The use of vaccination in the control of bluetongue in southern Africa

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Summary

The eradication of bluetongue virus (BTV) from endemic regions of Africa is virtually impossible due to the role played by widely distributed *Culicoides* spp. midge vectors and the ubiquitous distribution of vertebrate reservoir species. In endemic areas, attempts can only be made to limit the occurrence of bluetongue (BT) disease and its economic impact through vaccination. Despite several potential problems (teratogenicity, risk of reassortment, and reversion to virulence of the attenuated viral strains), the current live-attenuated vaccine, produced by Onderstepoort Biological Products (OBP), South Africa, has been used for decades in enzootic regions, and has been shown to provide a safe and efficacious means to control the disease in regions of southern Africa, as well as other areas of the world.

Keywords

Africa – Bluetongue – Cattle – Control – *Culicoides* – Live-attenuated vaccine – Sheep – South Africa – Southern Africa – Vaccination – Vaccine.

Bluetongue (BT) virus (BTV) is the prototype species of the genus *Orbivirus* in the family *Reoviridae*. The viral genome consists of 10 double-stranded RNA segments that encode four non-structural (NS1, 2, 3 and 3A) and seven structural (VP1-VP7) proteins (31, 39). Currently, there are 24 known serotypes of BTV worldwide (25). BT is an Office International des Épizooties (OIE) 'List A' disease, and is thus of serious socio-economic concern and of major importance in the international trade of animals and animal products. BTV has been recognised as an important aetiological agent of disease in sheep in South Africa for over a century, and for many years was believed to be restricted to Africa south of the Sahara (14). However, since 1943, BTV has been identified in several countries outside Africa, such as Argentina, Australia, Bulgaria, China, Cyprus, France, India, Israel, Italy, Malaysia, Pakistan, Portugal, Spain, the United States of America (16) and, most recently, Kazakhstan (24). BTV commonly occurs between latitudes 35°S and 40°N, but the virus has also been detected further north beyond 48°N in Xinjiang, China, western North America and Kazakhstan (12, 24, 29).

Possible factors that have contributed to the spread of BTV include animal migration and importation, extension in the distribution of its major vector, *Culicoides* spp., involvement of newly identified or as yet unidentified vector(s), the apparent ability of the virus to overwinter in the absence of adult vectors, and its occurrence in healthy reservoir hosts, such as cattle and some wild ruminants. On account of the wide host range of BTV and its biological transmission by insects, control of BT in an endemic region is based primarily on the active immunisation of susceptible animals, as well as on the prevention or limitation of contact between the susceptible host and insect vectors.

Bluetongue endemicity in southern Africa

The enzootic nature of BTV in large regions of the African continent and more specifically southern Africa is supported by climatic factors that favour the maintenance and recirculation of the virus in its vertebrate and non-vertebrate hosts. Reservoir and amplifying hosts, such as game, cattle and goats, compounded by the ubiquitous distribution of suitable midge species, contribute to the persistence and transmission of BTV. In areas where the winter is mild, BTV may be transmitted throughout the year.

Most African indigenous sheep breeds are resistant to or show only mild clinical symptoms of BT, which is generally not considered serious in many sheep-rearing communities. This has resulted in the limited use of effective control measures, including vaccination, in many African countries despite evidence of BTV infection through serology and virus isolation (23). In South Africa, however, where the majority of the sheep population consists of originally exotic wool breeds, outbreaks of clinical disease are common and result in economical losses, either through direct mortality, or indirectly as a result of the loss in condition, compromised breeding efficiency and reduction in wool quality. Factors such as sheep breed susceptibility, variation within the breed, virulence of the virus strains, and year-to-year variation in climatic conditions, such as rainfall, make it difficult to envisage control measures other than vaccination. Due to the large number of circulating serotypes, it is generally impossible to predict the serotype for a specific season or area. Furthermore, several serotypes tend to circulate simultaneously (40).

