Exploring Variation in Infection of Different Bluetongue Virus Particle Types in *Culicoides* Biting Midges

Figure 1. A *Culicoides* biting midge

Rosie Hobbs[1], Dr Christopher Sanders[2], Dr Laura Jones[2], Dr Marc Guimerà Busquets[2]

1. The University of Sheffield, Western Bank Sheffield S10 2TN. 2. The Pirbright Institute, Ash Road, Pirbright, Woking, GU24 0NF, Email: rhobbs3579@gmail.com

Introduction

Culicoides biting midges (Diptera: Ceratopogonidae) are small hematophagous insects. They serve as viral vectors for over 50 viruses including the economically important *Orbiviruses* (Family: *Sedoreoviridae*) of livestock, bluetongue virus (BTV), epizootic haemorrhagic disease virus (EHDV) and African horse sickness virus (AHSV). However, not all species of *Culicoides* are competent vectors and even within a competent species many individuals are unable to transmit disease due to barriers to infection and dissemination within the insect. BTV has over 29+ serotypes which may have different levels of infectivity in *Culicoides*. BTV has also been shown to have different virus particle types. Infectious subviral particles (ISVPs) are formed when the outer proteins of viral particles are cleaved by interaction with serine proteases (Fig. 2). BTV ISVPs have been reported to be approximately 100-fold more infectious than whole virus particles (WP) to insect cell lines (*Culicoides*, KC cells). In addition, ISVPs are generated in the presence of the saliva of a competent vector but not that of non-vector species. The role proteases and ISVPs play in determining susceptibility of vectors to infection with different BTV strains is not clear. Here we examine the effect of viral particle type of different BTV serotypes on the infection of a known vector of BTV, *Culicoides sonorensis,* and explore the interaction between virus serotype, insect proteases and infection rates of *Culicoides* to increase understanding of the drivers of infection to help mitigate future outbreaks.

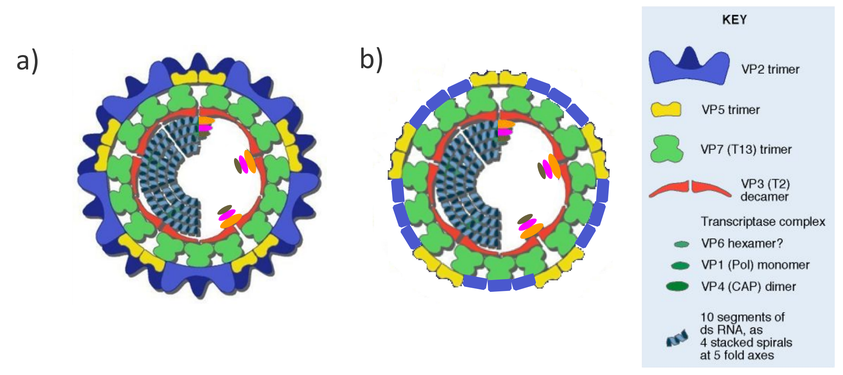
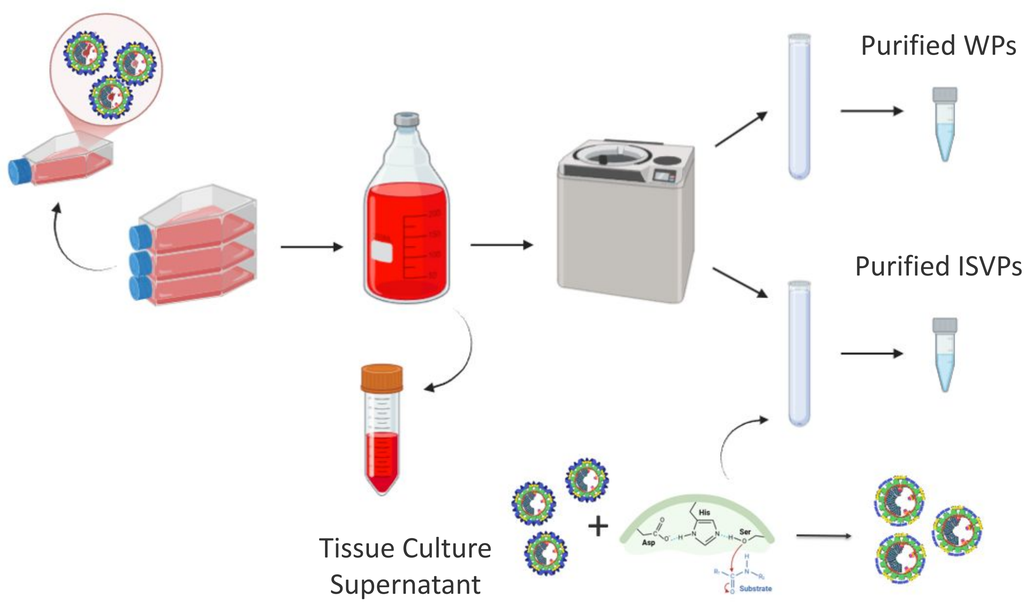


Figure 2. Virus Particles. a) Whole BTV viral particle. b) Infectious Subviral Particle where VP2 has been cleaved by treatment with serine protease such as trypsin and chymotrypsin

Methods

Generating different BTV particle types



BTV-3 and BTV-4 were grown in BSR cells, harvested and homogenised to generate tissue culture supernatant (TCS). The cell culture homogenate was purified through separation in a sucrose gradient to generate whole virus particles (WPs). ISVPs were generated through chymotrypsin treatment of each virus for 1h at 37°C.

