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Review

Bluetongue vaccines in Europe

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Abstract

The article reviews the history, present status and the future of BT vaccines in Europe. So far, an attenuated (modified live viruses, MLV) and inactivated virus vaccines against BT were developed and used in the field. Moreover, the virus-like particles (VLPs) produced from recombinant baculovirus, and live recombinant vaccinia or canarypox virus-vectored vaccines were tested in the laboratory. The main aims of BT vaccination strategy are: to prevent clinical disease, to reduce the spread of the BTV in the environment and to protect movement of susceptible animals between affected and free zones. Actually, all of the most recent European BT vaccination campaigns have used exclusively inactivated vaccines. The use of inactivated vaccines avoid risk associated with the use of live-attenuated vaccines, such as reversion to virulence, reassortment of genes with field strain, teratogenicity and insufficient attenuation leading to clinical disease. The mass vaccinations of all susceptible animals are the most efficient veterinary method to fight against BT and successful control of disease. The vaccination of livestock has had a major role in reducing BTV circulation and even in eradicating the virus from most areas of Europe.

Key words: bluetongue, vaccines, prophylactic vaccination, Europe

Introduction

Bluetongue (BT) is a vector-borne viral disease of ruminants, including sheep, goats and cattle that induces variable clinical signs depending on the species and the breed (MacLachlan 1994). The disease is caused by the bluetongue virus (BTV), which is the member of the genus *Orbivirus* within the family *Reoviridae*. On the basis of serotype-specific virus neutralization assays, 24 immunologically distinct serotypes (BTV1 to BTV24) have been identified worldwide (Gorman 1990). In 2008 an additional putative BTV serotype 25 (Toggenburg virus) was isolated from goats in Switzerland (Hofmann et al. 2008) and recently a novel BTV serotype 26 was identified in Kuwait (Maan et al. 2011). The virus is transmitted by specific species of

Culicoides, family *Ceratopogonidae* (MacLachlan 1994). An evidence for transplacental and contact transmission of BTV was also presented (Menzies et al. 2008).

The distribution of BT is determined by the geographical distribution of the arthropod vector and includes Africa, southern Asia, Australia, the Middle East, and the Americas (Walton 2003). Historically, Europe has experienced only sporadic incursions of BT, involving a single-virus serotype on each occasion. However, since 1998, BT outbreaks have occurred annually, involving strains from six distinct BTV serotypes – BTV1, 2, 4, 8, 9, and 16 (Mellor and Wittmann 2002). In August 2006, for the first time, the BTV passed the latitude 50° and BT outbreaks occurred in North-Western Europe: the Netherlands, Belgium, Germany,

France and Luxembourg (Wilson and Mellor 2009). In 2007-2008, the BT situation changed for the worse, BTV serotype 8 spread to other regions of Europe, the number of outbreaks increased rapidly and new BTV serotypes (1, 6, 11 and 16) were detected (Wilson and Mellor 2009, De Clercq et al. 2009). However, the implementation of BT compulsory vaccination programmes in Europe in spring 2008 resulted in reduction of BTV8 cases to 79 in the culicoides activity season 1st May 2009 – 31st December 2010. In the some season, 650, 6 and 17 cases of BTV serotypes 1, 4 and 16 were noticed, respectively (<http://eubtnet.izs.it/btnet/reports/Outbreaks.html>). The BTV serotypes and BT restriction zones in Europe as for 1st January 2011 are presented in Fig. 1.

binant vaccinia or canarypox virus-vectored vaccines (Savini et al. 2008, Noad and Roy 2009). The protective vaccines against BT in ruminants that are currently available commercially can be either attenuated or inactivated. Their protective activity is serotype-specific, probably due to the B and T cell mediated protective immunity. Thus, in endemic areas where multiple BTV serotypes may be present, efficient vaccines against infections with several BTV serotypes may be necessary. Furthermore, vaccines against BT need to be sterile, safe and should allow differentiating between infected and vaccinated animals (DIVA), in order to facilitate trade. The main aims of BT vaccination strategy are: to prevent clinical disease, to reduce the spread of the BT virus in the environment and to protect movement of susceptible animals between affected and free zones.

