Development of Novel Diagnostic Assays for Orbiviral Diseases of Domestic and Wild Animals

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The recent emergence and spread of bluetongue virus (BTV) across the whole of Europe suggests that other orbiviruses could also emerge to threaten livestock species and wildlife populations in Europe and other parts of the world. The genus *Orbivirus* is the largest within the family *Reoviridae*, containing 22 virus species, as well as 14 unclassified orbiviruses. The orbiviruses are transmitted primarily by arthropod vectors (e.g. *Culicoides*, mosquitoes or ticks) and several are associated with severe and economically important diseases of livestock, including BTV in cattle and sheep, African horse sickness virus (AHSV), equine encephalosis virus (EEV) and Peruvian horse sickness virus (PHSV) in equids, as well as epizootic haemorrhagic disease virus (EHDV) in wild ungulates or cattle.

Recent incursions of BTV in Europe, Southeastern USA, Australia and Asia, EHDV in North Africa, the Middle East and the Mediterranean region, AHSV in sub-Saharan Africa, and EEV in Israel and Gambia, indicate a need for the development of faster, more sensitive and more reliable diagnostic assays. These are required to detect and identify rapidly the viruses and virus types involved, monitor their incidence and movement, and identify infected animals. The *Orbivirus* genome is composed of 10 linear segments of double-stranded ribonucleic acid (dsRNA), each segment coding for at least one viral protein. The outer capsid proteins VP2 and VP5 are situated on or near the surface of the virus particle and are more variable than components of the virus core, or the non-structural proteins. VP2 (encoded by Seg-2) is the outermost of the BTV capsid proteins and represents the primary target antigen for neutralising antibodies, and hence Seg-2 is a target for the development of type-specific nucleic-acid-based diagnostic assays. In contrast, the genome segments coding for protein components of the virus core and/or the non-structural proteins can be used as targets for development of serogroup (virus-species) specific, reverse transcription - polymerase chain reaction (RT-PCR) based diagnostic assays.

Virus species-specific and type-specific conventional (gel based) RT-PCR diagnostic assays, for the detection, identification and typing of some of these viruses (BTV, EHDV and AHSV), have been developed using the sequence data for segments 7 and 2, respectively. Initial evaluation studies indicate that these assays are reliable, specific, do not cross-react with related orbiviruses (group/species specific) or with related types (type specific). Although they are labour intensive, the results obtained can be confirmed by sequence analyses of the resulting complementary deoxyribonucleic acid complementary (c) DNA amplicons, and phylogenetic comparisons to determine the strain of virus involved. However, conventional RT-PCR assays are prone to cross-contamination, potentially leading to false positive results.

The authors also describe group-specific real-time RT-PCR assays that use a 'closed-tube' format, which are therefore less susceptible to cross-contamination. These assays target the conserved genome segment 9, or genome segment 1, which can be used to detect all of the serotypes, as well as geographic variants (different topotypes) of BTV, EHDV, AHSV, EEV and PHSV. Type-specific real-time RT-PCR assays that target the most variable genome segment 2 can be used to differentiate 25 serotypes of BTV or the seven serotypes of EHDV. These diagnostic assays were found to be very sensitive, reproducible and suitable for rapid screening of field samples. Results will be presented from studies to optimise these RT-PCR assays.

Keywords: *Orbivirus – Bluetongue virus – Epizootic haemorrhagic disease virus – African horse sickness virus – Equine encephalosis virus – Peruvian horse sickness virus* – Diagnosis.

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