The generation of bluetongue virus reassortants in cattle

Introduction

Bluetongue is a non-contagious economically important disease of domestic and wild ruminants. The disease is caused by the bluetongue virus (BTV), a double stranded segmented RNA virus transmitted by *Culicoides* midges (Diptera: Ceratopoginidae). In South Africa sheep are susceptible to the disease, while cattle and goats are in general sub-clinically infected. Twenty seven serotypes of the virus occur worldwide and at least 22 occur in South Africa. When the host or vector is simultaneously infected with more than one serotype or strain of BTV, reassortment of genome segments may take place and viruses with different phenotypic characteristics may be generated. In South Africa the disease is mainly controlled by vaccination. The currently used vaccine consists of 3 bottles, each containing 5 different live attenuated serotypes, which are administered to sheep 3 weeks apart. Both vaccine and wild type strains circulate in South Africa. The possibility therefore exists that reassortment may occur between genetically and phenotypically diverse strains in either the insect or ruminant host. This has the potential for the emergence of viruses with different phenotypic characteristics i.e. virulence and/or the ability to cross the ruminant placenta. In general cattle are not vaccinated in South Africa however at least 97% have antibodies to BTV. Cattle may therefore act as important hosts for multiplication and possible reassortment of the virus.

Aim

The aim was to investigate the potential generation of reassortants in cattle simultaneously infected with BTV field and vaccine strains.

Materials and Methods

Six BTV negative cattle were infected with both vaccine strains present in Bottle 2 of the vaccine and a wild-type BTV-4 strain. Blood samples were collected daily for 21 days post infection. BTV was directly isolated from the buffy coat using a plaque forming assay. Viruses were typed by plaque neutralization assay using type specific antiserum (Figure 1), and further characterized by next generation sequencing (NGS) (Miseq sequencing). Genetic profile analysis of the isolated viruses was compared to the parental vaccine strains using PAGE gel electrophoresis. Viruses showing differences in their electrophoretic profiles were selected and sequenced using NGS. Sequences were compared to the parental strains (in progress). The different possible reassortant strains have also been tested for their ability to cause cytopathic effect in Vero cell cultures by means of a CellTiter-Blue Cell viability assay (Promega).

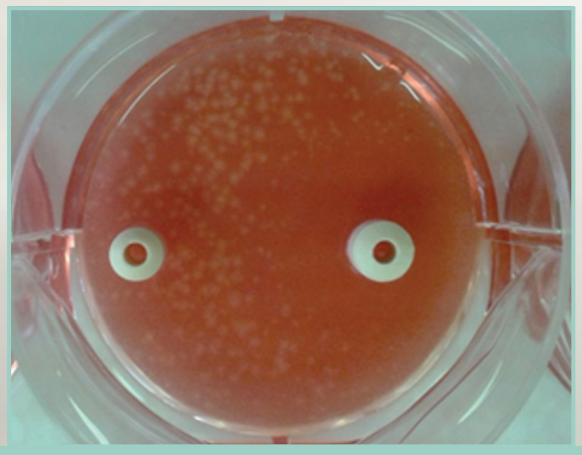

Figure 1: Parental strains were isolated using a serum neutralization assay.

Results

In total, 11 potential reassortant strains were isolated from the buffy coat of experimentally infected cattle. Clear mobility shifts were observed between the isolates grown directly from the buffy coat and the parental strains as indicated in Figures 2 and 3.

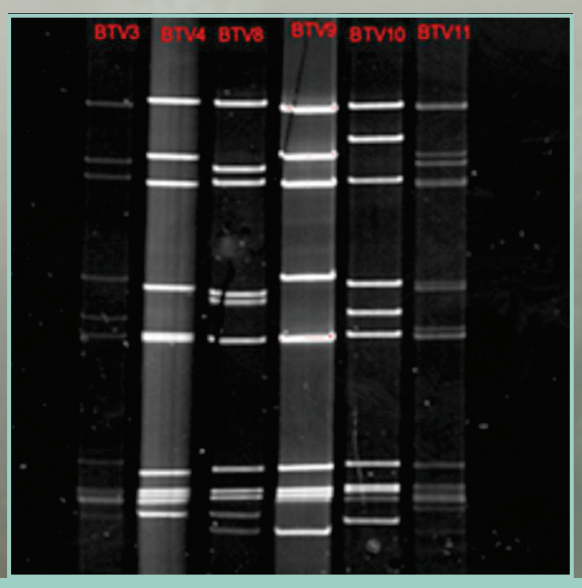

Figure 2: Parental strains were isolated from Bottle 2 of the BTV vaccine using plaque purification and typed using a serum neutralization assay. All 10 segments are visible and each of the serotypes has a unique profile. The serotypes include BTV serotype 3, 8, 9, 10, and 11. The wild-type BTV serotype 4 was also used as a parental strain.

