

REVIEW

Bluetongue virus vaccine: conventional to modern approachK. RANJAN¹, M. PRASAD², B. BRAR², U. LAMBE², R. KUMAR³, M. GHOSH⁴, G. PRASAD⁵

¹Department of Veterinary Physiology and Biochemistry, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, 250110, India; ²Department of Animal Biotechnology, LLR University of Veterinary and Animal Sciences, Hisar, Haryana, 125004, India; ³Department of Veterinary Physiology, College of Veterinary & Animal Sciences, Pookode, Wayanad 673576, India; ⁴APR Division, ICAR-Central Institute for Research on Buffaloes, Hisar-125 001, Haryana, India; ⁵Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, Uttar Pradesh, 250110, India

Received January 16, 2018; revised May, 11, 2018; accepted February 4, 2019

Summary. – Bluetongue (BT) is an economically important, infectious and non-contagious disease of ruminant animals. BT disease is caused by bluetongue virus (BTV) of the genus *Orbivirus* (the family *Reoviridae*). BTV is transmitted by certain species of biting midges of the genus *Culicoides*. Although originally BT was restricted to African continent, now it is present in all the continents except Antarctica. Conventional BT vaccines such as live attenuated and inactivated vaccines showed different degree of success in BT control. However, conventional vaccines have certain disadvantages of reversion to virulent strain and frequent booster dose requirement. Several BT outbreaks in India and the rest of the world open a new insight for development of better vaccines. The development in molecular biology techniques allowed the development and validation of several modern vaccines such as subunit vaccine, recombinant vector vaccine, disabled infections single cycle (DISC) vaccine, differentiating infected from vaccinated animals (DIVA) approach etc. Most of these vaccines are considered as safer, having better protective immune response and provided cross-protective immunization against more than one serotype.

Keywords: bluetongue virus; live vaccine; inactivated vaccine; DISC; recombinant vaccine

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E-mail: drkoushleshranjan@gmail.com; phone: +91-9729828914.

Abbreviations: BEI = binary ethylenimine; BT = bluetongue; BTV = bluetongue virus; CLP(s) = core-like particle(s); DISA = disabled infectious single animal; DISC = disabled infectious single cycle; DIVA = differentiating infected from vaccinated animals; LAV(s) = live attenuated vaccine(s); MVA = modified vaccinia virus Ankara; VLP(s) = virus-like particle(s); muNS-Mi = mu (μ) non-structural-microsphere

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1. Introduction

Bluetongue (BT) disease is caused by bluetongue virus (BTV) of the family *Reoviridae* under the genus *Orbivirus*. BTV is a non-enveloped, structurally complex virus having 10 dsRNA genome segments surrounded by three concentric layers of protein shells (Verwoerd, 1969). BT is highly infectious and non-contagious, arthropod (*Culicoides*) vector-borne viral disease of domestic (sheep, goat and cattle) and wild (samber, deer, antelope, elk, elephant, mithun, llama etc.) ruminants (Prasad *et al.*, 1998; Roy, 2005; Bommineni *et al.*, 2008; Rajkhowa *et al.*, 2008; Meyer *et al.*, 2009). The disease is more severe in sheep, mild in case of goat, while cattle and buffalo act as reservoir (Browne, 1971). However, BT outbreaks have also been reported in cattle (Mehlhorn *et al.*, 2008). The disease was also reported in white tailed deer (Verwoerd and Erasmus, 2004) and sporadically in South American camelids and even from carnivores (Meyer *et al.*, 2009). BTV is transmitted by biting midges of the genus *Culicoides* (the family *Ceratopogonidae*) (DuToit, 1944). BT virus replicates in salivary glands of *Culicoides* (Browne and Jones, 1966). There are more than 1400 different species of *Culicoides* but only few are considered as competent vectors of BTV (Meiswinkel *et al.*, 2008; Ranjan *et al.*, 2017a,b). The BTV-infected animals are treated with symptomatic therapy, which includes gentle handling of affected animals, their stabling and administration of non-steroidal antiphlogistic drugs (Tweedle and Mellor, 2002). Other measures such as monitoring of animals through clinical, serological and virological examinations as well as monitoring of insect vectors are also adapted. The prophylactic immunisation with suitable antiviral preparations and removal or prevention of vectors attack to animals can be ensured.

2. Bluetongue virus and its genome organization

The virion particle of BTV is icosahedral and non-enveloped. It consists of inner core and outer capsid. The whole genome of BTV is of 19 kbp and consists of ten dsRNA segments and encodes 7 structural (VP1 to VP7) and 5 non-structural (NS1, NS2, NS3/NS3A, NS4 and NS5) proteins (Mertens *et al.*, 1984; Belhouchet *et al.*, 2011; Stewart *et al.*, 2015). Segment 1-encoded VP1 protein forms transcription complex along with VP4 and VP6 proteins and acts as RNA-dependent RNA polymerase (Roy *et al.*, 1988). Segment 2-encoded VP2 protein forms triskelion motifs on outer capsid of virion particle. VP2 protein is responsible for he-

magglutination, receptor binding and serotype specificity of the virus (Hassan and Roy, 1999). VP5 protein is arranged as trimers that form outer-layer globular motifs of BTV particle (Nason *et al.*, 2004). VP5 protein is significantly conserved and acts as minor serotype-specific protein (Singh *et al.*, 2005). VP5 protein also assists in release of mature virion particle from the endosomal compartment to the cytoplasm of the host cell (Hassan *et al.*, 2001).

