EMIDA-ERANET-Project "OrbiNet": Molecular and reverse genetics studies of orbivirus transmission, host responses, epidemiology and diagnostic systems

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Key words
bluetongue, Toggenburg Orbivirus, reverse genetics, reassortants, recombinant proteins, VP2, ELISA,

Aim of the study
Toggenburg Orbivirus (TOV) is tentatively classified as bluetongue virus (BTV) serotype 25. To study the replication mechanisms of TOV which does not grow in cell culture it was planned to create reassortants of TOV and BTV-1. A second project aim was the development of tools for specific TOV antibody detection, i.e. ELISAs using recombinant VP2.

Material and methods
TOV-/BTV-1 reassortants were created by standard reverse genetics techniques. Growth properties of infectious reassortants were studied based on replication kinetics. VP2 of TOV was cloned and expressed in various expression systems. Both full-length as well as truncated forms of tagged VP2 were expressed and tested for their suitability as ELISA antigens. Furthermore an overlapping peptide library was synthesized in order to identify TOV-specific linear epitopes on VP2.

Results and significance
The BTV reverse genetics techniques could be established at the IVI. This allowed to rescue reassortants of TOV and BTV-1. In the context of a collaboration with P. van Rijn, Lelystad, Netherlands, it was shown that TOV genome segments S2, S6, S7 and S10 are functional in the context of BTV-1. However, the more BTV-1 segments were exchanged with the respective TOV segments, the less efficient was the replication of the reassortant viruses.
A VP2-based ELISA for the specific detection of TOV antibodies could not be developed. The main reasons were that (i) full-length VP2 expression failed, and (ii) TOV-specific epitopes on VP2 could not be identified, neither by using truncated versions of VP2, nor by
a microarray-based VP2 peptide scan.

Publications, posters and presentations
Müller, M.; Hofmann, M.A. (2011) Toggenburg Orbivirus, epidemiology and diagnosis. Oral presentation at the first EMIDA OrbiNet Meeting, Paris, 9th May, 2011.
Liniger, M.; Hofmann, M.A. (2012) Toggenburg Orbivirus: Towards the establishment of reverse genetics and the development of a novel VP2-based antibody ELISA. Oral presentation at the second EMIDA OrbiNet Meeting, Paris, 16th May, 2012.
Vögtlin, A.; Hofmann, M.A.; Nenniger, C.; Liniger, M.; Renzullo, S.; Steinrigl, A.; Loitsch, Y.; Schwermer, H.P.; Kaufmann, C.; Thür, B. (2013) Persistent infection of goats with BTV-25 (Toggenburg orbivirus). Oral presentation at the second EMIDA OrbiNet Meeting, Paris, 26th May, 2013.

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