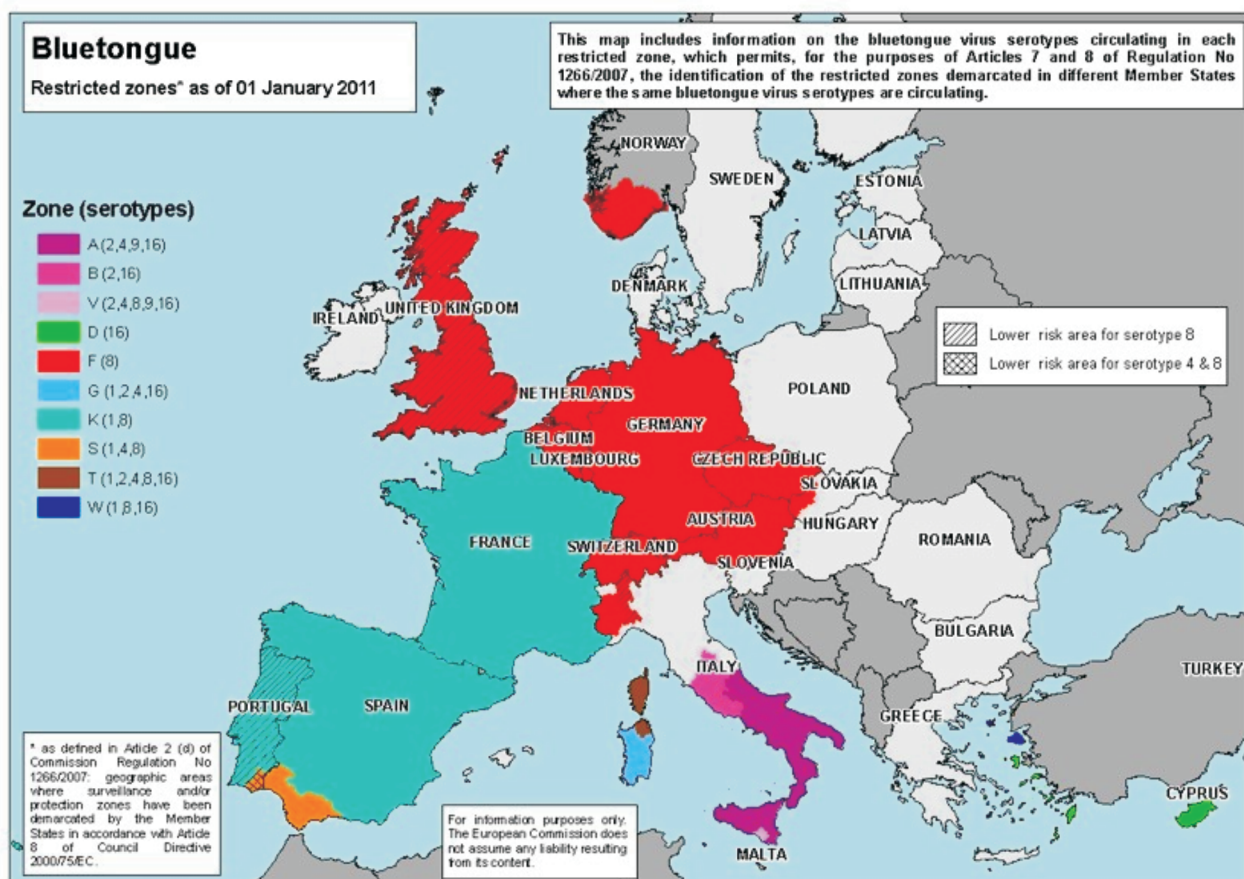


Fig. 1. BTV serotypes and BT restriction zones in Europe as for 1st January 2011 (http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_en.htm)

The article reviews the history, present status and the future of BT vaccines and prophylactic vaccinations against BT in Europe. The types of BT vaccines that have been developed include modified-live (attenuated) virus (MLV) vaccines, inactivated whole (killed) virus vaccines, virus-like particles (VLPs) produced from recombinant baculoviruses and recom-

Modified live (attenuated) virus (MLV) vaccines

MLV vaccines are available for many BTV serotypes and are used in several countries outside the EU but have also, in the past, been used within Europe (e.g., Spain, Italy and France). These vaccines are

produced by serial passage of BTV field isolates in tissue culture or in embryonated chicken eggs. MLV vaccines typically elicit a strong antibody response, which is directly correlated with their ability to replicate in the vaccinated animals. They are inexpensive and are capable of stimulating protective immunity after a single inoculation and have proven effective in preventing clinical BT disease (Patta et al. 2004, Savini et al. 2008). However, a variety of documented or potential drawbacks are attributed to BT MLV vaccines including under-attenuation, which may result in disease development that varies among sheep of different breeds. Potential adverse consequences are depressed milk production in lactating sheep, and abortion/embryonic death and teratogenesis in offspring when used on pregnant females (MacLachlan et al. 2009). Another risk associated with the use of MLV vaccines is derived from their potential of infecting vectors with possible reversion to virulence and/or reassortment of MLV genes with those of wild-type virus strains. These events have been well documented in Europe (BTV2 and 16 MLV vaccine strains) (Batten et al. 2008), but the frequency and significance of reassortment remain uncertain. Finally, MLV vaccines are not DIVA vaccines.

MLV vaccines produced by Onderstepoort Biological Products (Republic of South Africa) have been used since 2000 in Spain, France, Italy and Portugal to the compulsory vaccination campaigns against BT. Reports of the adverse events in the field vary with the strain of MLVs used for vaccination of the animals. The monovalent BTV2 MLV vaccine was used in Corsica (2001-2004) and Italy (from 2004) on more than 4 000 000 sheep and goats and no or negligible adverse reaction were reported after vaccination. However, when the same MLV vaccine was used in Menorca and Mallorca (2000-2001) on 320 000 sheep, adverse events were observed in 0.13% and abortion in 0.16% of the vaccinated animals (Savini et al. 2008). Similarly, no adverse reactions were observed after vaccination with BTV4 MLV in Corsica in 2004, bivalent MLV vaccine BTV2 and 4 in Menorca during 2003 vaccination campaign and trivalent vaccine containing BTV serotypes 2, 4 and 9 used since 2005 in some regions of Italy on more than 1 000 000 sheep, goats and cattle. However, the other trivalent BTV2, 4 and 16 MLV vaccine used in 2003 in Sardinia caused a typical BT signs in many vaccinated sheep and goats. These incidents were attributed to inadequate attenuation of the BTV serotype 16 present in the MLV vaccine (Savini et al. 2008). An important factor in confirming the efficacy of MLV vaccines is their ability to elicit neutralizing antibodies in vaccinated animals. Serological studies performed on cattle and sheep vaccinated with several MLV vaccines had shown that more than 80% of the vaccinated animals had specific BTV antibodies (Savini et al. 2004, 2004a). Experimental challenge studies have demon-

strated that vaccination with the BTV2 MLV strain prevented viremia in at least 90.5% of vaccinated cattle that were challenged at 7 months after vaccination with a dose of $2 \times 10^{5.8}$ TCID₅₀ of virulent homologous field isolate (Savini et al. 2004b). The efficacy of MLV vaccines has also been demonstrated in the field – following the 2000-2001 and 2003 BT vaccination campaign in the Balearic Islands, no outbreaks have been detected since December 2003 in the area.

Inactivated vaccines

The first inactivated vaccine used in the field in Europe was the vaccine against BTV serotype 2 used to vaccinate sheep in 2005 in Corsica (France). Then, in 2005-2006, a monovalent BTV4 and bivalent BTV2 and 4 vaccines were used in Corsica, Spain, Portugal and Italy (Rodriguez-Sanchez et al. 2008). BT inactivated vaccines were then developed and commercialized for BTV9, BTV1 and BTV8 and actually all of the most recent European BT vaccination campaigns have exclusively used inactivated vaccines (Wilson and Mellor 2009). The use of inactivated vaccines avoid risk associated with the use of live-attenuated vaccines, such as reversion to virulence, reassortment of genes with field strain, teratogenicity and insufficient attenuation leading to clinical disease (Roy et al. 2009). Although the hitherto available inactivated vaccines need to be administered twice (particularly in cattle), are rather expensive to produce and are primarily directed against only one serotype, they proved to be safe and effective (Schwartz-Cornil et al. 2008, Savini et al. 2009, Gethmann et al. 2009). Therefore, the European Food Safety Authority currently recommends using inactivated vaccines to protect animals from bluetongue disease (Enserink 2008), instead of MLV vaccines.

