# Evaluation of cross-protection of bluetongue virus serotype 4 with other serotypes in sheep

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Bluetongue (BT) is a non-contagious disease of sheep and other domestic and wild ruminants caused by the bluetongue virus (BTV). Currently 26 serotypes of the virus have been identified. In South Africa, 22 serotypes have been identified and BT is controlled mainly by annual vaccinations using a freeze-dried live attenuated polyvalent BTV vaccine. The vaccine is constituted of 15 BTV serotypes divided into three separate bottles and the aim is to develop a vaccine using fewer serotypes without compromising the immunity against the disease. This study is based on previously reported cross-neutralisation of specific BTV serotypes in *in vitro* studies. Bluetongue virus serotype 4 was selected for this trial and was tested for cross-protection against serotype 4 (control), 1 (unrelated serotype), 9, 10 and 11 in sheep using the serum neutralisation test. The purpose of the study was to determine possible cross-protection of different serotypes in sheep. Of those vaccinated with BTV-4 and challenged with BTV-1, which is not directly related to BTV-4, 20% were completely protected and 80% showed clinical signs, but the reaction was not as severe as amongst the unvaccinated animals. In the group challenged with BTV-10, some showed good protection and some became very sick. Those challenged with BTV-9 and BTV-11 had good protection. The results showed that BTV-4 does not only elicit a specific immune response but can also protect against other serotypes.

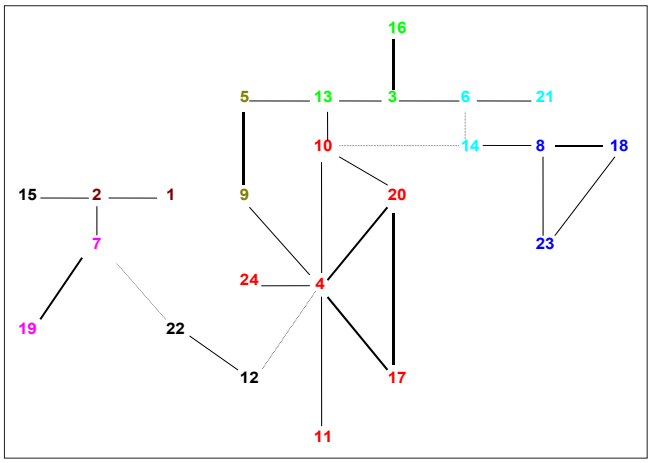
## Introduction

Bluetongue (BT) is an insect-borne viral disease of ruminants caused by the bluetongue virus (BTV). The disease has a major impact on the economy and animal welfare in affected countries and has the ability to spread to new geographical areas where naive animal populations are at risk**.** BTV has a wide host range, mostly affecting sheep, but also goats, cattle and some wild ruminants. Twenty-six serotypes are known of which 22, except BTV-20, BTV-21, BTV-25 and BTV-26, are endemic and co-circulate in southern Africa (Gerdes 2004; Mertens *et al.* 2007). Serological cross-neutralisation amongst BTV serotypes within specific groups using the serum neutralisation test (SNT) is well described (Erasmus 1990) (Figure 1). However, only a few studies exist where sheep were used to determine cross-protection. Jeggo (1986) inoculated sheep with BTV-4 and challenged them at various intervals with BTV-3, showing protection only for 14 days after the first inoculation. Simultaneous infection with three BTV serotypes also resulted in the replication of only two (Jeggo, Wardley & Taylor 1984). Using a recombinant vaccine, Perez de Diego *et al.* (2011) and Calvo-Pinilla *et al*. (2012) showed that BTV protection is serotype-specific and that homologous protection involves antibodies and a T-cell response, whilst heterologous protection is supported by a T-cell response directed to the NS1 encoding gene (Schwartz-Cornil *et al*. 2008).

The most effective method of controlling BT in endemic areas like South Africa is by prophylactic vaccination or protecting the animals from being bitten by the midge vector (Ilango 2006). Live attenuated vaccines (LAVs) and inactivated vaccines are mainly used in southern Africa and Europe, respectively (Savini *et al.* 2008). The LAV is constituted of 15 BTV serotypes divided into three separate bottles and the challenge is to shorten the time between the first vaccination and the time when the animal is fully protected (up to 9 weeks). During this lengthy period, animals are at risk of developing the disease due to infection with the serotypes which they have not yet been inoculated against (Dungu, Gerdes & Smit 2004). The purpose of the study was to determine whether the number of serotypes in the vaccine can be reduced without affecting efficacy, thus shortening the time taken for the development of complete immunity after vaccination of animals.

