**ABSTRACT**

Bluetongue virus (BTV) is an economically important member of the genus Orbivirus and closely related to African horse sickness virus (AHSV) and Epizootic hemorrhagic disease virus (EHDV). Currently, 26 different serotypes of BTV are known. The virus is transmitted by blood-feeding *Culicoides* midges and causes disease (bluetongue [BT]) in ruminants. In 2006/2007, BTV serotype 8 (BTV-8) caused widespread outbreaks of BT amongst livestock in Europe, which were eventually controlled employing a conventionally inactivated BTV vaccine. However, this vaccine did not allow the discrimination of infected from vaccinated animals (DIVA) by the commonly used VP7 cELISA.

RNA replicon vectors based on propagation-incompetent recombinant vesicular stomatitis virus (VSV) represent a novel vaccine platform that combines the efficacy of live attenuated vaccines with the safety of inactivated vaccines. Our goal was to generate an RNA replicon vaccine for BTV-8, which is safe, efficacious, adaptable to emerging orbivirus infections , and compliant with the DIVA principle.

The VP2, VP5, VP3 and VP7 genes encoding the BTV-8 capsid proteins, as well as the non-structural proteins NS1 and NS3 were inserted into a VSV vector genome lacking the essential VSV glycoprotein (G) gene. Infectious virus replicon particles (VRP) were produced on a transgenic helper cell line providing the VSV G protein *in trans*. Expression of antigens *in vitro* was analysed by immunofluorescence using monoclonal and polyclonal antibodies.

In a pilot study, sheep were immunized with two different VRP-based vaccine candidates, one comprising the BTV-8 antigens VP2, VP5, VP3, VP7, NS1, and NS3, the other one containing antigens VP3, VP7, NS1, and NS3. Control animals received VRPs containing an irrelevant antigen. Virus neutralizing antibodies and

protection after BTV-8 challenge were evaluated and compared to animals immunized with the conventionally inactivated vaccine. Full protection was induced only when the two antigens VP2 and VP5 were included in the vaccine.

To further evaluate if VP2 alone, a combination of VP2 and VP5 or VP5 alone were necessary for complete protection, we performed a second animal trial. Interestingly, VP2 as well as the combination of VP2 and VP5 but not VP5 alone conferred full protection in terms of neutralizing antibodies, and protection from clinical signs and viremia after BTV-8 challenge.

These results show that the VSV replicon system represents a safe, efficacious and DIVA-compliant vaccine against BTV as well as a possible platform for protection against other Orbiviruses, such as AHSV and EHDV.