In vivo infection of *Culicoides* with different particle types

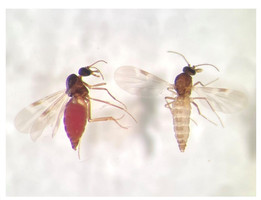


Figure 3.
Blood-fed vs unfed
Culicoides
Image provided by the Entomology group

Figure 4. Gravid female
Culicoides
Image provided by
Melanie Nicholls

Created in https://BioRender.com

Culicoides sonorensis were fed using a BTV WP, ISVP or TCS- spiked bloodmeal via Hemotek™. Engorged females (Fig. 3) were selected and maintained at 25°C for 8 days then gravid survivors (Fig. 4) were homogenised individually. RNA was extracted from each homogenate and BTV genome detected and quantified using qRT-PCR. Protease inhibitors were used in some bloodmeal treatments to prevent or reduce cleavage of viral proteins occurring in the midge.

Results

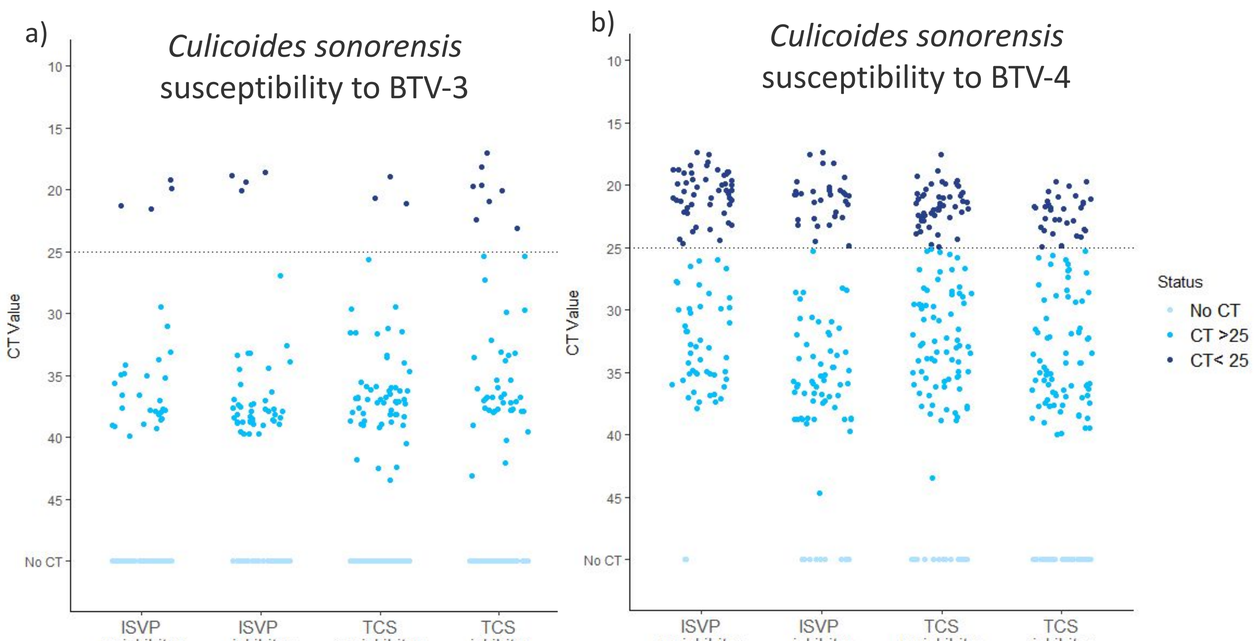


Figure 5. Detection of BTV genome in *C. sonorensis* fed tissue culture supernatant (TCS)-derived virus or generated ISVPs of BTV-3 (a) and BTV-4 (b). CT values from qRT-PCR of individuals are shown. Each BTV particle type group was further split into treatment with protease inhibitor and without. Those individuals with a CT value of below 25 (dotted line) can be considered to have a transmissible level of infection. Those with no CT were not infected whereas intermediate values suggest sub-transmissible infection of the individual.

C. sonorensis was highly susceptible to BTV-4 (85.63% infection) and less susceptible to BTV-3 (36.11% infection), regardless of the presence of ISVPs or protease treatment (Fig. 5). The greatest infection rate was observed in *Culicoides* fed BTV-4 ISVP in the absence of protease inhibitors ($P<0.05$). This treatment introduces the most opportunities for cleavage from the lab treatment and midge proteases. Inclusion of a protease inhibitor significantly reduced infection and dissemination rates for BTV-4 in both the ISVP group ($P = 0.037$) and the TCS groups ($P = 0.023$). Susceptibility to BTV-3, in contrast, was greatest in the group fed TCS derived BTV in the presence of the protease inhibitor where there was the lowest cleavage possibility, although this was not significant. *Culicoides* fed bloodmeals spiked with ISVPs in the presence of a protease inhibitor had similar infection levels as midges treated with TCS and no protease inhibitor ($P = 0.362$)

Conclusions

- Infection susceptibility of *Culicoides* to BTV of different serotypes may be differentially influenced by a complex interaction of BTV viral particles with proteases within *Culicoides.*
- Preventing interactions between vector proteases and viral particles **reduces** the number of individuals that become infected and competent vectors of BTV-4 but may **increase** susceptibility to BTV-3
- ISVPs may interact with midge proteases permitting a further increase in susceptibility to BTV-4 and reduction in BTV-3, suggesting further complexity in this interaction.
- Can we use our understanding of the role of ISVPs in vector infection to predict emergence and transmission of BTV strains within different vector populations?

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