## Materials and methods

BTV-specific antibody-free (BTV-specific ELISA negative) indigenous mutton-merino sheep were used. BTV serotype 4 at a titre of 4.2 x $10^6$ log10 $TCID_{50}$/mL was used as the vaccine and the standard Onderstepoort Biological Products challenge material for BTV-4 (control), BTV-1 (unrelated virus), BTV-9, BTV-10 and BTV-11 (related to BTV-4) were used as challenge material.




*Source*: Erasmus, B.J., 1990, 'Bluetongue virus', in Z. Dinter & B. Morein (eds.), *Virus infections in ruminants*, pp. 227–237, Elsevier Science Publishers, Amsterdam

**FIGURE 1:** Serological cross-neutralisation of bluetongue virus serotypes.

Serum was collected on day 0 and sheep were then grouped into vaccinated and unvaccinated as follows: 27 sheep were inoculated subcutaneously with 2 mL live attenuated BTV-4 vaccine and five were left unvaccinated as negative controls. The animals were monitored for clinical reactions twice daily for 14 days and were bled weekly to assess antibody reaction. On day 28, four groups of six animals each – five vaccinated animals and one unvaccinated animal – were challenged intravenously with different serotypes, namely BTV-1, BTV-9, BTV-10 and BTV-11. Two vaccinated animals were challenged with BTV-4. After vaccination and challenge the animals were monitored for BT-related clinical signs of fever (normal rectal temperature ranges between 37 °C and 40 °C), depression, dyspnoea, haemorrhages and for all clinical signs associated with endothelial cell damage. The BTV-4 cross-protection and degree of clinical reaction post-challenge was measured using percentage protection index (PPI) values with a cut-off value of 55% (Huismans *et al*. 1987). A PPI above 55% with no overt clinical signs was regarded as protective. The clinical reaction of the animals post-challenge was monitored serologically using SNT with a cut-off value of 1:16.

# Results

Significant clinical signs were observed in animals challenged with BTV-1 with low neutralising antibodies (below 1:16), which indicated the lack of protective antibodies. Animals challenged with BTV-9 showed no clinical signs and protective neutralising antibodies against the virus were present. Animals challenged with BTV-10 had neutralising antibody titres above 1:16, but some animals showed overt clinical signs. This indicates that BTV-4 neutralising antibodies were not sufficient to provide protection to animals against BTV-10. Some animals did not show any clinical signs although SNT titres were above the cut-off value (BTV-10). These animals could have been infected subclinically (Sperlova & Zendulkova 2011). The animals challenged with BTV-11 had a 100% PPI value but the SNT results were below the cut-off value of 1:16. A possible explanation is that the cell-mediated immune response, specifically T-cells directed to the NS1 non-structural and inner core proteins, played a role in the protection of the animals (Schwartz-Cornil *et al*. 2008). Therefore, BTV-4 vaccine should not be used as the primary vaccine for protection against BTV-1 and BTV-10; however, animals were protected against BTV-9 and BTV-11 by BTV-4 neutralising antibodies. According to Figure 1, BTV-1 is only distantly related to BTV-4, which might be the reason why there was poor cross-protection. *In vivo* vaccine studies should include different combinations of serotypes and investigate both humoral and cellular immune responses together with clinical signs. The immunogenicity of the different BTV serotypes differ; the different titres used in a vaccine and the ability to replicate well in host cells also play a role in eliciting a strong humoral immune response (Dungu *et al*. 2004; Modumo & Venter 2012).

# Acknowledgements

## Competing interests

The authors declare that they have no financial or personal relationship(s) which may have inappropriately influenced them in writing this article.

## Authors' contributions

E.H.V. (University of Pretoria) was the project leader and prepared the manuscript, whilst G.B.Z. (Onderstepoort Biological Products) performed the experiments and assisted in writing the manuscript.

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