In a surveillance programme initiated in 1979, BTV was detected in *Culicoides* midges from 12 different sites throughout South Africa (3). This study revealed that the total number of BTV serotypes isolated per season varied from 11 to 17, and varied in prevalence. In each season 2 to 5 serotypes dominated, but were replaced by different, highly prevalent serotypes the next year. These serotypes, which included BTV serotypes 1-8, 11, 12, 16, 19, and 24, generally had a high transmission potential. However not all the serotypes were highly pathogenic for sheep. It was speculated that serious outbreaks of BT were possibly caused by those serotypes possessing a high transmission potential as well as a high pathogenic index for sheep (3). In 1996, there were several outbreaks of BT and epizootic haemorrhagic disease (EHD) in South Africa, following heavy rainfalls. BTV serotypes 1-9 and 12 were isolated from sheep (G. Gerdes, personal communication) and serotypes 2, 3, 6, and 8 were isolated from cattle (4) in the same year, supporting the previous findings of the co-circulation of several serotypes during any BT season, and the long-term persistence of these serotypes in susceptible and reservoir hosts in an endemic area.

At present, 17 of the 24 known serotypes of BTV have been detected in South Africa. However, serotype 15 has only been isolated from sheep during an outbreak in 1976. Serotypes 1, 2, 3, 4, 6 and 10 are known to have a high pathogenic index and high epidemic potential.

Historical background to the current Onderstepoort Biological Products bluetongue vaccine

As it was clear at the turn of the last century that BTV was enzootic to South Africa, vaccination was recognised as a suitable means of controlling the disease. The attenuated blood vaccine developed by Theiler in 1906 (38) was used over almost 40 years, and was based on a virulent BTV strain (now known as serotype 4) that had been passaged until it lost its virulence. The realisation of the plurality of different BTV serotypes that were involved in outbreaks, and safety concerns, brought Alexander to develop an embryonated egg-passaged quadrivalent lyophilised BT vaccine (2). Isolates of BTV were subsequently attenuated by 100 passages in eggs, and showed a reduction in the severity of temperature reactions and incidence of post-vaccination clinical disease in sheep. Immunogenicity of these isolates was further improved by using plaque selection or purification to select strains at lower egg-passage levels. Ten plaques were selected at random and screened in sheep for low pathogenicity and good immunogenicity. Seventeen of the then 20 known serotypes of BTV were shown to be present in South Africa. Since it was also known that several serotypes could be involved in an outbreak, the use of a polyvalent vaccine was imperative. A single vaccine containing 14 serotypes was then developed and used for a time (3, 13, 14). Around 1977/1978, serotype 19 was added to the vaccine due to an outbreak in 1976 in the Orange Free State caused primarily by serotypes 18 and 19. However, this single dose, multivalent vaccine did not induce adequate protection in sheep to all serotypes (14). Later, the attenuation of the strains was further modified by a reduction in the number of egg passages, followed by plaque selection and further cell culture passage (3).

Since BTV is an RNA virus and exists as a quasispecies, there is a variation in the presence of virulent and avirulent strains present at any one time. Passaging of the virus allows this ratio to change and thereby creates the opportunity for the selection of avirulent or attenuated virus. On the basis of the febrile reaction and incubation period in sheep, which correlated with the rate of replication of the viral serotypes, the current Onderstepoort Biological Products (OBP) vaccine was developed. The present OBP vaccine (Reg. No. G 358 Act No. 36/1947) comprises three bottles (vaccines A, B, and C) comprised of five serotypes each (Fig. 1) administered separately at three-week intervals. The selection of the serotypes included in the current vaccine was based on the prevalence and pathogenic

