Nucleotide sequence comparison of the segments S10 of the nine African horsesickness virus serotypes

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Summary. Segments 10 (S10) of the double-stranded RNA (ds RNA) genomes from African horsesickness virus (AHSV) serotypes 2, 4, 5, 6 and 7 were cloned and sequenced. Direct sequencing of previously reverse transcribed amplified (RT)-PCR segments S10 was also performed. Nucleotide sequences of two strains (the virulent Moroccan strain and a vaccine strain) of the same serotype (4) were determined. Sequences of the viral serotypes were analysed and compared to each other. Two in-phase ATG codons were conserved in the S10 of AHSV-2, AHSV-4, AHSV-5, AHSV-6 and AHSV-7 and were considered candidate translation initiation codons of NS3 and NS3A respectively. A close relationship between serotypes 4, 5 and 9 and between serotypes 3 and 7 were described. Closer relationships of serotype 2 to the 1 and 8 group on one hand and of serotype 6 to the 4, 5 and 9 group on the other hand were observed.

Introduction

African horsesickness is an infectious disease of equidae associated with high morbidity and mortality. It is caused by an insect-borne orbivirus, African horsesickness virus (AHSV), which belongs to the Orbivirus genus in the family Reoviridae (bluetongue virus -BTV- is the prototype orbivirus) [3, 28]. The virus is transmitted by culicoides species viz. C. imicola [6] and possibly mechanically by other biting insects, e.g., mosquitoes. There are nine virus serotypes which can be distinguished in a neutralization assay [17]. AHS occurs mainly in southern, eastern, western and central Africa, although it has occurred in the Middle East and parts of Asia since 1944 and recently in Spain.

In July 1987, Spain experienced an outbreak following the importation of zebras (in which clinical signs are less obvious than in horses) from Namibia [25]. The disease caused by type 4 AHSV, spread to Portugal (1989) and Morocco (1989–1991).
The AHSV genome is composed of ten double-stranded RNA segments [1, 22]. There are seven structural proteins which form a double-shelled virus particle. The outer capsid is composed of the two major proteins (VP2 and VP5), which are responsible for viral neutralization and antigenic variability whereas the inner capsid is composed of two major (VP3 and VP7), and three minor (VP1, VP4 and VP6) proteins [2, 8]. VP3 and VP7 are highly conserved among the nine serotypes [2, 22]. At least three non-structural proteins NS1, NS2 and NS3 have been identified [9, 20, 26]. Two related protein products were synthesised from the smallest genome segment of AHSV, segment 10 (S10), in in vitro translation studies, and their counterparts identified in AHSV-infected cells. These proteins were termed NS3 and NS3A [25] but they have also been described as NS4 and NS4A [8] and P21 and P20 [15]. Very little is known about the role of NS3/NS3A during virus replication. Van Staden et al. [27] have proposed a model for the membrane-associated topology of NS3.

As De Sa et al. [5], Laviada et al. [17] and Van Staden et al. [27] reported, sequence data on the segment 10 genes of a number of AHSV serotypes (1, 3, 4, 8, 9) indicated that these genes were not as conserved as within the BTV serogroup but no comparison of the S10 gene products of all the AHSV serotypes has been done. In this study, we complete the work of De Sa et al. [5] by reporting nucleotide sequence information for segment 10 from AHSV serotypes 2, 4, 5, 6 and 7 and the nucleotide sequences of two strains, a virulent and a vaccine strain.

Materials and methods

Viruses and cell

The vaccine strains of the AHSV serotypes 2, 4, 5, 6 and 7 were kindly provided by Dr House (US Dept. of Agriculture, Foreign Animal Disease Diagnostic Laboratory, Greenport, New York). All other strains were obtained from South Africa (Dr. Erasmus, Onderstepoort Veterinary Research Institute, South Africa) where they had been passaged 100 times in suckling mice via intracranial inoculation in 1968.

All AHSV viruses were propagated on Vero (ref ATCC* CC1 81) cell lines in 175-cm² flasks in growth medium RPMI 1640 supplemented with 8% foetal calf serum, streptomycin (100 μg/ml) and penicillin (100 UI/ml). The infected monolayers showed cytopathic effects from 48 h to 96 h later depending on the strains. The titres, as determined by the Reed and Muench [24] method of estimating 50% end-points, varied from $10^5$ to $10^7$ TCID$_{50}$/ml.

The Moroccan strain was isolated from one splenic sample of an infected horse by inoculating monolayers of Vero cells (this sample was graciously provided by Dr. El-Harrack, Biopharma Laboratory, Rabat). The serotype was determined by neutralization test [24].

* American Type Culture Collection