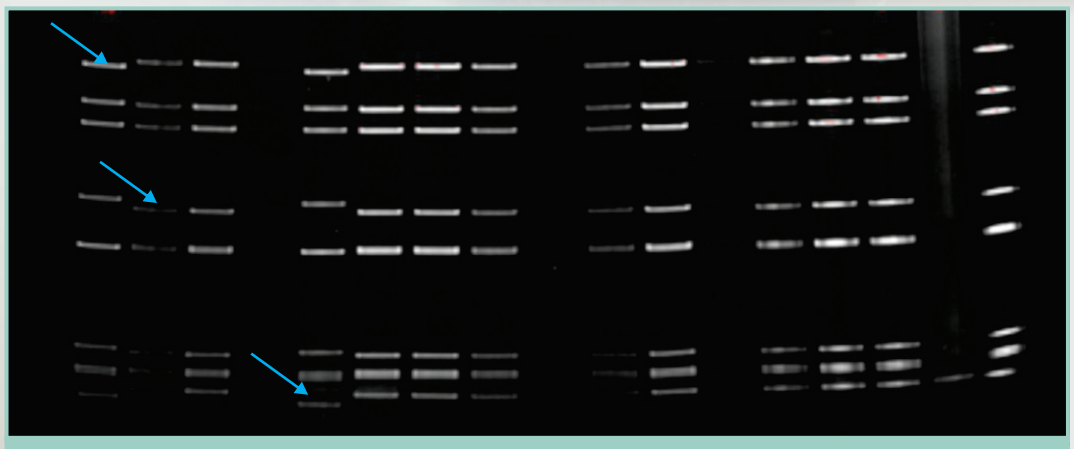

Figure 3: Electrophoretic profiles of BTV isolates using PAGE. Mobility shifts are indicated by the arrows.

The cell viability assay showed clear differences in the cytotoxic ability of the possible reassortant BTV isolates on Vero cell cultures. E.g. sample 2b is more cytotoxic than any one of the parental strains, while samples 9b and 11b are less cytotoxic than the parental strains (Figure 4).

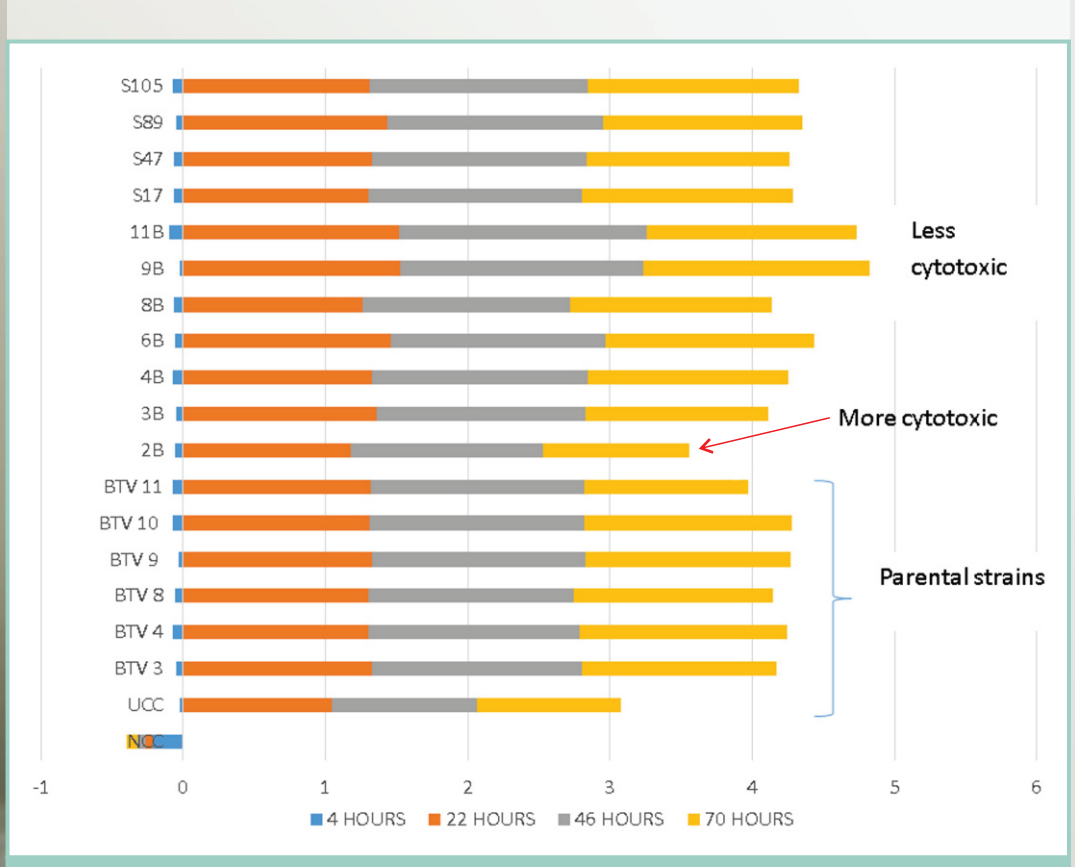

Figure 4: Cell viability assay measuring metabolic activity of the cell after infection of cells with potential reassortant viruses compared to the parental strains. Visible variation between the parental strains and potential reassortant strains can be observed with samples 2b, 9b and 11b.

Conclusion

The generation of reassortant viruses between vaccine and field strains of BTV was clearly demonstrated in cattle. Obtaining sequencing data confirming this is currently in progress. Some reassortants showed more cytotoxicity compare to the parental strains when using the cell viability test. Although the reassortment of live attenuated vaccines with field strains, contributing to their genotypic, and potentially phenotypic, variability has been reported, its frequency and biological consequences remain poorly understood, especially in cattle in South Africa. The data proves that there is the possibility of emerging viruses of unknown characteristics and a more structured surveillance strategy is required to identify these viruses in the field.

Carien van den Bergh, Peter Coetzee and Estelle Venter
Department of Veterinary Tropical Diseases

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA