Vaccines for Prevention of Bluetongue and Epizootic Hemorrhagic Disease in Livestock: A North American Perspective

D. Scott McVey¹ and N. James MacLachlan²

Abstract

Bluetongue (BT) and epizootic hemorrhagic disease (EHD) are noncontagious, insect-transmitted diseases of domestic and wild ruminants caused by related but distinct viruses. There are significant gaps in our scientific knowledge and available countermeasures to control an outbreak of orbivirus-induced disease, whether BT or EHD. Both BT virus (BTV) and EHD virus (EHDV) cause hemorrhagic fevers in susceptible ruminants; however, BT is principally a disease of domestic livestock whereas EHD is principally a disease of certain species of wild, non-African ungulates, notably white-tailed deer. The live-attenuated (modified live virus [MLV]) vaccines available in the United States for use in small ruminant livestock do provide good protection against clinical disease following infection with the homologous virus serotype. Although there is increasing justification that the use of MLV vaccines should be avoided if possible, these are the only vaccines currently available in the United States. Specifically, MLVs are used in California to protect sheep against infection with BTV serotypes 10, 11, and 17, and a MLV to BTV serotype 10 is licensed for use in sheep throughout the United States. These MLV vaccines may need to continue to be used in the immediate future for protective immunization of sheep and goats against BT. There are currently no licensed vaccines available for EHD in the United States other than autogenous vaccines. If there is a need to rapidly develop a vaccine to meet an emerging crisis associated with either BTV or EHDV infections, development of an inactivated virus vaccine in a conventional adjuvanted formulation will likely be required. With two doses of vaccine (and in some instances just one dose), inactivated vaccines can provide substantial immunity to the epizootic serotype of either BTV or EHDV. This strategy is similar to that used in the 2006–2008 BTV serotype 8 outbreaks in northern Europe that provided vaccine to the field within 2 years of the initial incursion (by 2008). Further research and development are warranted to provide more efficacious and effective vaccines for control of BTV and EHDV infections.

Key Words: Arbovirus—Orbivirus—Bluetongue—Vaccines—Epizootic hemorrhagic disease—Immunity.

Introduction and Historical Perspective

IN RESPONSE TO UNITED STATES Animal Health Association (USAHA) Resolution 16, the US Department of Agriculture (USDA) in collaboration with the Department of the Interior (DOI) organized a gap analysis workshop composed of international experts on orbiviruses. The workshop participants met at the Arthropod-Borne Animal Diseases Research Unit in Manhattan, Kansas, May 14–16, 2013, to assess the available scientific information and countermeasures to effectively control and mitigate the impact of an outbreak of an emerging *Orbivirus* with epizootic potential, with special emphasis given to bluetongue virus (BTV) and epizootic hemorrhagic disease virus (EHDV). This review is a summary with regard to immunization practices and vaccines and therefore summarizes the needs for continued research and development.

Bluetongue (BT) and epizootic hemorrhagic disease (EHD) are noncontagious, insect-transmitted diseases of domestic and wild ruminants caused by related but distinct viruses. Both BTV and EHDV cause hemorrhagic fevers in susceptible ruminants; however, BT is principally a disease of domestic livestock whereas EHD is principally a disease of certain species of wild, non-African ungulates, notably white-tailed deer. BTV is the

¹USDA, ARS, Arthropod-Borne Animal Disease Research Unit, Center for Grain and Animal Health Research, Manhattan, Kansas. ²Department of Pathology, Microbiology and Immunology, School of Veterinary Medicine, University of California, Davis, California. prototype member of the genus *Orbivirus*, family Reoviridae (Mertens et al. 2004).

BT principally is a disease of certain breeds of sheep, although disease sporadically occurs amongst cattle, goats, South American camelids, and some species of wild non-African ruminants (Verwoerd and Erasmus 2004, Maclachlan et al. 2009, Verwoerd 2012). BTV infection of ruminants occurs throughout much of the temperate and tropical regions of the world, coincident with the distribution of specific species of hematophagous *Culicoides* biting midges that are biological vectors of the virus (Gibbs and Greiner 1994, Tabachnick 2004, Tabachnick 2010, Maclachlan 2011). BT typically occurs when susceptible sheep are introduced into areas where virulent strains of BTV circulate, or when virulent strains of BTV extend their range into previously unexposed populations of ruminants. The global distribution of BTV has generally been between latitudes of approximately 40–50°N and 35°S. Although single BTV serotypes had incurred transiently into southern portions of Mediterranean Europe during the 20th century, since 1999 multiple serotypes of BTV have invaded and spread throughout virtually all of western Europe, further north than had ever previously been documented in the region (Mellor and Leake 2000, Gomez-Tejedor 2004, Mellor et al. 2008, Rodriguez-Sanchez et al. 2008). For example, during the recent European BTV serotype 8 (BTV-8) epidemic, the virus spread throughout virtually of western Europe (Purse et al. 2005, Toussaint et al. 2006, Purse et al. 2008, Wilson and Mellor 2008, Guis et al. 2012). The global epidemiology of EHDV infection is less precisely defined than that of BTV, but the global range of EHDV is predicted to occur between latitudes 35°S and 49°N, coincident with the distribution of competent biting midge vectors (Savini et al. 2011). Unlike BTV infection, EHDV has not been described to date in Europe, although the virus occurs throughout extensive portions of Asia, Southeast Asia, Australia, Africa, and the Americas (Savini et al. 2011).