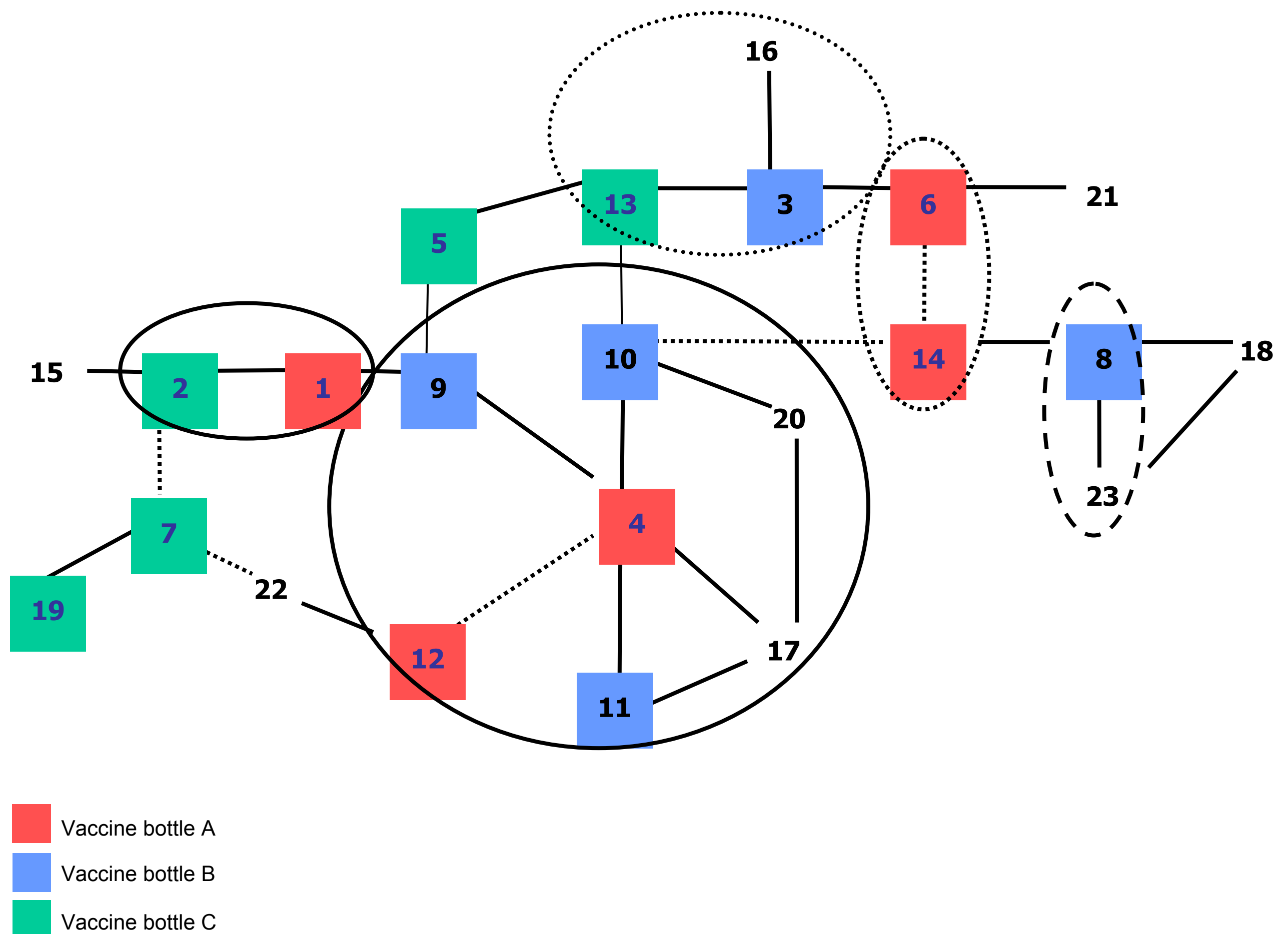

Figure 1
Serological cross-neutralisation of bluetongue virus serotypes
The serological cross-neutralisation between 23 of the 24 known BTV serotypes is represented graphically, as well as the component serotypes of the three vaccine bottles (bottles A, B and C) of the live-attenuated BT vaccine from Onderstepoort Biological Products, South Africa

index of the prevailing serotypes in South Africa at the time, as well as the ability of these serotypes to provide adequate cross-protection to other less dominant serotypes, as assessed by cross-neutralisation and cross-challenge studies (B.J. Erasmus, personal communication). The specific combination of the serotypes in each vaccine bottle is based on the replication rate of the different serotypes, which correlates with the degree of attenuation. The slower replicating serotypes are given first. The vaccine strains presently used by OBP were originally obtained from clinical cases of BT, and only serotype 10 of the original vaccine developed by Howell is still contained in the current vaccine.