Segment 3-encoded VP3 protein is highly conserved and forms inner most sub-core icosahedral symmetry of the virion particle (Loudon and Roy, 1992). Segment 4-encoded VP4 protein is guanylyl-transferase or transmethylase and acts as capping enzyme. Similarly, VP6 protein shows helicase and NTPase activity and binds with ssRNA and dsRNA. The VP7 is a serogroup-specific protein found on outer core surface of virion particle. The non-structural proteins are expressed in infected host cells and are not part of the virion particle. The NS1 protein forms tubules for translocation of progeny virus particles to the cell membrane in infected host cell (Owens *et al.*, 2004). The ssRNA-binding NS2 protein forms inclusion body in host cell cytoplasm (Owens *et al.*, 2004). NS3 and NS3a are expressed in insect cells only and assist in release of virion particles from infected cells (Hyatt *et al.*, 1993). Recently, two more NS proteins NS4 and NS5 have also been reported, whose actual functions are yet to be confirmed.

3. Bluetongue virus epidemiology

BT infection is stretched from 35°S to 40°–50°N of the globe. The restriction in geographical distribution of BTV can be correlated with favorable environmental and climatic conditions, which support the *Culicoides* breeding. *Culicoides* vectors can transmit BTV to long distances in new geographical areas (Burgin *et al.*, 2012). There are 27 distinct BTV serotypes have been identified worldwide so far (Hofmann *et al.*, 2008; Jenckel *et al.*, 2015). All these serotypes are determined based on specific interaction of viral outermost VP2 protein of a particular serotype with corresponding neutralizing antibodies in serum neutralization assays (Eaton *et al.*, 1990; Maan *et al.*, 2007).

India is endemic for BTV because the tropical rainy climate of country favors the breeding of *Culicoides* vector. It was estimated that India harbors 63 distinct *Culicoides* species in different geographical locations (Ranjan *et al.*, 2015). However, only a few species of *Culicoides* have been demonstrated as potent vector for BTV transmission. Recently, *Culicoides oxystoma* and *Culicoides peregrinus* were reported as potent vector of BTV 16 and 23 in India (Ranjan *et al.*, 2017a,b). Out of 27, a total of 24 different BTV serotypes (except BTV serotypes 25, 26 and 27) have been reported from different regions of India (Prasad *et al.*, 2009;

Minakshi *et al.*, 2012; Dadawala *et al.*, 2013; Kumar *et al.*, 2013; Prasad *et al.*, 2013; Ranjan *et al.*, 2013, 2014a,b, 2015; Vishwaradhya *et al.*, 2013; Maan *et al.*, 2015; Krishnajyothi *et al.*, 2016; Hemadri *et al.*, 2017; Reddy *et al.*, 2017).

Similarly, 12 BTV serotypes (BTV1, 2, 3, 5, 7, 9, 12, 15, 16, 20, 21 and 23) have been isolated in Australia (Melville *et al.*, 2015). BT virus is mostly endemic in northern Australia and its distribution extends down the east coast into New South Wales and south of Sydney, where climate is favorable for *Culicoides* vector. However, clinical form of BT disease is not much reported in Australia, possibly due to limited overlap between sheep farming regions and those places, where BTV is prevalent (Firth *et al.*, 2017). China is also endemic for several BTV serotypes but predominant are BTV1, 2, 3, 4, 12, 15 and 16 (Zhang *et al.*, 2010). BT has also showed considerable socioeconomic concern in European Union nations. Once it was considered an exotic disease in Europe. Now it is well established that at least 6 BTV serotypes (BTV1, 2, 4, 8, 9 and 16) were continuously present in Europe. Later on, BTV8 has caused a severe epizootic outbreak in northern Europe (Saegerman *et al.*, 2008).

Sequence data analysis showed that viruses of western lineages circulate in Africa, Caribbean and the Americas. However, eastern lineages of BTV are found endemic in Australia, Asia and Indonesia (Carpi *et al.*, 2010). The segmented nature of BTV genome allowed the reassortment of its genome, which results in BT virus with genome segments from different parent BT viruses (He *et al.*, 2010). Surveys have indicated that South Africa is endemic for BTV and 22 of the known 26 serotypes are present with serotypes 20, 21, 25 and 26 being considered as exotic (Coetzee *et al.*, 2012). Cattle, indigenous sheep breeds and wild ruminants are frequently infected by multiple serotypes but rarely demonstrate clinical signs of BT disease. However, European sheep breeds usually showed clinical form of disease (Coetzee *et al.*, 2012). USA is also endemic for BTV. Several studies have reported the prevalence of BTV serotypes 1, 2, 3, 4, 6, 8, 10, 11, 12, 13, 14, 17, 19 and 22 in USA (Schirtzinger *et al.*, 2018).

4. Prophylactic immunization

The prophylactic vaccination may prevent the clinical manifestation of BT in animals. Vaccination results in interruption of BTV cycle and ensures that lesser number of animals would be infected by BTV. Thus, it reduces the economic loss caused by BT by safer transfer and trading of animals from BTV enzootic regions to other countries (Savini *et al.*, 2008; Bhanuprakash *et al.*, 2009; Caporale and Giovannini, 2010). BTV serotypes usually show very poor cross-protective immunity. Moreover, BTV serotypes are not uniformly distributed in a geographical region. Therefore, during selection and formulation of BTV vaccine, serotype

specificity and local area BTV prevalence should be taken into account (Bhanuprakash *et al.*, 2009). In a region with a history of single serotype BT outbreaks, vaccination against that serotype is needed for protection against viremia. In contrary, in a region with multiple serotype prevalence, protection against all the circulating serotypes is required. However, the ideal single BT vaccine for all regions is not available (Feenstra *et al.*, 2017). For BT control, several types of vaccination approach such as live attenuated, inactivated, subunit and many modern vaccine formulations have been in use. Each of the vaccination approaches has its own advantages and disadvantages (Table 1).

5. Conventional vaccine approach

5.1 Live attenuated vaccines

Live attenuated vaccines (LAV) are prepared from living pathogens that have been weakened in laboratory. They are derived from wild type disease-causing microorganisms. LAVs lead to an excellent immune response because they provide continuous antigenic stimulation for memory cell production. In the case of viruses or other intracellular microorganisms, where mostly cell-mediated immunity is desired, LAV may be a choice because they are capable of replicating within host cells. For BT control, LAV is only the commercially available vaccine in endemic regions, where multiple serotypes of virus are prevalent (Caporale and Giovannini, 2010). In BT endemic regions such as South Africa, multivalent live attenuated vaccines against several BTV serotypes are still in use (Veronesi *et al.*, 2005). A single dose of LAV also elicits sufficient immune response and protective immunity against virulent strain of European BTV serotype 8 in sheep. Post vaccination, vaccinated animals didn't show any clinical signs, while the unvaccinated sheep showed severe clinical signs of BT (Dungu *et al.*, 2008).

LAVs are generally produced by either serial passages in cell lines followed by lyophilisation (freeze drying) process. The serial passages of BTV4, 9 and 16 in BHK-21 or Vero cell cultures reduced the viral replication capability in bovine fetal aorta (BFA) cell line as compared to virulent homologous strains (Franchi *et al.*, 2008). The vaccines produced from attenuated BTV9 or BTV4 strains were found safe, immunogenic and elicit sufficient immune response to prevent viremia and clinical disease symptoms in sheep following challenge with virulent homologous BTV strains (Franchi *et al.*, 2008). A freeze dried polyvalent Onderstepoort LAV for BT (Reg. No. G 358 Act No. 36/1947) was validated for prophylactic immunisation in sheep (OBP, 2013). The polyvalent LAV comprises of 3 bottles having several BTV serotypes such as Bottle A (BTV serotypes 1, 4, 6, 12 and 14), Bottle B (BTV serotypes 3, 8, 9, 10 and 11) and Bottle

Table 1. Comparison of different strategies of bluetongue vaccines

| S.n. | Vaccine | Vaccine formulation | Advantages | Disadvantages |
|------|---|---|---|---|
| 1 | Modified live vaccines (MLVs) | Live BT virus allowed for several passages in cell culture or host system. Post vaccination, viruses replicate in host animal without causing disease and elicit immunity against BTV. | Usually single dose vaccination is possible. Cost effective. Immunity can persist for several years. | Possibility of reversion back to virulent strain due to genetic reassortment with virulent BTV strains. Possibility of transmission by <i>Culicoides</i> vector. Several side effects such as abortion, decreased milk yield etc. reported. |
| 2 | Killed or inactivated vaccines | BT virus are inactivated by UV radiation, heat treatment and chemical agents and mixed with specific adjuvant, which stimulates the immune system against the antigens available on inactivated viruses. | Cannot reassort with virulent BTV strains. Considered as safe vaccine. | Multivaccine doses are required as immunity exists for short time. Hypersensitivity to adjuvant in host is possible. |
| 3 | Recombinant vector vaccines | BTV-specific antigenic genes are introduced in genetically modified non-pathogenic viruses to elicit immunity against BTV. Such modified viruses are used for vaccination to animals. | Usually single dose vaccination is used. Strong neutralizing immunity is observed. | Technically difficult to design. Expensive in nature. |
| 4 | Disabled infectious single cycle (DISC) vaccines | Deletion of an essential gene results in modified BTV virions, which will replicate one time post vaccination. Purified single cycle BTV viruses are used as vaccine. | Risk of reversion to virulence is lower. | Difficult to design the vaccine. Expensive in nature. Multiple doses are required. |
| 5 | Disabled infectious single animal (DISA) vaccine | BT virus without expressing non-structural proteins NS3/NS3a is used as DISA vaccine. It is a replicating vaccine, induces serotype-specific protection without causing detectable viremia. | Risk of reversion to virulence is lower. Chimeric vaccine against more than serotype is possible. | Difficult to design the vaccine. Expensive in nature. |
| 6 | Differentiating infected from vaccinated animals (DIVA) | In DIVA strategy vaccine is based on a different strain than the current field strain. A serological test is used to differentiate between vaccine-induced antibodies and antibodies against the field virus. | Can differentiate between infected and vaccinated animals. Special restrictions, necessary for infected animal's trade, can be avoided for vaccinated animals. | DIVA vaccine is not so effective in comparison to conventional vaccines. Special DIVA tests have to be developed, which are sometimes not as sensitive as conventional tests. |
| 7 | Virus-like particle vaccines | Insect cell-grown genetically modified baculovirus vectors express BTV structural proteins, which assemble into empty viral particles. Such purified viral particles with adjuvants are used as vaccine. | Lower possibility of side effects. High stability. No risk of disease. | Multiple doses required. Difficult to design the vaccine. Expensive in nature. Local reaction to adjuvant possible. |

C (BTV serotypes 2, 5, 7, 13 and 19) (Table 2) (OBP, 2013). The lyophilized strains of BTV4 and 16 used as live vaccine elicit sufficient protective immune response in sheep, which was evidenced by absence of illness in animals following challenge with virulent homologous strain (Table 2) (Zhungunissov *et al.*, 2015).

Although the production cost of LAVs is lower (Savini *et al.*, 2008; Bhanuprakash *et al.*, 2009), they may lose efficiency in tropical conditions, especially if the temperature is above 35°C (Hammoumi *et al.*, 2003). Certain concerns regarding safety of LAVs have also been raised. LAVs generally produce viremia, which lasts over two weeks in vaccinated sheep (Veronesi *et al.*, 2005). It may provide an opportunity for vectors to spread live vaccine virus to other healthy animals. Thus, within vector or host, live vaccine virus may either revert to virulent virus or produce a recombinant progeny virus after

reassortment of genes between LAV virus and wild type BTV strains (Tweedle and Mellor, 2002; Veronesi *et al.*, 2005; Savini *et al.*, 2008). Some of the Indian BT viruses such BTV2 (isolate IND2003/02) and BTV10 (isolate IND2004/01) showed >99% nucleotide identity with live attenuated vaccine strains of BTV2 from South Africa (Maan *et al.*, 2015) and BTV10 from USA (Maan *et al.*, 2012), respectively, which indicates their possible origin from LAV (Ranjan *et al.*, 2015). It is also observed that multivalent LAVs elicit poor immune response. Moreover, LAVs may lead to appearance of clinical signs of BT such as reduced milk production, abortion, still-birth, poor semen quality in rams (Breard *et al.*, 2007; Savini *et al.*, 2008) and fetal malformation in ewes (Tweedle and Mellor, 2002; Bhanuprakash *et al.*, 2009). The study by some of the researchers showed that BTV2 and BTV9 LAV strains can cross the placental barrier of cattle, sheep and goats and

Table 2. Live attenuated vaccines used for bluetongue disease

| Virus origin | BTV serotype | Strain | Remarks | Reference |
|------------------------|-------------------------|--|---------------------------|---|
| RSA, 1958 | BTV1 | Biggarsberg/8012 | | |
| RSA, 1958 | BTV2 | Vryheid/5036 | | |
| Cyprus, 1944 | BTV3 | Cyprus/8231 | | |
| RSA, ~1900 | BTV4 | Theiler/79043 | | |
| RSA, 1953 | BTV5 | Mossop/4868 | | |
| RSA, 1958 | BTV6 | Strathene/5011 | | |
| RSA, 1955 | BTV7 | Utrecht/1504 | | |
| RSA, 1937 | BTV8 | Camp/8438 | For use in sheep only | (European Commission, 2000; OBP, 2013) |
| RSA, 1942 | BTV9 | University Farm/2766 | | |
| Portugal, 1956 | BTV10 | Portugal/2627 | | |
| RSA, 1944 | BTV11 | Nelspoort/4575 | | |
| RSA, 1941 | BTV12 | Estantia/75005 | | |
| RSA, 1959 | BTV13 | Westlands/7238 | | |
| RSA, 1959 | BTV14 | Kolwani/89/59 | | |
| RSA, 1976 | BTV19 | 143/76 | | |
| Pakistan | BTV16 | Pakistan/7766 | | |
| China | BTV1 BTV16 | Live attenuated | For use in sheep and goat | Zhang <i>et al.</i> , 2004 |
| RSA | BTV2 BTV8 | Modified live vaccines | For use in sheep | Modumo and Venter, 2012 |
| Republic of Kazakhstan | BTV4 BTV16 | Khuroson-40/13/4 RT/ RIBSP40/13/16 | For use in sheep | Zhugunissov <i>et al.</i> , 2015 |
| USA | BTV10 | Modified live virus of BTV 10 | For use in sheep and goat | http://www.colorado-serum.com/pdf/csc_catalog.pdf |
| Turkey | BTV4 | SA BT/4, Freeze Dried, Monovalent, live attenuated | For use in sheep | http://www.cfsph.iastate.edu/Vaccines/manufacturer_list.php?manufacturer=126&lang=en |
| USA | BTV10 BTV11 BTV17 | Bluevac-10 Bluevac-11 Bluevac-17 | For use in sheep | California Wool Growers Association http://cawoolgrowers.org/vaccines/bluetongue.html |

RSA: Republic of South Africa; BTV: Bluetongue virus.

are able to infect fetuses leading to malformations or abortions depending on term of pregnancy (Savini *et al.*, 2014). Due to abortion and fetal malformation, LAVs in ewes are recommended nine to 15 weeks before mating and in rams after the mating period (Bhanuprakash *et al.*, 2009).

However, some of the previous reports claim that is difficult to prove the assumption that either LAVs may revert back to virulent strain or reassorted with field BTV strain which give rise to a new virus strain (European Commission, 2000). Due to difficulty in experiment and unfeasible nature of result, no virus transmission trials have been conducted between vaccinated sheep-vector-unvaccinated ruminants. It was reported that attenuated viruses that elicit titers of less than 1000 plaque forming units (PFU) per ml of blood at height of viremia in animals will not be transmitted by bloodsucking *Culicoides* vector (OIE, 1996). Thus most of the commercially produced LAVs may be treated as safe because they elicit viremia lower than 1000 PFU/ml of blood (European Commission, 2000).

5.2 Inactivated vaccine

The inactivated vaccines mostly produce humoral immune response. Inactivated vaccines have some added advantages over LAVs such as inactivated virus eliminates concerns about reversion to virulent strain, reassortment with field strains and viremia and vector transmission. Moreover, it also positively addresses the problems of abortion and danger of fetal infection, which often arise from live attenuated BT vaccines. The inactivated vaccines can also be utilised for rapid response against new emerging serotypes. The new serotypes are molecular characterized by cloning and sequencing techniques, which is a time-taking process. Thus, vaccine development after complete molecular characterization of pathogen may lead to severe economic losses. However, inactivated BT vaccines can be prepared within short time by inactivation of BTV using suitable agents such as gamma radiation (Campbell, 1985), β -propionalactone (Savini *et al.*, 2007) and binary ethylenimine (Ramakrishnan

