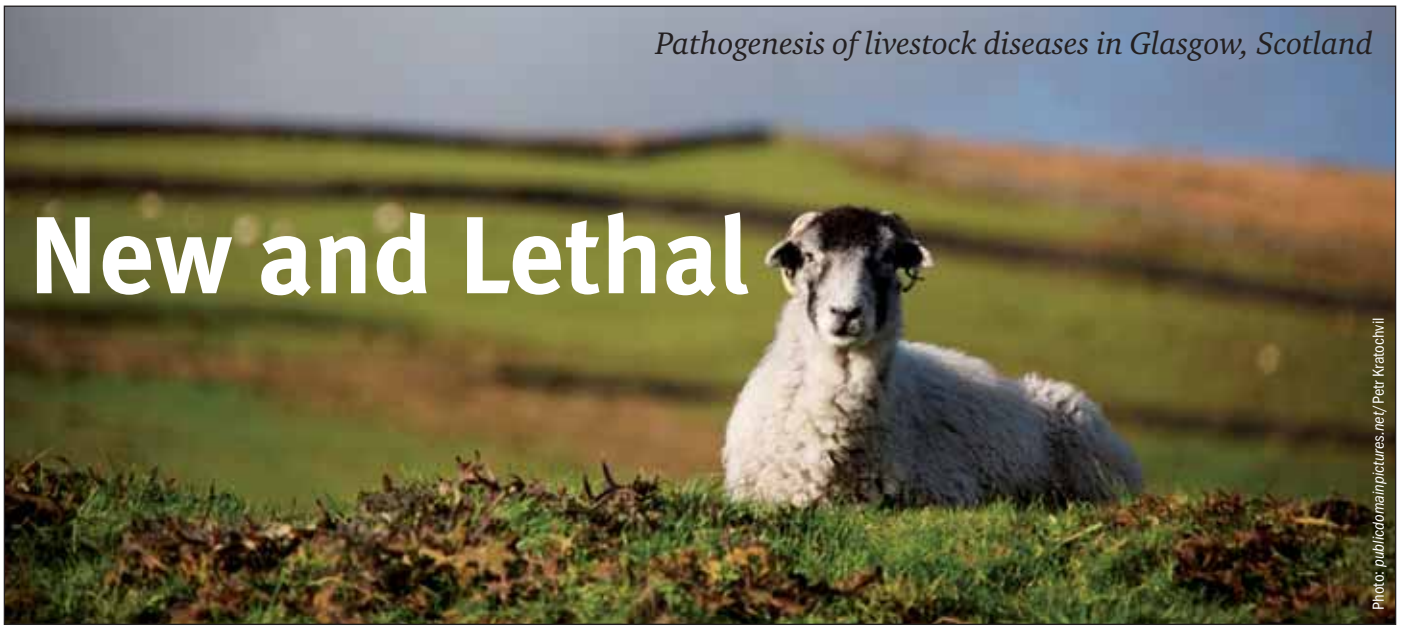


Photo: *publicdomainpictures.net*/Petr Kratochvil

First identified in November 2011, the Schmallenberg Virus has already conquered the whole of Europe, infecting cattle, sheep and goats. A joint effort from scientists in the UK, Italy and Germany has added substantial knowledge about the virus belonging to the family Bunyaviridae.

German towns have a history of donating their name to science, particularly to viruses. After Marburg gave its name to the Marburg virus, a small town in North Rhine Westphalia served as inspiration for the name of a new virus, the Schmallenberg virus (SBV). It's from there that the first virus samples derived: plasma samples from cows that suffered from fever and diarrhoea symptoms. According to the German Friedrich Loeffler Institute (FLI), there was no trace of this virus before November 2011 – its origin is still unclear. Although the virus made its first appearance in Germany, it quickly spread to the rest of Europe, most likely through infected midges.

Mariana Varela, Massimo Palmarini (chair of virology) and Alain Kohl from the MRC Centre for Virus Research at the University of Glasgow and their colleagues became interested in the SBV virus as early as January 2012, just two months after its discovery. Since then, they have made it their aim to develop a new platform, on which to study its pathogenesis, tropism and virus-host interaction. In their recent work, they describe molecular, serological and *in vivo* methods to understand the biology of this virus (*PLoS Pathog* 9(1): e1003133).

Fever, diarrhoea and malformations

Until now, SBV has only been detected in sheep, cattle and goats but antibodies against the virus have also been found in other animals such as bison, roe deer, red deer, mouflon and alpaca. There is currently no evidence that the virus is able to infect humans, too. A study conducted by the Robert Koch Institute in Berlin showed that North Rhine Westphalian sheep farmers had no antibodies against SBV in their blood and showed no signs of ill health.

But in farm animals, the infection spread quickly, made easy by light-winged midges, mosquitoes and ticks. Once infected with the virus, cattle develop fever, diarrhoea and reduce their milk secretion. What's worse, SBV induces late abortions or birth defects in newborn sheep, cattle and goats, including musculoskeletal and CNS malformations.

Since very little was known about the virus, Mariana and co. decided to first study host tropism or what cellular host the virus prefers most *in vitro*. "We used several cell lines from various animal species and humans, and found that SBV shows a very good *in vitro* tropism for cell lines derived from sheep, cow, dog, hamster and human," says Mariana. SBV replicated, for example, very comfortably in sheep CPT-Tert cells. Seventy-two hours post infection, these cell lines showed plaques, regions of cell destruction, of about 3 mm in diameter. Moreover, in another experiment, they found that SBV was easy to transfect in two different cell lines. "SBV is a multifaceted virus replicating in different host cell types," Mariana summarises their experiments.