Immune response to bluetongue vaccine

Studies have demonstrated that both the humoral and cellular immune responses play a role in immunity to BTV (20, 22). Both homologous and heterologous neutralising antibodies have been demonstrated, depending on whether the animals were experimentally inoculated simultaneously or sequentially with BTV. Cellular immune responses mediated by cytotoxic T lymphocytes (CTLs) generally give heterotypic protection, which is relatively short-lived. Although CTLs do not prevent virus infection, they act to clear the virus from an infected host, and have been shown to effectively clear homologous and heterologous serotypes of BTV (21). In addition, it has been shown that BTV-specific ovine CTLs are cross-reactive (37). More recently, it was shown that CTL recognition patterns in sheep are quite diverse, possibly due to the different distribution of CTL epitopes on different viral proteins (19). The NS1 and VP2 proteins are most frequently recognised by CTLs, and each contains more than one CTL epitope. The use of a multivalent whole live vaccine allows for a greater and more extensive induction of a CTL response due to the presence of multiple CTL epitopes. The heterologous cross-neutralisation between certain BTV serotypes that has been demonstrated by serological studies (Fig. 1), is further supported by partial sequence analysis of the VP2 (Fig. 2) and VP5 genes (C. Potgieter, personal communication), which shows a similar cross-relationship, and lends support