Table 3. Inactivated vaccines used for bluetongue disease

| Country | BTV serotype | Vaccine characteristics | Reference |
|-------------------------|---|--|--|
| India | BTV18 | Hydroxylamine inactivated and adjuvanted with aluminium hydroxide (Al(OH)(3)) gel, saponin, or Al(OH)(3) gel/saponin. | Ramakrishnan <i>et al.</i> , 2005 |
| | BTV1 | Inactivated with binary ethyleneimine and adjuvanted with saponin. | Pandey <i>et al.</i> , 2006 |
| | BTV1 | Binary ethyleneimine (BEI) inactivated vaccine showed cross protective immune response against heterologous BTV23 serotype challenge in Indian native sheep. | Umeshappa <i>et al.</i> , 2010 |
| | BTV2, 9, 15 | Inactivated vaccine against BTV2, 9 and 15, under development. | Balam <i>et al.</i> , 2011 |
| | Pentavalent (BTV1, 2,10, 16, 23) | Inactivated pentavalent BTV vaccine consisting of BTV1, 2, 10, 16 and 23 with Montanide ISA50 adjuvant. | Reddy <i>et al.</i> , 2010 |
| China | BTV1, 16 | Inactivated BTV vaccine. | Zhang <i>et al.</i> , 2004 |
| USA | BTV11,17 | BEI-inactivated and Al(OH)3 adjuvanted. | Berry <i>et al.</i> , 1982 |
| | BTV11,17 | Inactivated vaccine. | Stevens <i>et al.</i> , 1985 |
| European Union | BTV8 (BTVPUR AlSap 8*) BTV8 (BOVILIS® BTV8) BTV1 (BTVPUR AlSap™ 1) BTV 1 and 8 (BTVPUR AlSap™ 1-8) BTV 2 and 4 (BTVPUR AlSap™ 2-4) BTV8 (Zulvac 8 Bovis) BTV8 (Zulvac 8 Ovis) BTV1 (Zulvac 1 Bovis) BTV1 (Zulvac 1 Ovis) BTV1 and 8 (Zulvac 1+8 Bovis) BTV1 and 8 (Zulvac 1+8 Ovis) | Purified virus inactivated in Al(OH)3 and saponin adjuvants supplemented. | Center for Food Security and Public Health, Iowa State University http://www.cfsph.iastate.edu/Vaccines/disease_list.php?disease=bluetongue&lang=en |
| France, Spain | BTV1 (BLUEVAC® 1) | Adjuvanted with Aluminum hydroxide and saponin. | |
| Spain | BTV4 (BLUEVAC® 4) | Adjuvanted with oil. | |
| | BTV8 (BLUEVAC® 8) | Mixed with aqueous adjuvant. | |
| | BTV1 and 4 (BLUEVAC® 1+4) | Mixed with aqueous adjuvant. | |
| | BTV1 and 8 (BLUEVAC® 1+8) | Mixed with aqueous adjuvant. | |
| Several countries | BTV8 (BIOBOS BTV 8) | Adjuvanted with oil. | |
| Italy | BTV2 | Beta-propiolactone inactivated and adjuvanted with ISA 206. | Emidio <i>et al.</i> , 2004 |
| | BTV2, 4 | Bivalent inactivated bluetongue vaccine and adjuvanted with ISA 206. | Savini <i>et al.</i> , 2009 |
| | BTV16 | Beta-propiolactone inactivated and adjuvanted with ISA 206. | Savini <i>et al.</i> , 2007 |
| Mexico, Portugal, Spain | BTV1 (Syvazul 1) BTV4 (Syvazul 4) BTV8 (Syvazul 8) BTV1 and 8 (Syvazul 1+8) | Adjuvanted with oil. | Center for Food Security and Public Health, Iowa State University http://www.cfsph.iastate.edu/Vaccines/disease_list.php?disease=bluetongue&lang=en |

BEI: Binary ethyleneimine; BTV: Bluetongue virus; ISA: Incomplete seppic adjuvant.

et al., 2006) (Table 3). These vaccines are administered along with suitable adjuvants. Some of the inactivated commercial BT vaccines have shown good safety and immunogenicity potency (Savini *et al.*, 2008). The monovalent inactivated vaccines against BTV2 and BTV4 and also bivalent vaccines against BTV2 and 4 were prepared in Southern Europe (Sa-

vini *et al.*, 2009). However, now monovalent vaccines against BTV1, 8 and 9 are also available (Zientara *et al.*, 2010). The vaccination using commercial inactivated vaccine against BTV serotype 8 (BTVPUR AlSap 8) with recommended schedule can effectively protect from wild type virus (Spedicato *et al.*, 2017). It was reported that first vaccination with

this vaccine (BTVPUR A1Sap 8), two weeks prior to challenge was able to prevent viremia (Spedicato *et al.*, 2017). Thus, it can prevent an unexpected BTV 8 outbreak in sheep, which has significant impact on management and legislation on sheep trade from BTV endemic areas.

The inactivated vaccines also have added advantage of DIVA strategies, which can easily differentiate between infected and vaccinated animals (Bhanuprakash *et al.*, 2009). However, it is difficult to predict the protective immune response of inactivated vaccine against heterologous serotypes. Although, protection against heterologous serotype may occur, it is difficult to say whether the protection is partial or complete (Breard *et al.*, 2015). Moreover, the production costs of inactivated vaccines are higher in comparison to attenuated vaccines. They elicit immunity for a short time and also require few booster doses with an adjuvant to generate a protective immune response.

6. Modern vaccination approach

Most of the currently used BT vaccines are either live attenuated or inactivated virus vaccines. Although such approach is useful in many instances, it has certain limitations. The multiple serotypes and evolution of reassortant viruses due to exchange of genome segments lead to major problems in control of BT. Thus, new generation vaccines are required for proper BT control. Several approaches have been made for development of new generation vaccines against BTV such as subunit vaccines, recombinant vector vaccines, virus-like particles (VLP) etc. They offer added advantages over traditional vaccines such as rapid onset of immune response, no risk of virus transmission, options to make them polyvalent and DIVA vaccines approach (Savini *et al.*, 2008; Bhanuprakash *et al.*, 2009; Roy *et al.*, 2009). However, they possess some of the major disadvantages such as technical expertise in production, which leads to higher cost of the vaccine (Bhanuprakash *et al.*, 2009).