BT has been recognized in the United States since at least the late 1940s. BTV-10 was first isolated and characterized in California during the early 1950s (McKercher et al. 1953), and BTV-11, -13, and -17 were identified later (Barber 1979). BTV-2 was first reported in Florida in 1982, and since 1998 at least 10 additional serotypes of BTV have been isolated in the southeastern United States (Gibbs et al. 1983, Johnson 2007, MacLachlan 2011, 2013b). BTV-2, which was long considered to be confined to the extreme southeastern United States, was recently isolated from cattle in California (Maclachlan et al. 2013).

An attenuated, sheep-adapted monovalent BTV vaccine was developed and first used in 1906 in South Africa by Sir Arnold Theiler (Theiler 1908). By the 1950s, multiple BTV serotypes were isolated, attenuated, and developed as liveattenuated (modified live virus [MLV]) vaccines by serial passage in embryonated chicken eggs (Alexander and Haig 1951). The attenuation process was later modified to include plaque selection (purification) and propagation in cell culture rather than in embryonated eggs, as reviewed by Dungu and colleagues (Dungu et al. 2004a, b). These MLV vaccines have been produced and used for many years in southern Africa, and a polyvalent vaccine containing some 15 serotypes was eventually developed. The current MLV vaccine formulation used in southern Africa delivers a series of three pentavalent immunizations (five serotypes per immunization) individually administered at 3-week intervals, although immunity to all serotypes is incomplete (when delivered as a combination vaccine) (Dungu et al. 2004a, b).

Similar MLV vaccines have been produced and used since the 1950s in the United States and Israel. The embryonated egg-passaged BTV vaccine that was originally developed in California during the 1950s (McKercher et al. 1957) was withdrawn from the marketplace some 20 years later because of the ability of the vaccine virus to cross the placenta and infect the fetus and subsequently induce teratogenic defects in fetal ruminants (Shultz and DeLay 1955, Maclachlan et al. 2000, MacLachlan and Osburn 2008). Attenuated South African vaccine viruses were used briefly (until 2005) in portions of the Mediterranean basin following the incursion of BTV to the region in the late 1990s (Savini et al. 2008, Zientara and Sanchez-Vizcaino 2013). However, there were serious concerns related to potential reversion to virulence of these vaccines, notably the BTV-16 MLV, as well as their documented potential to be abortigenic, to be naturally transmitted by vector midges, and to reassort gene segments with wild-type BTV in the field (Ferrari et al. 2005, Batten et al. 2008, Savini et al. 2008, Savini et al. 2014). Although these vaccines were generally efficacious and effective, safety concerns led to development and use throughout much of Europe of inactivated vaccines to several BTV serotypes during the recent BT epidemic, notably BTV-8 after incursion into northern Europe (Szmaragd et al. 2007, Savini et al. 2008, Eschbaumer et al. 2009, Hamers et al. 2009a, Szmaragd et al. 2010b, Wackerlin et al. 2010, Eschbaumer et al. 2012, Moulin et al. 2012, Zientara and Sanchez-Vizcaino 2013).

Attenuated (MLV) BTV vaccines continue to be used in South Africa, the United States, and Israel. For the United States, the only BTV vaccine approved for national use in domestic livestock (sheep and goats) is against serotype 10, and produced by the Colorado Serum Company (Denver, CO). The BTV-10 vaccine strain used in this product was initially attenuated by passage in embryonated eggs using the traditional methodology of the Onderstepoort Veterinary Research Institute of South Africa. Attenuated vaccines against BTV-10, -11, and -17 are produced on behalf of the California Wool Growers by Poultry Health Laboratories (Davis, CA), and use of these vaccines is limited to sheep in California. No vaccine is currently available for immunization of livestock against BTV-13 in the United States nor to BTV-2 or the 10 additional BTV serotypes that were recently isolated in the southeastern Uniteed States. These MLV vaccines are capable of generating an effective immune response with one dose and prevent clinical BT disease in properly vaccinated animals subsequently infected with the homologous BTV serotype. There are numerous potential adverse consequences to the use of live-attenuated (MLV) BTV vaccines in livestock, including reduced milk production in lactating ewes, mild signs of disease, and abortion, early embryonic death, and teratogenesis when used in pregnant females, particularly in the first half of gestation (Schultz and Delay 1955). The risk of spread through vectors with potential reversion to virulence and gene reassortment is also considerable (Osburn et al. 1996, Ferrari et al. 2005, Batten et al. 2008, Savini et al. 2008).

Inactivated vaccines have also been used extensively in some regions of the world, and there has been recent (since 2005) commercial development of these vaccines during the European BT epidemic, but only against a limited number of virus serotypes (e.g., 1, 2, 4, 8, and 9) (Savini et al. 2008, Zientara and Sanchez-Vizcaino 2013). These inactivated vaccines have been used extensively in Europe and are generally safe. Two doses of the vaccines are often, but not invariably, required to induce a complete and long-lasting immunity (Savini et al. 2008). Licensed inactivated vaccines have not been commercially available in the United States, presumably because the estimated market has been small as it is limited to sheep. The combination of perceived efficacy issues (cross-serotype protection and incomplete immunity with polyvalent preparations) and safety issues (reversion to virulence, incomplete attenuation, and vector spread with gene reassortment) also contribute to the preferred use of inactivated vaccines as compared to MLV (MacLachlan, et al. 1985, MacLachlan and Osburn 1983, MacLachlan and Osburn 1988, Flanagan and Johnson 1995, Murphy et al. 2005).

Autogenous vaccines have been produced in the United States using inactivated BTV and EHDV antigens. These vaccines have been used extensively in sheep and the captive cervid industry with mixed, anecdotal reports of effectiveness. There are no published peer-reviewed data available for evaluation of these autogenous vaccines. Most of the autogenous vaccines contain multiple serotypes of EHDV and BTV as well as mixed bacterial antigen fractions/toxoids. Although the autogenous vaccines are perceived to be relatively safe, efficacy and effectiveness are questionable at best.

There are few commercial vaccines available to protect livestock against EHDV infections. Both attenuated and inactivated vaccines are commercially available in Japan for Ibaraki disease, which is caused by genetically distinct strains of EHDV—serotype 2 (EHDV-2, formerly EHDV-7) (Inaba et al. 1970, Inaba 1975, Kitano 2004). As related but different strains of EHDV-2 are clearly present in North America, use of similar vaccines could be considered for the United States. Ibaraki, although a distinct strain, is otherwise a typical EHDV that is virulent in cattle and capable of causing fatal infections (Campbell et al. 1975, Kitano 2004).

Immunity to BTV and EHDV, the Basis of Vaccination Strategies

Vaccine-induced and convalescent, infection-driven immune responses to orbiviruses such as BTV and EHDV include neutralizing antibody responses (directed at the VP2 protein) and nonneutralizing antibody to other structural and nonstructural viral proteins. The neutralizing antigenic determinants of orbiviruses reside on the VP2 outer capsid protein, but the other outer capsid protein (VP5) can influence the conformational nature of individual epitopes on VP2 (White and Eaton 1990, MacLachlan et al. 1992, Rossitto and MacLachlan 1992, DeMaula et al. 2000). The different serotypes of BTV segregate into clusters that sometimes demonstrate at least partial cross-neutralization by sera from naturally exposed animals. The degree of cross-recognition may increase with subsequent exposure of animals to multiple serotypes of BTV (Thomas 1985, Heidner et al. 1990, MacLachlan, et al. 1992, Dungu et al. 2004a b). In addition to humoral immune responses to BTV, there is also stimulation of multiple classes of T lymphocytes that generate cytokines, interferons and chemokines that regulate and facilitate effective immunological maturation and memory (Stott et al. 1985, Stott et al. 1990, MAPA 2006, Perez de Diego et al. 2012). These T lymphocytes include regulatory (helper) and effector (killer cell) populations.

Further study of this aspect of host responses to orbiviral infections is warranted to allow continued improvement of vaccines and to potentially overcome the inherent problem of serotype specificity of protective immunity (Calvo-Pinilla et al. 2014). Further, additional characterization of the response of dermal macrophages, lymph node, and blood dendritic cells as well as plasmacytoid dendritic cells as part of the early pathogenesis of orbivirus infection is warranted. Very early host responses are mediated by these cells and they include type I interferons, chemokines, and other cytokines (Chauveau et al. 2012, Darpel et al. 2012, Ruscanu et al. 2012, Ruscanu et al. 2013). One important consideration of the host immune response to vaccination is that immunization should reduce the extent and duration of peak viremia to prevent infection of feeding midges, which essentially prevents transmission of virus to susceptible animals (Savini et al. 2006a, b).

Inactivated Vaccines

Although not initially adopted for widespread commercial use, potentially efficacious inactivated BTV vaccines were first described in the 1970s (Parker et al. 1975, Stott et al. 1979, Campbell et al. 1985, Stevens et al. 1985, Stott et al. 1985). The first inactivated vaccine that was developed and used in the field after the recent emergence of BT in Mediterranean Europe was against BTV-2. Subsequently, a monovalent BTV-4 and a bivalent BTV-2 and -4 vaccine were developed and used in Corsica, Spain, Portugal, and Italy. Subsequently, inactivated vaccines against BTV-1 and BTV-9 were developed and commercialized, and several different commercial inactivated vaccines were also produced and widely used to protect livestock following the appearance of virulent BTV-8 in northern Europe in 2006 (Szmaragd et al. 2007, Savini et al. 2008, Eschbaumer et al. 2009, Hamers et al. 2009a, b, Szmaragd et al. 2010, Wackerlin et al. 2010, Moulin et al. 2012, Eschbaumer et al. 2012, Zientara and Sanchez-Vizcaino 2013).

Inactivated whole virus vaccines are very safe if produced properly. They can be highly efficacious (Stott et al. 1985, Di Emidio et al. 2004). Although not yet available, strategies for differentiating infected from vaccinated animals (DIVA) are theoretically possible with these types of vaccines. Their inherent potential disadvantages include: (1) High costs of production, because vaccine formulation usually requires a large antigen mass; and (2) need for multiple priming doses of vaccine as well as booster immunizations. Inactivated vaccines generally induce a relatively transient immunity with only one dose of vaccine. Inactivated vaccines also have inherent potential limitations due to stability and product "shelf-life," and these limitations may limit their utility in outbreak situations. Complex formulations that involve multiple component emulsions may require extended development which in turn may delay and limit availability.

Safety

In Europe, several studies have been conducted on sheep to evaluate the safety of the subcutaneous injection of inactivated monovalent and polyvalent vaccines against multiple serotypes of BTV in either simple, repeated, or overdose trials (reviewed by Savini et al. 2008, Zientara et al. 2010, Zientara and Sanchez-Vizcaino 2013). In all conditions, the inactivated BTV vaccines were very well tolerated, as demonstrated by the absence of systemic reaction (fever, weight loss, reproductive dysfunction) related to vaccination. Some vaccines induced transient local reactions of variable severity (mild to moderate) with different frequency (unusual to common). These usually disappeared within 3 days but, in a single case, a moderate local reaction persisted for 2 weeks (Hamers et al. 2009b). Anaphylactic shock was also reported in 0.02% of sheep following vaccination with a BTV-4 inactivated vaccine. This event was observed only in areas where BTV-4 MLV had previously been used.

Similar formulations of inactivated vaccines were also tested for safety in cattle (MAPA 2006). The vaccines were well tolerated, and no side effects or local reactions were observed, even when five doses of the BTV-4 inactivated vaccine were administered to the same animal (Savini et al. 2006a, b). In general, the numbers of adverse event reports from the field have been very low in Europe where these vaccines have been extensively used.

Efficacy

Consideration of both immunogenicity and efficacy data is required to evaluate vaccine-associated immunity. Immunogenicity is generally evaluated by analysis of antibody responses by virus-neutralizing activity in cell culture or by enzyme-linked immunosorbent assay (ELISA). With regard to BTV, efficacy is determined by challenge, *i.e.*, inoculating immunized animals with virulent virus. The resulting reductions in the peak and/or duration of viremia (or viral nucleic acid levels as assessed by quantitative [real time] PCR assay) are considered indicative of disease protection.

The inactivated prototype BTV vaccines used in Europe recently induced significant titers of neutralizing antibodies after one or two injections in sheep. A booster effect was observed after the second immunization (Savini 2006a, b, Hamers et al. 2006a, b). In cattle, one dose of BTV-4 or BTV-2 and BT-4 inactivated vaccines induced a weak humoral response that rapidly declined to be undetectable 21 days following vaccination. However, the second dose of vaccine elicited high and stable titers of neutralizing antibodies (MAPA 2006, Savini et al. 2006a, b). Inactivated commercial BTV-8 vaccines protect challenged animals, as reflected in increasing neutralizing antibody titers and reduced clinical signs, fever, and viremia (Moulin et al. 2012, Zanella et al. 2013a). Protection of the fetus in gestating animals has been demonstrated in mid-term ewes and heifers (van der Sluijs et al. 2013).

In cattle, efficacy studies have been performed on several inactivated BTV vaccines used during the recent European outbreak. Two doses of the inactivated BTV-4 vaccine administered at 24-day intervals prevented viremia in vaccinated animals challenged with the homologous virulent serotype. Similarly, none of the animals vaccinated with two doses of BTV-inactivated vaccine developed detectable viremia following challenge with virulent field strains of BTV-2 and/or BTV-4 that were performed up to 1 month after the second vaccination (Savini et al. 2006a, b). Although a single dose of inactivated vaccine prevented viremia in vaccinated animals challenged 2 weeks after vaccination, a single vac-

cination did not fully prevent viremia in animals challenged 7 months after vaccination (MAPA 2006). Neutralizing antibodies persist in cattle for at least 3 years in one study and even out to 4 years after immunization in another study (Oura et al. 2012, Batten et al. 2013).

A similar duration of antibody and cellular immune responses was detected at 2 years postimmunization in BTV-8-vaccinated sheep and cattle (Hund et al. 2012). This persistence of serum neutralizing antibody titers is similar to that observed after natural exposure or infection with BTV-8 (Eschbaumer et al. 2012). In another study, approximately 50% of the vaccinated sheep and cattle did not seroconvert and remained antibody negative 1 year after immunization (Zanella et al. 2013a). Colostral antibody from immune ewes can interfere with the immune response of immunized lambs if the lambs are vaccinated before 5 months of age (Leemans et al. 2013). The field efficacy and effectiveness of the inactivated BTV vaccine was indirectly confirmed when all but two of more than 40,000 seasonally migrating vaccinated Spanish cattle remained negative for BTV by RT-PCR after staying in a restricted area in the presence of BTV circulation (Jimenez-Clavero et al. 2006).

The role of inactivated vaccines in control of BTV-8 in Europe

BTV-8 emerged in northern and northwestern Europe in 2006 and 2007 causing devastating disease outbreaks among both cattle and sheep (Saegerman et al. 2008, Wilson and Mellor 2009). As this epidemic progressed, officials recognized that vaccination would be a critical component of control programs (Szmaragd et al. 2010a, b). As there had been significant development of inactivated vaccines for other BTV serotypes (see above), commercial inactivated vaccines for BTV-8 were developed and made available relatively quickly (by 2008). Inactivated BTV-8 viral antigen in adjuvanted formulations provided good immunogenicity in both cattle and sheep, although multiple doses and higherpotency formulations were required (especially in cattle) (Oura et al. 2009, Calistri et al. 2010, Hund et al. 2012). In fact, optimized immunization protocols using a single dose of vaccine generated strong antibody responses that persisted up to 4 years in cattle and for greater than 2 years in sheep (Oura et al. 2010, Bartram et al. 2011, Vitour et al. 2011, Oura et al. 2012, Batten et al. 2013). Although there is variability in neutralizing antibody responses (Zanella et al. 2013a, b), greater than 80% seroconversion of vaccinates has consistently been achieved (Hulten et al. 2012). The observed consistent immunogenicity has been supported by experimental efficacy studies in cattle and sheep (Eschbaumer et al. 2009, Hamers et al. 2009a, b, Wackerlin et al. 2010, Eschbaumer et al. 2012, Moulin et al. 2012). Furthermore, in addition to proven efficacy in the field, these vaccines also were effective at reducing BTV transmission (Szmaragd et al. 2007, Szmaragd et al. 2010a, b). Specifically, these vaccines generally affected a robust reduction of viremia so that vector transmission and transplacental transmission were prevented (Moulin et al. 2012, van der Sluijs et al. 2012, Batten et al. 2013, van der Sluijs et al. 2013). Inactivated BTV-8 vaccines are also very safe (Gethmann et al. 2009, Probst et al. 2011, Leemans et al. 2012) and herd and flock immunization compliance has been high (Probst et al. 2011).

