Comparative nuclear localisation of African horse sickness virus non-structural protein NS4 across all serotypes

Boughan, S.[1], Venter, E.[2], Potgieter, C.[3] and van Staden, V.[1]

[1] University of Pretoria, South Africa, [2] Laboratory for Microscopy and Microanalysis, South Africa, [3] Deltamune (Pty) Ltd, Pretoria, South Africa

The disease African horse sickness is caused by African horse sickness virus (AHSV), with mortality rates of up to 90% of infected horses. AHSV remains an important virus in Southern Africa, and is listed as a notifiable animal disease by the World Organisation for Animal Health. AHSV has nine serotypes, and a viral genome comprised of ten double stranded RNA (dsRNA) segments encoding seven structural and four non-structural proteins. The recently discovered non-structural protein NS4, the smallest of the AHSV proteins, is the focus of this study. NS4 has been shown to localise to both the nucleus and the cytoplasm of AHSV infected cells, even though all replication processes of AHSV occur exclusively in the cytoplasm (Zwart *et al.*, 2015). It is hypothesised that NS4 from the related bluetongue virus (BTV) may inhibit host immune response pathways, thereby giving BTV a replication advantage (Ratinier *et al.*, 2016). Whereas BTV NS4 is highly conserved across all serotypes, AHSV NS4 displays two forms of NS4 (NS4-I and NS4-II) which differ in sequence and length. NS4-II also exists as a sub-type named NLS-NS4-II which contains an N-terminal nuclear localisation signal. Little is known about the functional importance of AHSV NS4 in the nucleus, therefore the aim of this study was to compare the localisation and role of NS4 in different nuclear compartments across all AHSV serotypes.

A total of 18 AHSV strains were amplified, representing the reference strains of the nine serotypes plus one recent field isolate from each. Genomic dsRNA was isolated from all strains, and the NS4 gene sequences determined. Virus-infected cells were harvested and the sizes of the expressed NS4 proteins compared by SDS-PAGE and Western blot using monospecific anti-NS4 antibodies. To visualize the intracellular localisation of NS4, AHSV-infected mammalian cells were immunolabelled with anti-NS4 serum and viewed with a Zeiss LSM880 confocal laser scanning microscope (CLSM) with Airyscan detector for super resolution microscopy.

Three different NS4 intracellular distribution profiles were observed across all samples, in each case corresponding to the NS4 gene sequences harboured by that specific virus. The smallest of the proteins, NS4-I, mainly showed a homogenous distribution throughout the nucleus. NS4-II was homogenous in the cytoplasm and the nucleus, while NLS-NS4-II displayed distinct punctate nuclear foci (Fig. 1). Different nuclear compartments are sometimes hijacked by viruses to inhibit the host machinery or immune response pathways. CLSM was used to determine if NS4 co-localises with any subnuclear compartments. We observed that AHSV infection of certain serotypes resulted in relocation of a component of nuclear speckles to virus-specific inclusions in the cytoplasm (Fig. 1). This is the first study to show that a nuclear compartment relocates to the cytoplasm of AHSV-infected cells. NS4 localised to another nuclear compartment, which in turn may suggest a role in inhibiting the immune response pathway. In conclusion, this study shows that serotype-specific differences exist for AHSV NS4. Because NS4 may localise to nuclear compartments involved in the immune response, NS4 may act as a virulence factor which is important in vaccine development.

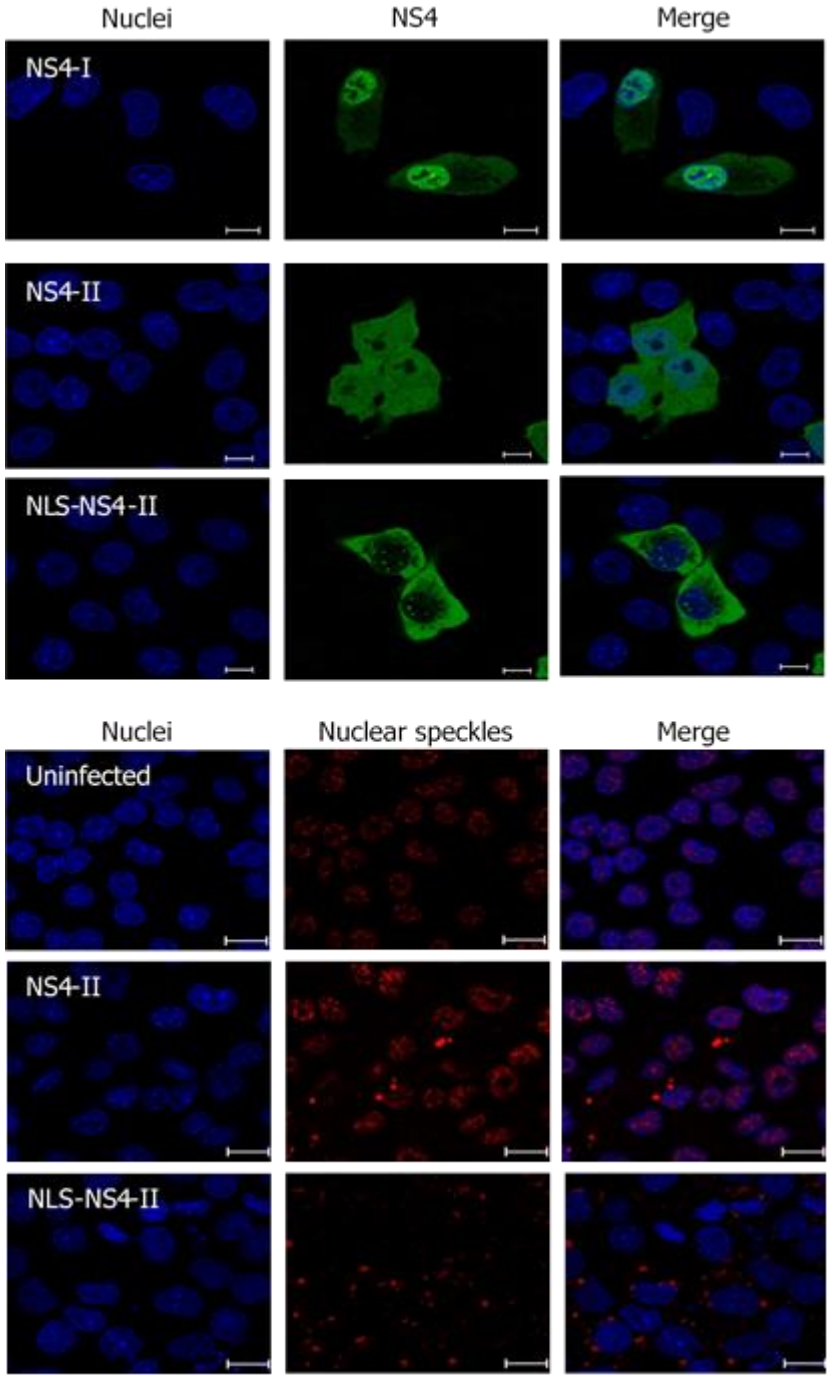

Figure 1. AHSV infected mammalian cells. BSR-T7 cells were uninfected or infected with specific AHSV serotypes expressing NS4-I, NS4-II or NLS-NS4-II as indicated and fixed to coverslips at 24 hours post infection. Cells were labelled with primary anti-NS4 (NS4) or anti-SC35 (nuclear speckles) and Alexa Fluor 488 or 594, respectively. The nuclei were labelled with DAPI (Nuclei), merged images are shown and scale bars represent 10µm.