During African horse sickness virus infection the virus inclusion bodies contribute to host translation suppression by sequestering ribosomes and compartmentalising viral translation.

Venter, E.[1], Yssel, L.[2] and van Staden, V.[3]

[1] Laboratory for Microscopy and Microanalysis, University of Pretoria, South Africa, [2] Department of Genetics, University of Pretoria, South Africa, [3] Department of Biochemistry, Genetics and Microbiology, University of Pretoria, South Africa

African horse sickness virus (AHSV) is an arthropod-borne virus endemic to sub-Saharan Africa. Infected equids develop African horse sickness, an often lethal disease in susceptible horses. AHSV is a member of the *Orbivirus* genus in the *Reoviridae* family and has a double stranded RNA genome. Ten genome segments encode structural proteins responsible for virion formation, and non-structural (NS) proteins which play a major role in viral morphogenesis (Roy *et al.,* 1994). The virus is reliant on different host machineries throughout its life-cycle. Often these host machineries are subverted to favour viral replication, whilst suppressing host cell functionalities. For *Reoviridae* members, virus replication results in the formation of characteristic cytoplasmic inclusions. In AHSV, NS2 oligomers aggregate to form these virus inclusion bodies (VIBs) (Brookes *et al.,* 1993). Until recently, it was believed that the sole purpose of the *Reoviridae* inclusions was to recruit viral RNA and structural proteins required for the replication and assembly of new viral particles. However, it has recently been found that these reovirus viral factories recruit cellular translation machinery and thereby become the site of viral protein synthesis (Desmet *et al.* 2014).

The aim of this study was to investigate mechanisms by which AHSV down-regulates host translation whilst up-regulating viral protein synthesis. To this end, AHSV VIB maturation (abundance and morphology) through the infection cycle, as well as the ability of the VIBs to recruit host cell translation apparatus and house protein synthesis, was investigated. To test whether these properties were solely influenced by NS2, or act in combination with other AHSV proteins, a recombinant baculovirus expressing NS2 (BacNS2) was used as control. Confocal laser scanning microscopy was used on BSR and Sf9 cells infected with AHSV-4 and BacNS2 respectively. Cells were labelled with anti-NS2, in combination with antibodies directed against other cellular markers or components as required, and visualised with a Zeiss LSM 880.

In AHSV-infected cells, NS2 was detected as early as 4 hpi, initially showing a combination of diffuse distribution and small cytoplasmic foci (Fig. 1A). These foci seemed to coalesce as infection progressed, with on average five large foci (VIBs) detected per cell late in infection (Fig. 1B). Some of these larger foci showed increased NS2 signal on the periphery, indicating the potential for altered NS2 distribution during the maturation process. Similar NS2 foci were also present in BacNS2-infected cells. In AHSV-infected cells, a subset of VIBs was shown to exhibit colocalization between NS2 and L11, a protein component of the large ribosomal subunit (Fig. 1C - 1E). Following this a puromycylation assay was conducted, where only actively translating ribosomes are detected by immunolabelling. The puromycylated ribosomes colocalized with NS2 inside the large VIBs, confirming that active translation occurred in some foci. Preliminary results therefore indicate that ribosomal components are recruited to the VIBs, compartmentalizing and up-regulating viral translation thereby suppressing host protein synthesis. These results indicate the potential for AHSV VIBs to have additional functions unreported thus far. Also, AHSV VIBs might have different functions based on their maturity and/or NS2 distribution.