MLV vaccines

MLV BTV vaccines continue to be used in the United States, Turkey, the Republic of South Africa, India, Israel, and elsewhere, and were used recently in portions of Europe. MLVs are produced by adapting field isolates of BTV to growth *in vitro* through serial passages in tissue culture or in embryonated chicken eggs (Erasmus 1975). Stimulation of a strong antibody response by these vaccines is directly correlated with their ability to replicate in the vaccinated host. MLVs are relatively inexpensive to produce in large quantities, they generate protective immunity after a single inoculation, and have proven effective in preventing clinical BT disease in the areas where they are used (Dungu et al. 2004a, b, Patta et al. 2004).

Safety

BTV MLVs suffer from a variety of documented or potential drawbacks, including underattenuation, with occurrence of disease in vaccinated sheep that is potentially breed specific (Veronesi et al. 2005, 2010). MLVs have different potential adverse impacts according to the specific formulation used, the specific virus serotype and strain, and the number of serotypes included in the vaccine. Potential adverse consequences are clinical disease with depressed milk production in lactating sheep and abortion/embryonic death and teratogenesis in offspring when used on pregnant females, especially those in the first half of gestation (MacLachlan et al. 1988, Monaco et al. 2004b, Monaco et al. 2004c, Savini et al. 2006a, b, Venter et al. 2004, Ferrari et al. 2005). Another risk associated with the use of MLVs is their potential for spread by vectors, with potential reversion to virulence and/or reassortment of MLV genes with those of wild-type virus strains. The frequency and significance of these events remain poorly defined but have been demonstrated in both North America and in Europe (Ferrari et al. 2005, Osburn et al. 1996, Batten et al. 2008). Natural dissemination of MLV strains of BTV (or reassortants thereof) is potentially responsible for the sporadic incidence of teratogenic defects in unvaccinated cattle in South Africa and North America (Maclachlan and Osburn 2008). Finally, the intrinsic inability to serologically distinguish naturally infected from MLV-vaccinated animals precludes the possibility of developing a serological DIVA strategy with the MLV vaccines.

Viremia after MLV immunization

Immunization with the attenuated viruses (MLVs) results in viremia similar to that which occurs following natural infection. Thus, the MLV vaccine virus potentially can infect competent vectors and be transmitted to other susceptible hosts. Therefore, MLV vaccination should be performed in the cooler months when few active *Culicoides* vectors are present. This practice will limit the possibility of transmission of the vaccine strains by biting midges while immunizing susceptible animal populations before the next epidemic season. However, MLVs often have been used during outbreaks, which is the peak vector season.

Transmission of MLV strains of BTV to insects would occur from viremic animals that are introduced into any new regions, including infection-free areas, where competent *Culicoides* species are present and highly active. Thus, the magnitude and duration of viremia in vaccinated animals is clearly important in determining whether or not MLV strains of BTV could be acquired and transmitted by local vectors (Gethmann et al. 2009, Probst et al. 2011, Leemans et al. 2012, Moulin et al. 2012, van der Sluijs et al. 2012, Batten et al. 2013, van der Sluijs et al. 2013).

Although virus titers in blood less than $10^3 50\%$ tissue culture infective dose (TCID₅₀)/mL have traditionally been considered a "safe" threshold, this level is speculative and authentic instances of insects acquiring BTV from animals with viremia titers less than 10^3 TCID₅₀/mL have been reported (Bonneau et al. 2002). Given the complex interaction of BTV, *Culicoides* vectors, and animal hosts in the life cycle of infection, virus titers induced by MLV should be kept to an absolute minimum, especially if field transmission of MLV strains is a concern.

Information pertaining to the MLV strains used in Europe is limited. Available data suggest that cattle vaccinated with BTV-2 and BTV-9 MLVs can be moved safely 32 days after vaccination (Monaco et al. 2004c), whereas sheep vaccinated with the same strains can be moved 28 days following immunization (Monaco et al. 2004c). From the viremia data obtained in cattle following BTV-2, -4, -9, and -16 MLV vaccination, it was determined that cattle could be moved safely (risk of infection < 0.01%) at 60 days after vaccination (Savini et al. 2006a, b). This observation, however, is most likely related to the inadequate attenuation of the BTV-16 MLV strain and cannot be extrapolated to MLV vaccines that do not include this serotype. Apart from some BTV-2 MLV vaccination studies on sheep and cows where virus titers were never found to be higher than 10^3 TCID₅₀/mL (Hammoumi et al. 2003, Monaco et al. 2004a, c), all other MLV combinations that have been studied in sheep (BTV-2, BTV-9, BTV-16; BTV-2 and -9; BTV-2 and -4; BTV-2, -4, and -16; BTV-2, -4, -9, and -16) and cattle (BTV-2 and -9; BTV-2, -4, -9, and -16) gave rise, for a brief period of 2-4 days, to viremic titers above the infecting threshold in at least some of the vaccinated animals (Monaco et al. 2004b, Cannas et al. 2005, Veronesi et al. 2005, Monaco et al. 2006, Savini et al. 2006a, b). There are no reports on the duration and titers of viremia in animals vaccinated with these MLV in the field, but local transmission of BTV-2 and BTV-16 vaccine strains in the field has been demonstrated (Ferrari et al. 2005, Monaco et al. 2006).

Efficacy

After the incursion of BTV into Mediterranean Europe, the Spanish, French, Italian, and Portuguese authorities all carried out compulsory vaccination campaigns (after 2000) using South African MLVs produced by Onderstepoort Biological Products, in an attempt to reduce direct losses due to disease and indirect losses due to trade embargoes caused by the presence of BTV. At that time, these were the only commercially available BTV vaccines. On the basis of the serotype(s) present in a given country/area, various MLV monovalent serotype formulations were used.