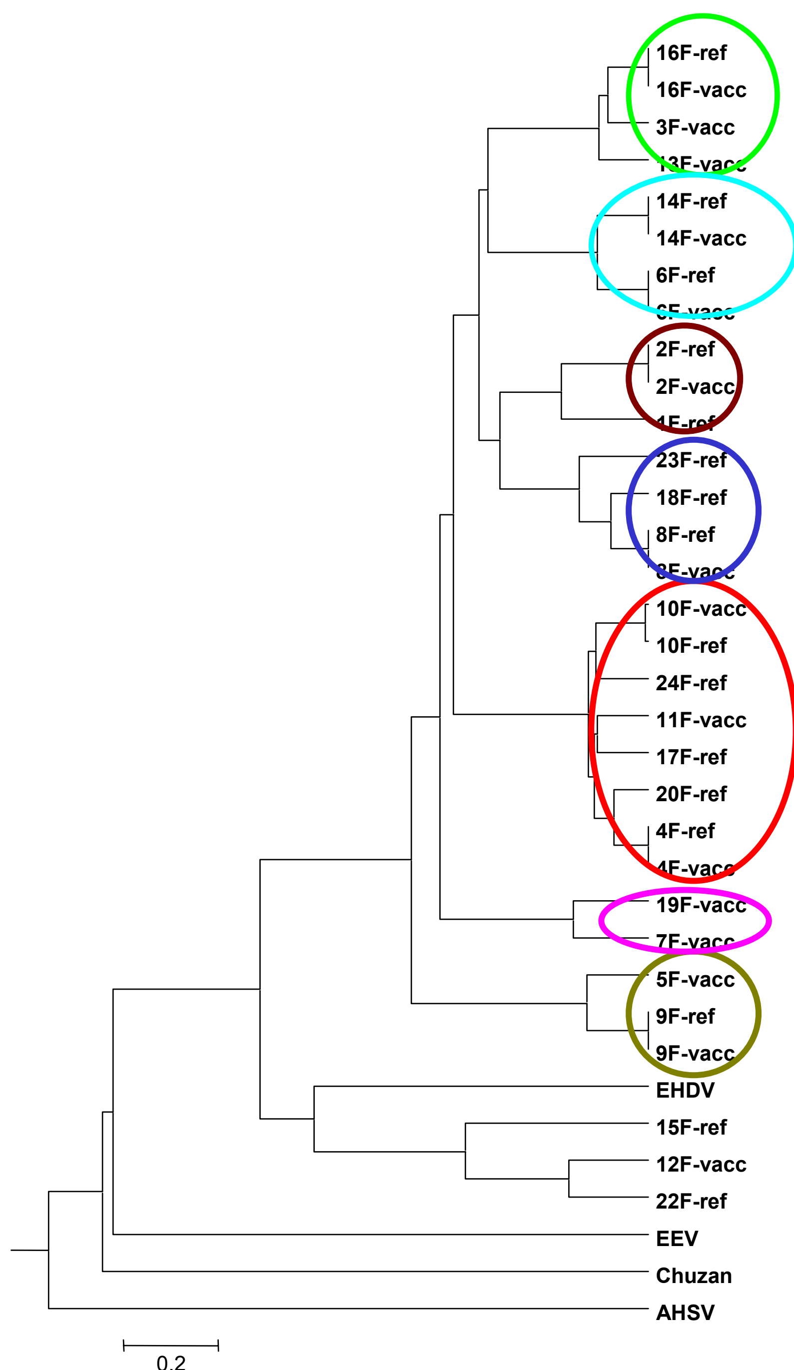

Figure 2
Phylogenetic relatedness of bluetongue virus serotypes
Phylogenetic tree of the cloned partial VP2 genes from reference and vaccine strains of BTV serotypes indicating a similar grouping as seen by cross-neutralisation (Fig. 1)
Courtesy: C. Potgieter, Onderstepoort Veterinary Institute

to the broad-spectrum coverage that the existing vaccine components contribute to BTV serotypes occurring in South Africa.

Bluetongue vaccine use and production in South Africa

An average of eight million doses of the OBP tri-pentavalent vaccine is sold and used annually in South Africa. However, this only affords protection to approximately one third of the commercial sheep population in the country (Fig. 3). The Eastern Cape Province is the biggest consumer of the vaccine as it is home to the largest number of wool-producing sheep. A further one million doses are sold to some of the neighbouring countries annually. In southern Africa, sheep should be vaccinated from August to October with 1 ml of each of the three vaccine

bottles at three-weekly intervals. The immunisation of ewes should commence 9-12 weeks before mating. However, it is not advisable to immunise pregnant ewes during the first half of pregnancy. Rams should be inoculated after the mating season. Lambs born of vaccinated ewes should be vaccinated at 6 months or older, or if administered earlier in heavily infected areas, they should be re-vaccinated at 6 months of age. A reasonable protection against most of the serotypes is achieved within 3 to 4 weeks after the last vaccine bottle is administered, but cannot be guaranteed in all vaccinated animals. Thus sheep must be vaccinated annually, to ensure adequate immunity to all serotypes.

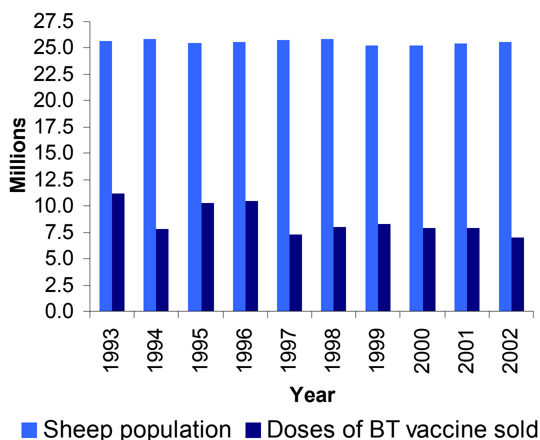

Figure 3
Annual doses of Onderstepoort Biological Products bluetongue vaccine sold from 1993 to 2002, in relation to the total commercial sheep population in South Africa

Safety aspects of the Onderstepoort Biological Products bluetongue vaccine

All batches of vaccines are produced according to national and international guidelines, and extensively tested to comply with purity, safety, efficacy and potency standards. Nonetheless, there are several concerns regarding the use of live-attenuated vaccines for the immunisation of sheep against BT. One potential safety problem relating to the use of live vaccines is the release and transmission of attenuated virus strains into the environment, which may result in a reversion to virulence through reassortment with a wild-type strain. However, vaccine strains, which produce less than 1×10^3 pfu/ml of blood at the height of viraemia in test animals, and elicit neutralising antibodies, are selected for vaccine production. Viraemias below 1×10^3 pfu/ml are thus considered to be safe and will ensure that the virus is not transmitted by vector midges. Preliminary studies conducted at the OBP to determine the level of viraemia in sheep post vaccination have shown that for serotypes 1, 2 and 4, no clinical reactions, elevated temperatures or virus were detected. Animals vaccinated with serotypes 10 and 16 demonstrated no clinical reactions but had a mild and brief fever that persisted for 5-6 days. However, viraemia levels in sheep vaccinated with serotypes 10 and 16 (1.25×10^2–7.5×102^2 pfu/ml) peaked below the desired minimum of 1×10^3pfu/ml of blood, and declined as the febrile reaction waned.