6.1 Subunit vaccines

Subunit vaccines contain only the antigenic parts of the pathogen, which are necessary to elicit a protective immune response in host. The high precision of subunit vaccines comes at a cost, because various subunits of pathogen are examined in detail to determine particular combinations, which will elicit effective immune response (WHO, 2013). Although purified VP2 protein elicits sufficient protective immune response in sheep, this strategy could not be progressed further to prepare subunit vaccine due to requirement of large amount of VP2 protein for each individual virus serotype. The purified VP2 protein can be used for vaccine development against specific BTV serotypes only

(Huismans *et al.*, 1987). Later on, subunit vaccine was prepared using expression of BTV proteins in recombinant baculovirus systems (Roy, 1990). The individual BTV10 proteins purified from baculovirus vectors were administered in sheep in different combinations and doses and challenged with homologous virus (Roy *et al.*, 1990). A combination of 50 µg of VP2 and 20 µg of VP5 elicit sufficient protective immune response against homologous virus. However, alone of VP2 protein 100 µg was required to elicit protective immune response. This indicates the protective role of VP5 protein in generation of immune response along with VP2 protein. However, the addition of other BTV-specific proteins such as VP3, VP7, VP1, VP6, NS1, NS2 and NS3 to VP2/VP5 did not show any increase in protective immune response (Roy *et al.*, 1990).

The recombinant VP2 protein of BTV8 along with NS1 and NS2 of BTV2 mixed with immune stimulating complex (ISCOM) adjuvant was used as subunit vaccine against BTV8 in cattle calves (Anderson *et al.*, 2014). BTV antigenic proteins were also expressed in bacterial and yeast expression systems to produce recombinant BTV proteins (Gould *et al.*, 1994; Pathak *et al.*, 2008). The VP2, VP5 and VP7 proteins of BTV8 were also expressed in bacteria. The VP2 protein was expressed as either three separate overlapping fragments or a single full length complete protein, whereas VP5 and VP7 were expressed as full length proteins. These proteins were tested as subunit vaccine in IFNAR (-/-) mice (Jabbar *et al.*, 2013). The fragmented VP2 protein along with VP5 and VP7 in adjuvant Montanide ISA-50V preparation did not show any neutralizing antibodies against BTV8. In addition, complete VP2 protein along with VP5 and VP7 in adjuvant Montanide ISA-50V preparation showed only partial protection against BTV8 (Jabbar *et al.*, 2013). An experimental subunit vaccine against BTV4 was also formulated and tested in guinea pigs and cattle (Legisa *et al.*, 2015). The VP2 protein of BTV4 from Argentina was expressed in baculovirus insect cell expression system either alone or fused with antigen-presenting cell homing (APCH) molecules (Legisa *et al.*, 2015). The VP2 or APCH-VP2 vaccinated guinea pigs and cattle elicited high neutralizing antibody titers similar to conventional inactivated vaccines. However, IFNAR(-/-) mouse model immunized with APCH-VP2 showed enhanced cellular immune response and neutralizing activity compared to that induced by VP2 alone (Legisa *et al.*, 2015). A new approach of subunit vaccine preparation was also developed using incorporation of VP2, VP7 and NS1 protein of BTV4 into avian reovirus (ARV), muNS-Mi (mu (µ) non-structural-microsphere) microspheres (MS-VP2/MS-VP7/MS-NS1) (Marín-López *et al.*, 2014). The IFNAR(-/-) mice vaccinated with muNS-Mi microspheres showed complete protection against challenge with homologous BTV4 and a partial protection against heterologous BTV1 serotype. The microspheres-based subunit vaccine has additional ad-

vantage of potent intrinsic adjuvant activity and stimulates specific T cell responses (CD4+) against the virus (Marín-López *et al.*, 2014).

6.2 Virus-like particles (VLPs)

VLPs have been successfully utilised against BTV for subunit vaccine formulation. Although VLPs are non-infectious particles, they resemble viruses and elicit protective immune response because they express viral structural proteins such as capsid or envelope. The vp2 and vp5 genes of BTV10 were inserted into a recombinant baculovirus along with baculovirus polyhedrin promoter (French *et al.*, 1990). The insect cells (*S. frugiperda*) co-infected with this recombinant virus along with another recombinant baculovirus expressing major core proteins VP3 and VP7 of BTV17 and BTV10, respectively, (French and Roy, 1990) yielded double-shelled, non-infectious VLPs. These VLPs were found very similar in size and appearance to BTV virion particles. Moreover, antibodies raised against these VLPs showed significantly higher neutralizing activity against homologous BTV serotype (French *et al.*, 1990). VLPs are considered as safe and have shown effective immune response against BTV (Roy and Noad, 2008; Barr and Tamms, 2007). VLPs are the only vaccines allowed for intensive clinical trials in different countries. Two doses of VLPs are sufficient for long-lasting protection. The production costs of VLPs are also reasonable due to technical advancement in insect cell culture manufacturing.

The first approach of VLP vaccination against BTV10 was performed when co-expression of four major structural proteins (VP2, VP5, VP3 and VP7) was used to assemble the VLP (Roy, 1990). Later on VLPs against several BTV serotypes such as BTV1, 2, 10, 13 and 17 in combination were prepared, which elicit sufficient protective immune response against homologous serotype in sheep (Roy *et al.*, 1994). VLPs against BTV8 in combination with BTV1 and 2 were validated, which also showed protective immunity against homologous BTV serotypes (Stewart *et al.*, 2013). VLPs in combination with adjuvant may elicit sufficient immune response at lower dose of VLP antigen. It was reported that only 10 µg of BTV VLPs in combination with adjuvant such as Freund's adjuvant or an incomplete Montanide ISA-50 elicit sufficient immune response when challenged with homologous serotype (Roy *et al.*, 1992). Moreover, the baculovirus expression system was used for co-expression of VP3 and VP7 proteins, which assembled as core-like particles (CLPs). However, these CLPs did not elicit complete immune response against BTV (Stewart *et al.*, 2012; Thuenemann *et al.*, 2013). It suggests that VP2 and VP5 are essential components of a subunit vaccine against BTV.