Interestingly, immunity derived from MLV immunization is not unlike immunity derived from inactivated vaccines. An important factor in confirming the immunogenicity of MLV vaccines is their ability to elicit neutralizing antibodies in vaccinated animals. Neutralizing antibodies play a key role in protecting animals from disease and viremia following reinfection with the homologous wild-type BTV. Knowing the duration of the immune status derived from vaccination is of paramount importance for both planning the frequency of vaccine booster immunizations to adequately protect the animals against disease and to facilitate the safe movement of vaccinated animals (OIE Terrestrial Health Code 2013). Experimental challenge studies have demonstrated that vaccination with the BTV-2 MLV strain prevented viremia in greater than 90% 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. 2004a, b). In that trial, the control animals were viremic through the 35th day with titers above $10^{5.8}$ TCID₅₀/mL. More than 80% of cattle and sheep that were vaccinated with MLV combinations had detectable BTV antibody titers several months after immunization (Hammoumi et al. 2003, Gerbier et al. 2004, Savini et al. 2004a, b, Gerbier et al. 2008). Colostral antibodies were found in calves born from vaccinated dams until 39 days of age (Savini et al. 2004a).

The efficacy and effectiveness of MLV vaccination has widely been demonstrated in the field. Following the 2000-2001 and 2003 BT vaccination campaigns in the Balearic Islands, no outbreaks have been detected since December, 2003, in the area. With regard to the vaccination strategy in Italy, several points warrant attention. First, on the basis of a risk assessment (Calistri et al. 2004, Giovannini et al. 2004a, b, c) and considering the encouraging results of preliminary studies, the Italian Authorities decided to vaccinate all susceptible domestic ruminant species (*i.e.*, sheep, goats, cattle, and water buffalo) in the infected and at risk areas, with the aim of limiting direct losses and reducing virus BTV circulation (Patta et al. 2004). Mass vaccination of susceptible populations started in January, 2002, although the starting dates and the percentages of vaccinated population achieved varied greatly among regions (Calistri et al. 2004, Giovannini et al. 2004b, c, Patta et al. 2004). In those areas where more than 80% of the target population was properly vaccinated before the new epidemic peak, clinical disease in sheep disappeared almost completely and virus circulation was significantly reduced (Patta et al. 2004), with substantial benefit to internal animal trade/movement.

The results obtained in some Italian regions with mass vaccination of all susceptible domestic ruminants and the experience gained during the vaccination campaigns contributed to the modifications of BT international standards. Specifically, risk analysis can be used as an alternative to individual testing to assess immunity level in the population of origin and determine the risk of spreading infection to free areas by movement of vaccinated animals from infected territories (Giovannini et al. 2004b, c). In particular, the analysis performed by Giovannini et al. (2004c) indicates that when more than 80% of the susceptible population in the territory of origin was vaccinated, the risk associated with the movement of vaccinated animals to free areas appeared acceptable and could further be mitigated by ancillary control measures.

In the absence of effective inactivated or recombinant or subunit vaccines, in an emergency MLVs still represent a valid option for vaccination, provided that the quality, safety, and efficacy of the MLV strains are appropriate. However, vaccination with MLVs should always be done during periods when vectors are absent or present in low numbers to minimize the potential for MLV vaccine viruses (or genes thereof) being incorporated into the gene pool of field strains of BTV. These vaccines can be an alternative also in a nonemergency situation when local conditions (*e.g.*, in cases where a large population of animals must be immunized in a very short period of time) indicate their use. In distinct contrast to the abundant data describing the efficacy and potential disadvantages regarding the use of BTV MLVs in Europe, there is virtually no recent objective published data on the impact of MLVs in the United States.

Recombinant Vaccine Technologies and Other Experimental Candidate Vaccines

Several experimental recombinant vaccines have been described and they clearly have numerous inherent potential benefits, including rapid onset of immunity, lack of transmissibility, potential for DIVA, and even a potential for a polyvalent strategy (Noad and Roy 2009, Maclachlan and Mayo 2013, Calvo-Pinilla et al. 2014). A recombinant vaccinia virus that expressed both VP2 and VP5 of Australian BTV serotype 1 induced variable titers of neutralizing antibody in sheep and afforded protection against homologous challenge (Lobato et al. 1997). There is a report of other similar experimental vaccines (Calvo-Pinilla et al. 2009). Both virus-like and core-like particle vaccine candidates have been efficacious (Lourenco and Roy 2011, Perez de Diego et al. 2011, Stewart et al. 2012, 2013). A recombinant capripoxvirus expressing VP7 was shown to provide partial protection against heterologous BTV challenge (Wade-Evans et al. 1996, Perrin et al. 2007), but like the recombinant vaccinia BTV vaccine, its development was not continued apparently. Finally, a recombinant canarypox virus-VP2/VP5 vaccine was recently described that induced highly effective protective immunity in sheep (Boone et al. 2007). This vaccine has a major inherent advantage in that the existing VP7 competitive ELISA assay would distinguish vaccinated from naturally infected animals (DIVA), and it uses an expression vector that is incorporated in several vaccines already in use in the European Union and elsewhere, although not in ruminant livestock. The vaccine still is in development. Other recombinant viruses have been evaluated experimentally for antigen delivery of BTV antigens (Ma et al. 2012).

There are other vaccine candidates in developmental research evaluation. They include replication-deficient monovalent and multivalent viruses, combinatorial subunit antigens for prime-boost delivery, VLP vaccines using cowpea mosaic virus capsid proteins and multiple recombinant subunits in adjuvants, recombinant antigens as subunit vaccines as well as expressed in plants (Calvo-Pinilla et al. 2009, 2012, Anderson et al. 2013, Celma et al. 2013, Jabbar et al. 2013, Thuenemann et al. 2013). Continued research and development of these approaches are warranted, particularly as some candidate vaccines provide a strategy for solutions to issues such as crossserotype protection, DIVA compatibility, nonreplicating immunogens (no subsequent transmission of viral RNA), and rapid onset of immunity with one dose of vaccine.