The studies conducted on sheep and cattle to evaluate the safety of inactivated vaccines shown that these were very well tolerated as demonstrated by the absence of the systemic reaction (fever, weigh loss, reproductive dysfunction, etc.) related to vaccination. Some vaccines induced transient local reactions of variable severity with different frequency (Savini et al. 2009). Assessment of efficacy is based on clinical and virological data as well as on immunogenicity. The level of viremia after virus challenge is considered the most objective way to assess the efficacy of the virus-induced immunity. The level of viremia is analyzed by either a BTV-specific quantitative real-time RT-PCR assay or by virus isolation in susceptible cell lines. The inactivated BTV serotype 2, 16 and 8 vaccines induced significant titres of neutralizing antibodies in sheep (Savini et al. 2007, Hamers et al. 2009a, 2009b). All sheep vaccinated with these inactivated vaccine developed high titres of neutralizing antibodies and were protected from clinical disease and viremia was completely

prevented after subcutaneous inoculation with a suspension of homologous BTV. Similarly, all cattle vaccinated with BTV8 vaccine were subjected to a virulent BTV challenge, and safety and antibody responses were detected. All control animals developed disease and viremia, while vaccinated cattle were clinically protected (Hamers et al. 2009b). In cattle, an efficacy studies have also been performed on bivalent (BTV serotypes 2 and 4) BT vaccine. Two doses of bivalent BT vaccine administered at a 28-day interval prevented viremia in vaccinated animals challenged with the homologous virulent serotype and no animals showed signs of disease. At the time of the challenge, only one of 16 vaccinated animals did not have neutralizing antibodies against BTV4, while the remaining 15 animals showed titres of at least 1:10 for either BTV serotypes 2 or 4. However, the BTV2 component of the bivalent BT vaccine elicited a stronger immune response in terms of both the number of virus neutralization (VN)-positive animals and antibody titres (Savini et al. 2009). The long-term efficacy of three commercially available inactivated vaccines against bluetongue virus serotype 8 (BLUEVAC 8, Zulvac 8 and BTVPUR Al-Sap 8) was evaluated in a seroprevalence study and challenge experiments by Wackerlin et al. (2010). Seroprevalences one year after vaccination ranged from 75% to 100%. In two infection experiments, groups of vaccinated sheep and cattle were challenged with a BTV8 twelve months after vaccination. With two exceptions, all animals, including those with low antibody levels prior to challenge, were protected from viral replication and clinical disease even at low initial antibody levels.

New generation BT vaccines

In addition to the MLV and inactivated BT vaccines, there is a number of other viable alternatives that promise to address the DIVA-compliance concerns of the current vaccines. Protein-based and recombinant vaccines are the most prominent among these candidate vaccines. Protein-based vaccines are based on the initial observation that the serotype determining outer capsid protein of the virus, VP2, elicits protective immunity in vaccinated sheep (Roy et al. 1990). This approach was taken to its logical conclusion by combining all four major structural proteins of the virus (VP2, VP5, VP7 and VP3) into a single, non-infectious, virus-like particle (VLP) immunogen that mimicked the virus structure but do not contain any of the virus nucleic acid (Roy et al. 1992). VLP are promising vaccine tools to generate multivalent BT vaccines and have been shown to be efficacious in protecting against homologous BTV challenge and partially protecting against heterologous BTV challenge in laboratory trial (Roy et al. 1992). Since VLP do not contain any of the

virus non-structural proteins (NSP) it is possible to distinguish between vaccinated and infected animals. A further advantage of the VLP is that, since the immunogen is entirely protein-based, there is no genetically modified virus component that requires expression of foreign genes in the vaccinated animals.