6.3 Plant-based BTV vaccine

The plants can be used as molecular farming to produce desirable recombinant proteins, which can be used as vaccine products. Initially, plant based-vaccine was developed with the idea that these plant products can be directly used as edible vaccine. However, this concept is now obsolete because vaccine products require some kind of purification, standardisation of dose, quality control and formulation in suitable delivery form (Rybicki, 2009, 2014). Plants have a potential for production of heteromultimeric protein complexes because plant expression systems can simultaneously express multiple desirable genes. This strategy can be used for production of BTV-like particles (VLPs) using desirable genes. The antigenic proteins VP2 and VP5 and major core proteins VP3 and VP7 of BTV8 were assembled using cowpea mosaic virus-based HyperTrans (CPMV-HT) and pEAQ plant expression vector system (Thuenemann *et al.*, 2013). The recombinant cowpea mosaic viruses were allowed to propagate in *Nicotiana benthamiana* plant. The purified virion particles elicited a strong antibody response in sheep and provided protective immunity against a South African BTV8 challenge infection. It was also found safe and without any clinical manifestation in sheep (Thuenemann *et al.*, 2013). In another approach, bluetongue VLPs and core like-particle (CLP) were expressed in *N. benthamiana* plant by infection with *Agrobacterium tumefaciens* using recombinant pEAQ-HT plasmid separately encoding four BTV8-specific (VP2, VP3, VP5 and VP7) capsid proteins (van Zyl *et al.*, 2016). The pEAQ-HT vector allows the expression of VLPs and CLPs in plant expression system. This is a more flexible approach to express recombinant BTV VLPs, which were aggregated in paracrystalline arrays in the cytoplasm of plant cells (van Zyl *et al.*, 2016).

6.4 DIVA (differentiating infected from vaccinated animals) and vaccination

The vaccine should act in such a way that infected and vaccinated animals should be differentiated easily (Table 4). The attenuated live vaccine viruses usually replicate in host body. Therefore, the antibody raised in infected and vaccinated animals cannot be differentiated. However, for inactivated vaccines several ELISA formats are in progress to detect non-structural (NS1, NS2 and NS3) proteins. Such approach is called DIVA strategy, where antibodies against non-structural proteins in sera of animals immunized with inactivated vaccine are either completely absent or present at very low titer. The subunit vaccine can also be used for DIVA strategy. A subunit vaccine having VP2 protein of BTV8 and NS1 and NS2 protein of BTV2 with an immune-stimulating complex (ISCOM) matrix adjuvant elicit differential level of antibody against VP7 protein in vaccinated and infected

Table 4. DIVA strategy vaccines for bluetongue disease

| DIVA strategy | Particulars | Remarks | Reference |
|-------------------------|---|--|-------------------------------------|
| NS3-ELISA | Recombinant NS3 protein of BTV4/22045/PT04 strain expressed in <i>E. coli</i> strain JM109 using expression vector pET-28a was used as antigen for ELISA. | Cattle and sheep vaccinated with bivalent inactivated BTV 2,4 vaccine didn't develop NS3 specific antibody in ELISA. | Barros <i>et al.</i> , 2009 |
| VP7-ELISA | The VP7 protein based subunit vaccine against BTV8 for differentiation between vaccinated and infected animals developed. | VP7 seroconversion in cattle was observed after natural infection, but not after experimental vaccination regardless of BTV serotype. | Anderson <i>et al.</i> , 2013, 2014 |
| Segment 10 genetic DIVA | Eight silent mutations in genome segment 10 of BTV were generated by reverse genetics. The modified virus was used as modified live vaccine. | These mutations are not detected by a segment10 pan BTV PCR test. However, BTV infected animals can be easily diagnosed with segment10 pan BTV PCR test (DIVA-test). | van Rijn <i>et al.</i> , 2013 |
| NS3/NS3a knockout | A NS3/NS3a knockout vaccine based on the backbone of laboratory adapted strain of BTV1, avirulent BTV6 or virulent BTV8 was prepared. | The lack of NS3/NS3a expression in vaccine enables the differentiation of infected from vaccinated animals. | Feenstra <i>et al.</i> , 2014b |
| DISA vaccine | Live BTV vaccine lacking NS3/NS3a protein prepared. | The NS3-specific competitive ELISA (NS3 cELISA) enable the differentiation of vaccinated and infected animals. | Tacken <i>et al.</i> , 2015 |

(challenged) animals. The differential level of VP7 antibodies detection in vaccinated and infected (challenged) animals suggests the DIVA compatibility of vaccine (Anderson *et al.*, 2014). However, such approach is still under progress and no sustainable data is available to support it.

Live modified viruses can also be used for DIVA strategy. BTV non-structural proteins NS3/NS3a are essential for virus release from insect cells *in vitro*; however, they are not essential for virus replication. The live attenuated virus BTV6 and 8 without NS3/NS3a protein is non-virulent, gave protection against challenge infection without viremia in sheep. Upon DIVA analysis using ELISA, vaccinated sheep will show negative result for NS3 antibodies, whereas infected animal will show positive result (Feenstra *et al.*, 2014a). Another DIVA approach using modified virus vaccines such as CLPs (VP3 and VP7) or VLPs (VP2, 3, 5 and 7) have also been in use. The vaccinated animals will have antibodies against certain protein available in VLPs or CLPs only. The detection of antibodies in animals against other than these proteins may differentiate the infected and vaccinated animals. Alternatively, virus vector vaccine against VP2 gene of BTV can also be used (Boone *et al.*, 2007). Other molecular techniques such as PCR and qPCR can also be used to differentiate the segment 2, 5, 6 and 10 of vaccine and field BTV isolate (Monaco *et al.*, 2006; Toussaint *et al.*, 2007).