Wild-type BTV does not appear to be able to cross the placenta to cause teratogenicity, or the production of physical defects in offspring *in utero*. Previous cases of teratogenic defects in sheep attributable to BTV were related to the use of chick-embryo propagated BTV vaccine, and foetuses were shown to be most susceptible at 5 to 6 weeks *in utero* (34). Current vaccine strains are derived from virus propagated first on chick embryos, then plaque purified and adapted on cell culture. A study performed with Australian BTV serotype 23 has shown that BTV adapted to cell culture is capable of crossing the placenta and inducing teratogenesis (15). Live BTV vaccine strains may thus be responsible for spontaneous cases of BTV-induced malformation in both sheep and cattle. However, the Australian study was conducted using only serotype 23, which is not highly pathogenic or prevalent in South Africa. Different serotypes of BTV differ in their pathogenesis, transmissibility and growth characteristics. Although no study has yet been conducted, there is a possibility that different attenuated serotypes with different passage history will vary in their teratogenicity; this should be further investigated in the field. However, for safety reasons and as a precaution, it is thus advised that pregnant ewes are not vaccinated in the first half of pregnancy. If an annual vaccination programme is implemented, as is advised, then both ewes and foetuses should be adequately protected during pregnancy. All lambs should be vaccinated at six months of age.

The 24 BTV serotypes have been shown to have considerable strain variation in the different gene segments within each serotype (5, 6, 7, 8, 9, 10, 17, 28, 30, 41). The reassortment of gene segments has been reported among strains of BTV (9, 18, 27, 32, 33). Recently it was demonstrated that variation of gene segments encoding the VP2 and NS3/NS3A proteins also occurred through genetic drift (7). As BTV is an RNA virus, it is likely to exist as a heterogeneous population of closely related variants characterised by one or more dominant nucleotide sequence(s) (quasispecies) (11). Arthropod-borne RNA viruses generally evolve more slowly than do non-arthropod-borne RNA viruses, most probably because of the restrictive pressures imposed during

alternating passaging in their vertebrate and invertebrate hosts (35). Nonetheless, the quasispecies trait bestows significant adaptive ability on RNA viruses, through the selection of mutants with highest fitness in a new environment, allowing for rapid evolution (26).

Although the reassortment of BTV genes has been demonstrated in the laboratory, only rarely has it been reported to occur in the field (36). Despite being an RNA virus, BTV is relatively stable and thus reassortment events are likely to be rare – unless driven by a specific or environmental factor. The risk of reassortment in the field is minimised by the long interval between the recommended vaccination period (late winter, early spring) and the BT season (summer), which would make the incidence of co-circulating vaccine and virulent wild-type viruses highly unlikely. During reassortment, progeny viruses receive one of each of the genome segments, but probably not from a single parent. Thus in the case of BTV which has 10 segments, in a mixed infection of only two serotypes there could be 2^{10} (= 1 024) possible progeny viruses. Where more serotypes are present, the possible reassortment combinations rapidly increase. Due to the high recombination possibilities, the mixing of a wild-type virulent and an attenuated vaccine strain is highly unlikely to result in the generation, and persistence, of a new virulent strain, or its subsequent effective transmission via the insect vector, and persistence and survival particularly where it competes with existing virulent wild-type strains.

In an epidemiological surveillance study conducted in the Balearic islands, BTV from cattle and goats was specifically found by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis to be field virus, while that from vaccinated sheep was a vaccine strain (1). This finding supports the highly unlikely possibility of midges transmitting vaccine viruses from vaccinated to unvaccinated animals under field conditions.

Conclusion

The eradication of BTV from endemic regions of Africa and certain parts of the world is impossible due to its ubiquity, broad host range, the multiplicity of serotypes that may be circulating at any point in time, and the role played by the widely distributed *Culicoides* vectors.

The vaccination of sheep with the OBP live-attenuated polyvalent vaccine is presently still the most effective and practical control measure against BTV in South Africa, as has been demonstrated by laboratory and field trials, and the extensive use of the vaccine over many years.

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