Investigation of Saliva Proteins from Different *Culicoides* Species and their Interaction with the Bluetongue Virus Particle

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The transmission, infectivity and virulence of many arthropod transmitted pathogens can be influenced by components of saliva from their arthropod vector. *Culicoides* saliva is therefore considered likely to play an important role in orbivirus transmission mechanisms. Difficulties in collecting saliva from such small insects have until recently limited our ability to analyse and identify *Culicoides* saliva proteins, or determine their functions and effects on the transmission efficiency, infectivity or virulence of these viruses. However, we have recently developed an efficient method to collect relatively large quantities of uncontaminated *Culicoides* saliva proteins, using protein binding filters. Saliva proteins were collected from *C. nubeculosus* [an inefficient bluetongue virus (BTV) vector from Europe] and *C. sonorensis* (a North American vector of BTV and epizootic haemorrhagic disease virus) for direct protein sequencing by electrophoresis and mass spectrometry. These analyses have identified a trypsin-like protease in *C. sonorensis* saliva, which was significantly reduced or absent from *C. nubeculosus* saliva. Previous studies have shown that the larger of the BTV serotype 1 (BTV-1) outer-capsid components (the cell attachment protein 'VP2') can be cleaved by proteases, forming infectious subviral particles (ISVP) that have an enhanced infectivity for adult *Culicoides*, or KC cells (a cell-line derived from *C. sonorensis*). We show that treatment with saliva from adult *Culicoides* also cleaved VP2 from several different BTV strains. This cleavage appears to be a step-wise process, with intermediate cleavage products being generated at lower temperature or lower saliva protein concentrations. Incubating purified BTV-1 particles with *C. sonorensis* saliva also increased their infectivity for KC cells about 10 fold, while infectivity for baby hamster kidney (BHK) cells was reduced by 2-4 fold. The saliva proteins from *C. sonorensis* cleaved BTV-VP2 more efficiently than those from *C. nubeculosus*, suggesting that the level of protease activity in the insect saliva could help determine the efficiency of BTV infection in the insect. The saliva protein collection method is now being applied to wild caught *C. imicola* (the South African BTV vector) to investigate whether the composition of the saliva protein is involved in the determination of vector competence.

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