Recombinant vectors could be developed as BT vaccines, if they are safe, inexpensive, DIVA, flexible for multi-serotype inclusions and if they provide long-term protective immunity in one injection. The initial work showed that co-injection of vaccinia virus encoding for VP2 and VP5 proteins of BTV serotype 1 could confer protective immunity in sheep (Lobato et al. 1997). More recently, a canarypox-based vector that expressed optimized synthetic genes for VP2 and VP5 elicited high levels of neutralizing antibodies and induced high level protection in sheep (Boone et al. 2007). Finally, a replicative capripox encoding for VP2, VP7, NS1 and NS3 (one injection) was partially protective in sheep (Perrin et al. 2007).

The other modern approach can be the future BT vaccines such as disabled infectious single cycle (DISC) vaccines (Schleiss et al. 2006). These immunogens are based on the production of modified bluetongue virus which has a deletion in one or more genes essential for virus replication. This virus is then propagated in cells that express a version of the deleted gene(s), and inserted into the genomic DNA of the cell to complement the defective virus, which then purified and used as an immunogen. As cells in the vaccinated animals will not express the viral protein necessary to complete a full replication cycle, the DISC vaccine will work at two levels. At the first, virus particles will act as a direct immunogen, as with VLPs, to elicit B and T cell responses. At a second level, DISC virus will enter cells and produce some, but not all, viral proteins, as with pox-vectored immunogens. Since deletion in the virus genome is in an essential protein, cells expressing viral proteins will not produce any infectious virus. Proteins that are deleted in the DISC strain can then be used as a basis for development of immunological tests to make the vaccine DIVA-compliant. However, at present the formal proof that a DISC vaccine is possible for BT has not been demonstrated but there is a number of recent advances that demonstrate the feasibility of some of the key steps towards the development of such vaccine (Boyce and Roy 2007).

Vaccination against BTV serotype 8 in Europe

The incursion of BTV serotype 8 to North-Western Europe in 2006 has had a considerable negative economic impact, partly due to direct losses from mortality and reduced production in affected livestock

but, more importantly, as a result of the ban of ruminant trade between BTV-affected and BTV free areas. To limit of economic losses and in an effort to minimize the circulation of virus and facilitate safe trade in live animals a vaccination strategy was agreed among EU member states (European Commission, 2008). Several inactivated BTV8 vaccines became commercially available early in 2008, and vaccination programmes were rapidly initiated throughout most of affected area. The authorities from BT-infected countries undertook vaccination of livestock according to their individual national policies, the geographic distribution of BTV, and the availability of appropriate vaccines. As the results, since spring 2008 more than 100 million animals have been vaccinated and the incidence of BT disease has decreased rapidly.

The success of vaccination varied considerably from one country to the other. In countries such as a Belgium and the Netherlands which were hit hard by BTV8 in both 2006 and 2007, transmission during the 2008 season was restricted to a handful of cases, but it is difficult to estimate the importance of vaccination in achieving the results as the vast majority of susceptible animals in these areas would be expected to already possess BTV antibodies as a result of natural infection and recovery. In the Netherlands more than 80% susceptible animals were vaccinated during 2008-2009 and the number of BTV8 cases decreased to 60 in 2008 and no cases of disease were detected in 2009-2010. In Germany, approximately 70% of cattle and 90% of sheep in the infected areas were vaccinated by the end of August 2008, more than 80% in 2009 and about 40% in 2010 (<http://www.bluetonguevirus.org/crl/ringtrial/2010/presentations>). The implementation of BT compulsory vaccination resulted in reduction of BTV serotype 8-borne cases to 5104 in 2008 and 142 in 2009. Similarly to the Netherlands, no cases of disease were detected in Germany in BT season 2010. The compulsory vaccination of about 80% population of susceptible animals in France resulted in reduction of BT cases to 83 in 2009 and only 1 in 2010. The implementation of BT prophylactic vaccination in Sweden resulted in no cases of BTV8 since 2009. Finally, despite the voluntary nature of the vaccination programme in the United Kingdom, sales data suggest that a coverage of 80% or higher was achieved within areas where BTV8 had been confirmed in 2007, although coverage in areas where no BTV had been reported and that were brought within the protection zone (PZ) for trading reason was as low as 40% in some areas (Wilson and Mellor 2009). The disease was eradicated successfully and very quickly, no circulation of BTV in United Kingdom was reported in 2008-2010 (<http://www.bluetonguevirus.org/crl/ringtrial/2010/presentations>).

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