Identification of regions of nonstructural protein NS3 involved in membrane destabilization in different Orbiviruses.

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Bluetongue virus (BTV), African horsesickness virus (AHSV) and equine encephalosis virus (EEV) are members of the Orbivirus genus within the family Reoviridae. Pathological features of orbiviral diseases include haemorrhages and oedema, associated with permeability changes in endothelial cells. These Orbiviruses have genomes comprised of ten segments of double-stranded RNA, the smallest segment (S10) encoding NS3. BTV and AHSV NS3 are membrane associated proteins involved in virus release. AHSV NS3 is a cytotoxic protein with membrane damaging capabilities potentially involved in virus virulence and pathogenesis. To analyze and compare changes in membrane permeability full-length or truncated Orbiviral NS3 genes were cloned and inducibly expressed in BL21(DE3) cells using the pET vector system. Optical density and Hygromycin B assays were used to monitor growth and membrane permeability of induced cultures. Significant differences in the degree of cytotoxicity of the different orbivirus NS3 proteins were observed, with the inhibition of cell growth ranging from 80.16% to only 10.4%. Expression and analysis of mutants of AHSV NS3 identified two conserved hydrophobic domains, postulated to form transmembrane regions, as essential for increased membrane permeability and cell lysis. In contrast, EEV NS3 mutants that contained either one or both of the hydrophobic domains had membrane-active properties, suggesting different functional constraints for these proteins. N-terminal and C-terminal fragments of AHSV NS3 where furthermore expressed as GST fusion proteins. Affinity purification and tag cleavage provided sufficient protein for further functional characterization and future production of antibodies specifically targeted to these regions of NS3.