Development of an animal model for bluetongue virus serotype 8

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In 2006, bluetongue virus serotype 8 BTV8\net06 (IAH collection nr. BTV-8 NET2006/04, Maan et al., 2008) was first detected in the Limburg province, the southern panhandle, of the Netherlands bordering Germany and Belgium. BTV8 has invaded Belgium, Netherlands, Germany, Luxemburg and the northern France. After overwintering, BTV8 reoccurred in several countries in 2007 and following years resulting in the largest BT-outbreak ever recorded. In many aspects, this outbreak strain is different from other BTV-strains, like spread by N-W European species of *Culicoides*, transplacental and oral transmission, and cause of severe disease in cattle (Backx et al., 2007, 2009, Meiswinkel 2007, Dijkstra et al., 2008). The BTV serogroup, family *Reoviridae*, genus *Orbivirus*, contains at least 24 serotypes defined as inducing no or very low levels of cross neutralization. Bluetongue (BT) is an arthropod-borne disease; transmission to ruminants, including cattle, sheep, and goats, occurs by bites of species of *Culicoides*. Since 1998, BTV serotypes 1, 2, 4, 9, and 16 have invaded European countries around the Mediterranean Basin, but these BTV serotypes have not been spread northwards so far.
We have performed research on the basis of BTV8\net07 (IAH collection nr. BTV-8 NET2007/01), the 1st reported BTV-infection in 2007. Extensive sequencing studies were performed on virus passaged once on embryonated eggs, the 3rd passage on BHK21 or KC cells, and directly from blood. All ten complete genome segments were reversely transcribed, PCR-amplified (Potgieter et al., 2009), and sequenced with 454 Roche GS FLX technology. No differences were found after one passage in eggs and three passages in BHK21, and only two differences were found after passage in KC cells. This demonstrated that BTV8\net07 is genetically stable in BHK21 cells, and thus can be reproducibly produced for challenge experiments.
In parallel, we have performed several animal trials with BTV8\net07 in order to develop a reproducible animal model for vaccination/challenge experiments. For this purpose, an infection experiment was performed in sheep and cattle with vireamic blood, and with the above described passages. The dose/animal was normalized by quantitative PCR-signals, therewith realizing that the infective dose/animal could be different between these inocula, in particular with respect to the vireamic blood.
From the summarizing results, we concluded that the 3rd passage in BHK21 was suitable as challenge inoculum, and that sheep show the most obvious clinical signs. In following experiments, this 3rd passage in BHK21 was further investigated in dose-response trials. In addition, virus titers were determined to define the dose of the optimized challenge inoculum. Details and results of these animal trials will be presented and discussed. In summary, a satisfactory and reproducible sheep model is developed in order to test and compare the efficacy and safety of vaccine (candidates) for Bluetongue.

References

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