Summary and Conclusions

A variety of vaccine strategies are potentially available for immunization of ruminant livestock against orbiviral diseases; however, of these options, only MLVs and inactivated vaccines have been produced commercially and used widely to prevent BT. Of these two strategies, inactivated vaccines appear to be the safer option, and one or two doses of inactivated vaccine provide protection against viremia. However, two injections of these vaccines will provide stable neutralizing antibody titers and protection from viremia. Studies of experimental efficacy (that is, by virulent virus challenge of immunity) have been supported by field observations that immunized animals were not infected by BTV even though exposed to vector-transmitted circulating virus.

BT vaccines may be used for different purposes or strategies, depending on the epidemiological situation of the affected area and strategy desired. The main purposes of BT vaccination strategies are to: (1) Prevent clinical disease, (2) limit the regional extension of BTV infection through reduction of the spread of the virus, (3) allow regional or country eradication of the disease based on the reduction of virus circulation, and (4) authorize the safe movement of susceptible animals between affected and free zones. With MLV (live-attenuated) BTV vaccines, viremia will be prevented in >90% of vaccinated animals with one dose of vaccine. The duration of protection is presumably quite long because >80% of vaccinated animals have stable neutralizing antibody titers. Furthermore, field use of attenuated vaccines has been associated with near complete clinical protection, and virus circulation was reduced. Further analysis of these studies reveals that immunization of approximately 80% of a susceptible population will provide effective herd immunity. The inherent shortcomings and potential disadvantages of MLVs are well documented.

In BT endemic regions, vaccines have been used to prevent clinical disease and death losses in sheep. In these regions, vector *Culicoides* spp. vectors may be present year round with continuous circulation of different BTV serotypes. This has led to the design of multivalent vaccines containing different MLV serotypes, as done in South Africa, where BTV infection is endemic. The South African MLVs were developed only to control clinical BT disease in sheep, because cattle and other ruminants, although susceptible to BTV infection, usually do not generally suffer clinical disease.

Since the incursion of BTV into previously nonendemic zones, as in some Mediterranean countries, BTV vaccines are used as an aid to prevent further extension of the infection to border zones. This provides local/regional reduction of virus circulation and safe movements of animals, which play an important role in the European livestock industry. Depending on their availability, MLV or inactivated serotype-specific vaccines can be used. Climatic and geographic factors as well as abundance of suitable BTV insect vectors are probably all important for the outcome and persistence (reemergence) of BTV infection in an area. It is commonly accepted that vaccines can help limit the spread of the disease. Ideally, for the purpose of eradication, a successful vaccination campaign should cover all susceptible ruminant species, attain a high degree of herd immunity, and encompass extensive areas surrounding any active BT outbreak. Successful control also requires restriction of animal movements between BT-affected and BT-free zones. It is to be stressed that vaccination is more likely to be effective in controlling BT outbreaks when only a single BTV serotype invades a previously free region, as occurred with BTV-8 in northern Europe. In contrast, it is clear that BTV has remained endemic in countries where MLV BTV vaccines are/were used (*e.g.*, Israel, Italy, South Africa, United States) and where more than one BTV serotype is present (Maclachlan 2011).

Limitations to vaccine-associated immunity and clinical use

There are three major technical concerns associated with the further development and use of BTV vaccines. As such, these concerns are the obstacles that must be overcome to provide effective and available vaccines. They are:

- 1. The onset of immunity with any particular vaccine formulation must be defined. The rapid antibody response kinetics and field protection observed with both inactivated and attenuated vaccines suggests a very reasonable and acceptable onset of immunity. But the onset of immunity data could be very useful with regard to constructing immunization programs in the field. This is particularly important in the case of a vector-borne disease where the *Culicoides* vectors may spread disease (in terms of distance of spread and numbers of animal exposures) very rapidly.
- 2. Immunity to BTV is immunologically complex. With both live-attenuated (MLV) and inactivated vaccines, sustained protection from viremia, specificity of virus-neutralizing antibody responses, and field effective-ness are effectively serotype specific. Antibody and cellular interactions with critical viral structures require interactions with a complex set of linear and conformational epitopes. This problem will require continued research to define the nature of protective antigen structures and development of unique formulations and methods for delivery. This research will also define better serological and cellular assays as correlates of protective immunity.
- 3. On a global basis, support for developing BTV vaccines has been lacking. Most regions of the world deal with endemic BTV infection with no or few attempts to vaccinate susceptible animals. Occasional bursts of disease activity revive some interest in related research. Funding is required for developmental research to improve vaccine efficacy and safety profiles, which will in turn increase the availability of relevant vaccines. The likelihood of future disease outbreaks is high, especially with climate change, increased global commerce, and other anthropogenic drivers of infection (Maclachlan and Mayo 2013a).
- 4. These concerns are, in part, technical issues associated with the biology of the disease agent-host interaction and/or a need for additional research and development. In addition, there are some restrictions on vaccine use that are in place because of market restrictions and the use of serological methods to ascertain exposure. Improved efficacy and safety profiles along with DIVA technology would facilitate removal of these regulatory restrictions (Tables 1 and 2).