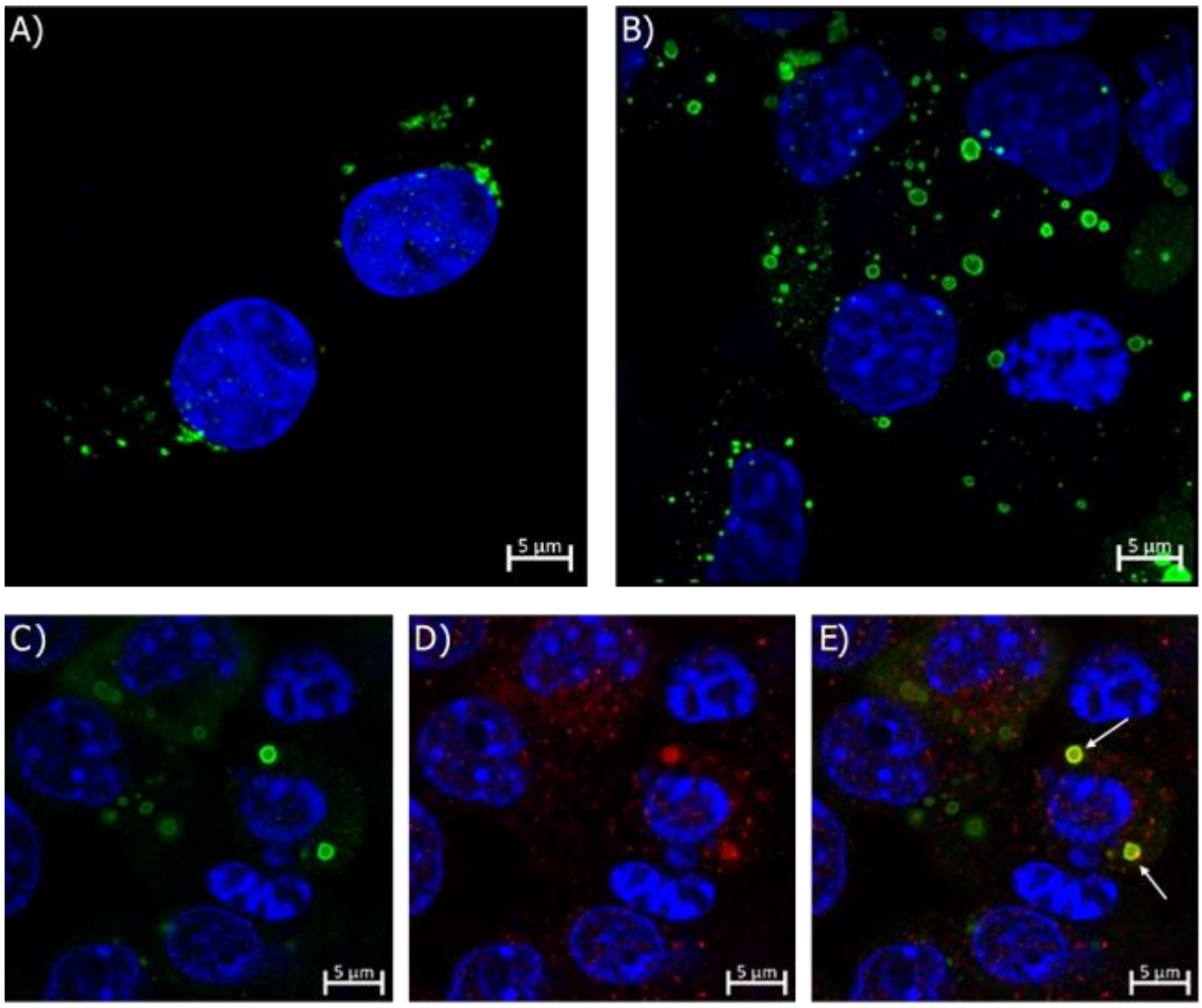

Figure 1. CLSM images of AHSV-4 infected BSR cells. The size and distribution of NS2 foci were compared early (A) and late (B) in the infection cycle. Co-labelling was done with viral and cellular ribosome-specific antibodies to indicate potential interaction of ribosomal subunits with AHSV VIBs (C - E). Ribosome-containing NS2 foci indicated by arrows. Fluorescent stains: DAPI for nuclei, anti-NS2/AF488 for NS2 foci, anti-L11/AF594 for ribosomes.