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: Ceratopogonidae). 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).

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.

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 compared to the parental strains. Visible variation between the parental strains and potential reassortant strains can be observed with samples 2b, 9b and 11b.