Recommendations

Vaccination is currently central to the response of most atrisk countries to any BT outbreak. Vaccination, however, can be problematic given the plurality of BTV serotypes, coupled

TABLE 1. SUMMARY OF CURRENT STATUS AND OBSTACLES TO VACCINATING AGAINST ORBIVIRUSES

- 1. Whereas a limited number of vaccines are available internationally for bluetongue virus (BTV), there are none for epizootic hemorrhagic disease virus (EHDV). There are substantial issues regarding availability of inactivated BTV vaccines, because some no longer are produced commercially (*e.g.*, those to BTV serotype 8).
- 2. Inactivated vaccines provide only serotype-specific protection but are reasonably efficacious and effective. However, they currently are the best option to provide vaccines in the face of an epizootic emergency.
- 3. Autogenous vaccines have been used in the captive cervid industry to immunize deer against EHDV infection. Success has been limited at best. No peer-reviewed objective data are available to assess immunogenicity, efficacy, or effectiveness.
- 4. A focused effort to identify potential master seed stocks of North American serotypes of BTV and EHDV should be initiated. Inactivated vaccines have been produced against only a limited number of BTV serotypes. Revamping production of an existing commercial vaccine can take several months, but creation of an entirely new one can take 2 years or longer, so the presence of available seed stocks to all 26 serotypes of BTV and all nine serotypes of EHDV would potentially expedite creation of new vaccines.
- 5. Current inactivated vaccines do not typically provide "sterilizing immunity," that is, current vaccines may not prevent virus transmission following infection.
- 6. Attenuated, modified live vaccines have very significant safety issues associated with their dissemination by insect vectors and reassortment of genes with those of circulating wild-type virus in the field, vertical transmission, and inherent issues related to either under- or overattenuation of the vaccine virus.
- 7. Neither inactivated nor live-attenuated BTV vaccines are differentiating infected from vaccinated animals (DIVA) compatible, whereas new-generation products could be (similarly for EHDV).
- 8. Standardization of diagnostic procedures within diagnostic laboratories and establishment of routine surveillance are critical components of any orbivirus control program.

with apparent serotype-specific immunity in livestock. Thus, effective vaccines potentially must be developed to all 26 currently recognized BTV serotypes. Furthermore, there is a glaring lack of choices in terms of currently available commercial vaccines for BTV. This is particularly true in the United States, and almost all recent data for evaluation of *Orbivirus* vaccines comes from Europe, Africa, and the Middle East. Live, attenuated (MLV) vaccines are routinely used to prevent BT among sheep in the United States, South

TABLE 2. THE WORKSHOP PARTICIPANTS DETERMINEDTHAT THE FOLLOWING CRITERIA CONSTITUTEDTHE IDEAL VACCINE PROFILES FOR BLUETONGUEVIRUS AND EPIZOOTIC HEMORRHAGIC DISEASE

- Efficacy
 - Development of new generation vaccines for both epizootic hemorrhagic disease virus (EHDV) and bluetongue virus (BTV) that provide:
 - Rapid onset of immunity in the face of an emerging outbreak
 - No transmission by insect vectors
 - Cross-serotype protection
- Cross-protection among serotypes
- <1 week onset of immunity
- No maternal antibody interference
- Two-year shelf life
- Safe vaccine
- No high containment required
- Differentiating infected from vaccinated animals (DIVA) compatible
- Rapid scale-up
- Reasonable cost
- Short withdrawal period
- Feasibility of registration
- Add new antigens
- Accelerated delivery

Africa, and Israel, and MLV vaccines were used for compulsory vaccination of cattle and sheep in Italy (and some other countries of Mediterranean Europe) following the incursion of BTV into that country in 1999. MLV vaccine viruses clearly can be acquired and transmitted by insect vectors, then circulate as field strains, and they can reassort gene segments with field viruses to generate novel progeny. MLV vaccines also have the capacity to cross the placenta to infect the fetus. Last, MLV vaccines have never been successfully used to eradicate BTV infection in countries where more than one BTV serotype is present.

Inactivated BTV vaccines, which were not commercially available at the beginning of the recent European epizootic, enjoy several potential advantages over MLV vaccines. Specifically, inactivated vaccines cannot revert to virulence, reassort genes with field or MLV viruses, or cross the placenta to cause reproductive losses. Inactivated vaccines were exclusively used in response to the outbreak of BTV-8 in Europe. However, inactivated vaccines suffer from their relative slow onset of immunity, as compared to MLV vaccines, and the lack of commercial products for most serotypes. Newgeneration products such as baculovirus-expressed virus-like particles (VLPs) and vectored recombinant vaccines, including a canarypox virus recombinant expressing the VP2 and VP5 outer capsid proteins of BTV, have been shown to be effective experimentally, but their inherent cost and limited market potential have prevented their commercial use to date. Subunit vaccination strategies are clearly viable for BTV as the neutralization epitopes are clustered on VP2, although the expression of immunogenic VP2 can be challenging, given the conformational nature of individual epitopes.

In summary:

1. Use of attenuated (MLV) vaccines should be avoided if possible, although these are the only vaccines available currently in the United States and their use to protect

against serotypes 10, 11, and 17 may need to continue for the near future in sheep. These vaccines do provide good protection from clinical disease with homologous serotype infections. There are no currently licensed vaccines available for EHDV in the United States.

- 2. If there is a need to rapidly develop a vaccine to meet an emerging crisis associated with an especially virulent orbivirus of a single serotype, development of an inactivated virus vaccine in a conventional adjuvanted formulation will be required. With two doses of vaccine, inactivated vaccines can provide substantial immunity to the epizootic serotype. This strategy is similar to that used in the 2006–2008 BTV-8 outbreak in northern Europe that provided vaccine to the field by 2008. There is a need to explore and develop regulatory mechanisms to deploy such vaccines in an emergency situation.
- 3. There are significant gaps in our scientific knowledge and available countermeasures to control a disease outbreak that will require improvements that can only be achieved through a coordinated research agenda to achieve a more optimal vaccine profile (see Table 2).

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Address correspondence to: D. Scott McVey USDA ARS ABADRU 115 College Avenue Manhattan, KS 66502

E-mail: scott.mcvey@ars.usda.gov