Reverse genetics for recent field strains of bluetongue virus

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Bluetongue virus (BTV), family *Reoviridae*, genus *Orbivirus*, contains ten double stranded RNA segments encoding at least ten viral proteins. 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. In 2006, BTV8\net06 (IAH collection nr. BTV-8 NET2006/04, Maan et al., 2008) invaded North-Western Europe resulting in the largest BT-outbreak ever recorded. BTV research has a long record (reviewed by Roy 2005), but molecular virology studies desperately needs methods to genetically modify BTV to study features in more detail. Recently, a genetic modification system for bluetongue virus based on uptake of synthetic genome segments was developed, and was used to isolate several reassortants of BTV6 and BTV8 (van Gennip et al., 2010). BTV-rescue with mutated genome segments was expected to be more difficult due to less viability of these mutant BTVs in the presence of wild type BTV. Classical reverse genetics has been published for BTV1 (Boyce et al., 2008), but reverse genetics is not published for virulent BTV, such as BTV8\net06 and other related orbiviruses.
The first attempts to develop reverse genetics for BTV8\net06 based the published sequence (Maan et al., 2008) have failed. Extensive sequencing studies were performed on BTV8\net07 (IAH collection nr. BTV-8 NET2007/01) from a 1st passage on embryonated eggs, BHK21 and KC cell cultures and directly from blood. All ten complete genome segments were reversely transcribed, PCR-amplified (Potgieter et al., 2009), and sequenced with 454 technology (Roche GS FLX technology). Generated sequences were compared with published data to determine the consensus sequences. Subsequently, previously derived cDNAs based on the published sequences were corrected with respect to the consensus amino acid sequences. In parallel, reverse genetics for BTV1 (Boyce et al., 2008) was reproduced, and optimized. Therefore genome segments were cloned under control of the T7 RNA-polymerase promoter and were flanked by a restriction enzyme site at the 3'-terminus. Capped positive stranded run-off RNAs were synthesized *in vitro*. BSR-monolayers were transfected twice with mixtures of purified *in vitro* synthesized RNAs. The mixture of the first transfection contains positive sense capped RNAs of six segments, whereas after 18 hours all ten RNAs were transfected. A similar approach was followed to develop reverse genetics for virulent BTV8\net06, and nonvirulent BTV6\net08. For the latter, the consensus sequence was determined as described above (Maan et al., 2010). In summary, classical reverse genetics was developed for cell-adapted BTV1, virulent BTV8\net07, and nonvirulent or vaccine strain BTV6\net08. The presented reverse genetics for BTV is very promising as genetic modification system as demonstrated in several presentations/posters. These results further show the feasibility of genetic modification of related orbiviruses, like African horse sickness virus and enzootic hemorrhagic disease virus.

References

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