The emerging Schmallenberg virus might be quick but Mariana Varela, Massimo Palmarini and Alain Kohl are quicker.

Creating a synthetic virus

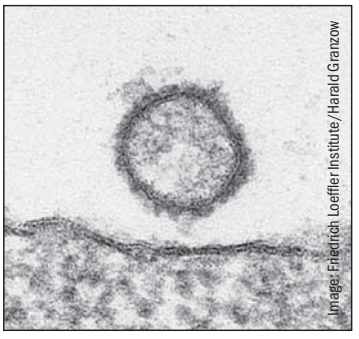
Apart from contributing to the understanding of virus biology, the team worked on developing a novel platform based on reverse genetics to "unravel the underpinning mechanism of viral replication and pathogenesis". The authors believe that by constructing individual viral mutants, it will be easy to identify the role of each viral protein. For their new SBV rescue protocol, they first constructed three plasmids containing full-length antigenome RNA of every viral segment, which was previously synthesised *in vitro*. Then they transfected two different cell lines (human 293-T and hamster BSR-T7 cells) with these plasmids and assessed the presence of the virus in cellular supernatants by the standard plaque assay five days later. "Using this method, we successfully rescued the virus and created 'synthetic' SBV (sSBV) under laboratory conditions," Mariana says.

Curiosity killed the cat claims an old British adage but in the lab it's not the "master" cat that has its tail on the line but the "prey" mouse. A crucial part of all *in vitro* findings is translating them to *in vivo*, using an animal model. Since SBV is a novel virus, Mariana and her colleagues were more than curious to test its virulence in the mouse. "We infected newborn mice intracerebrally with SBV and sSBV and found that sSBV is as virulent as SBV, at least under lab conditions," she reveals. Brain tis-

sue from infected mice displayed severe symptoms of SBV infection such as haemorrhage and necrosis in the cerebral cortex 72 hours after infection. And vacuolation of the white matter of cerebrum with nuclear debris 120 hours post infection. Viral presence was confirmed by immunostaining and showed that the virus exclusively infected neurons.

Proper labelling saves lives

It is important to note that SBV replicates in the central nervous system of infected animals. But infection can be transmitted vertically from pregnant animals to their offspring. Thus, the Glasgow vets studied the neurotropism of SBV *in utero* in naturally-infected lambs and calves. "We collected our samples from SBV-endemic regions in Germany and analysed brain sections for signs of SBV infection. As a control, we used tissue sections from animals we knew died of a different disease." As an interesting lesson, the team used tissue sections from animals that had died before 2001, underlining the importance of proper sample labelling and storage, which is part of good laboratory practice (GLP). By doing this, they did not sacrifice extra animals.



The viral malefactor in electron-microscopical detail.

So, how is the new guest behaving towards its host? To study the molecular determinants and to learn what the virus does to the host immune system, Mariana and co. passaged SBV 32 times in cell culture, to attenuate the virus. They called their clone SBVp32. At the same time, they created a SBV mutant without the non-structural (NSs) protein by reverse genetics and called it SBVΔNS. The NSs protein of several Bunyaviruses is known to play an important role in viral replication and pathogenesis.

Understanding Nature's new gift

Next, Mariana and co. used the attenuated and reverse genetically-designed SBV to find out more about the host immune response. They inoculated mice with the two virus versions intracerebrally. Both sSBV and SBVp32 were lethal but SBVp32 proved to be more virulent, killing all mice a few days after infection. SBVΔNS, on the other hand, delayed death and about half of all mice even survived. "This clearly indicates that the NSs protein has an important role during viral pathogenesis. Our gut feeling tells us that this phenomenon could be due to the inability of the virus to act against the innate immune system of the host," says Mariana.

A microbiological study is only complete when the exact mechanism of the immune response to a particular pathogen is explored. It is known that the NSs proteins of related viruses indirectly inhibit the synthesis of IFN-α and β. Thus, the team wanted to find out whether SBV and SBVΔNS do something similar. To this end, they infected seven-day old IFN receptor null mice intercerebrally and saw all mice succumb to the viral attack, latest by day six post infection. Indeed, the NSs protein seems to modulate the IFN response.

Quick as a flash

Astonishingly, the Glasgow scientists completed their work in less than a year. "It was truly a team effort and each person in the list of authors did something critical. All the pieces came together at the same time, enabling us to create the complete story in such a short amount of time. It would have been impossible to generate all this data with only a single lab or if just a few people would have collaborated," Mariana proudly explains.

As a great bonus, this virus research has attracted the attention of several funding agencies. Since this virus affects livestock and is a common problem in major countries in Europe, the EU has allocated about three million euros for scientific studies on SBV. The Glasgow study, for example, received funding from both the Wellcome Trust and the Medical Research Council (MRC, UK).

As yet, there is no vaccine on the horizon, making it important to protect the animals from infection and thus avert further spread of the disease. According to the FLI, one smart way to do this is to schedule the insemination date to take place before midges become very active in the summer. But this strategy won't bar scientists like Mariana Varela, Massimo Palmarini and Alain Kohl from developing a powerful vaccine. And with their reverse genetics approach together with their *in vivo* infection model, they are on the best path to achieving success.

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