6.5 Recombinant viral vector vaccines

Recombinant viral vector vaccines are using an attenuated virus to introduce microbial DNA to the cells of the body. These are recombinant vaccines, where recombinant viruses carry BTV antigen-specific genes for their expression in the host (Table 5). The viral vectors (carrier viruses) used for

this purpose are considered safe because they are attenuated. Moreover, they possess BTV antigen-specific genes (transgenes) without any molecular regulatory elements of the parental BTV. Thus, it greatly reduces the risk of gene segment reassortment with field BTV strains. Viruses, which can express VP2 gene of BTV, such as capripox, canarypox, vaccinia and herpes virus have been utilised for preparation of recombinant viral vector vaccine with different level of success (Boone *et al.*, 2007; Savini *et al.*, 2008; Franceschi *et al.*, 2011; Ma *et al.*, 2012).

6.5.1 Herpesviruses

Herpesviruses have dsDNA genome, which can accommodate large size of transgene insert. Recombinant-vectored vaccines against VP2 and VP5 gene of BTV8 were developed using equine herpes viruses construct. These vaccines provided only partial protection during challenge in experimental vaccination in IFNAR (-/-) mice (Ma *et al.*, 2012). Similarly, VP2 gene of BTV was expressed in a non-pathogenic bovine herpes virus 4 strain, and a recombinant BoHV4 -VP2 construct was prepared. The IFNAR (-/-) mouse used as an experimental model showed only partial protection from BoHV4 -VP2 construct (Franceschi *et al.*, 2011).

6.5.2 Adenovirus

Recombinant adenovirus vectors can induce T cell immunity; hence they are used as potent vaccine candidates against several viral diseases. The replication-defective recombinant human adenovirus serotype 5 (Ad5) expressing VP2, VP7 or NS3 BTV proteins were used for vaccination trial in

Table 5. Recombinant viral vector vaccines for bluetongue disease (Calvo-Pinilla *et al.*, 2014)

| Viral vector used for delivery | BTV serotype and proteins expressed | Protection against homologous virus | Protection against heterologous virus | Vaccine tested in species |
|--------------------------------|-------------------------------------|-------------------------------------|---------------------------------------|---------------------------|
| Bovine herpes virus | BTV8 VP2 | Partial | Not analyzed | IFNAR (-/-) mice |
| Equine herpes virus | BTV8 VP2 | No | Not analyzed | IFNAR (-/-) mice |
| Equine herpes virus | BTV8 VP2, VP5 | Partial | Not analyzed | IFNAR (-/-) mice |
| MVA virus | BTV4 VP2, VP5 | Partial | Not analyzed | IFNAR (-/-) mice |
| MVA virus | BTV4 VP2, VP5, VP7 | Complete | No | IFNAR (-/-) mice |
| MVA virus | BTV4 VP2, VP5, VP7 | Complete | Complete | IFNAR (-/-) mice |
| MVA virus | BTV4 VP7, NS1 | Partial | Partial | IFNAR (-/-) mice |
| MVA virus | BTV8 VP2, VP5, VP7 | Complete | Not analyzed | IFNAR (-/-) mice |
| MVA virus | BTV8 VP2 | Complete | Not analyzed | IFNAR (-/-) mice |
| MVA virus | BTV4 VP2, VP7, NS1 | Not analyzed | Partial | sheep |
| Capripox virus | BTV1 VP7 | Not analyzed | Partial | sheep |
| Capripox virus | BTV2 VP2, VP7, NS1, NS3 | Partial | Not analyzed | sheep |
| Canary pox virus | VP2, VP5 | Complete | Not analyzed | sheep |
| Vaccinia virus WR | VP2 | Partial | Not analyzed | sheep |
| Vaccinia virus WR | VP2, VP5 | Partial | Not analyzed | sheep |

BTV: Bluetongue virus; MVA: Modified vaccinia virus Ankara.

IFNAR(-/-) mice and sheep (Martín *et al.*, 2015). The mice vaccinated with different rAd5 showed complete protection against BTV challenge as evidenced by humoral as well cellular (BTV-specific CD8+ and CD4+ T cell) immune response. However, sheep showed mild disease symptoms and reduced viremia after vaccination with Ad5-BTV-VP2 and Ad5-BTV-VP7 or only with Ad5-BTV-VP7 followed by BTV challenge (Martín *et al.*, 2015). Sheep vaccinated with Ad5-BTV-VP7 elicited sufficient BTV-specific CD8+ T cell response but no neutralizing antibodies response.

In other study, the VP7 core protein of BTV2 was expressed either in a non-replicative canine adenovirus type 2 (Cav-VP7 R⁰) or a leporipoxvirus (SG33-VP7) to elicit immune response in sheep (Bouet-Cararo *et al.*, 2014). Both of the recombinant antigens elicited the humoral immune response in sheep. Although both SG33-VP7 and Cav-VP7 R⁰ stimulated sufficient antigen-specific CD4+ response, only Cav-VP7 R⁰ significantly stimulated the antigen-specific CD8+ cells. Later on, Cav-VP7 R⁰ vaccine-immunized sheep were challenged with either homologous serotype BTV2 or the heterologous BTV8. The viremia estimation by real-time PCR in plasma showed that the immune response triggered by Cav-VP7 R⁰ was insufficient to provide protective immunity against BTV (Bouet-Cararo *et al.*, 2014). It only generated partial protection against homologous serotype. It indicates the need for further study of the role of BTV core proteins in cross-protective immune response.

6.5.3 Poxviruses

Poxviruses replicate in the cytoplasm of infected cells, hence they eliminate the risk of genomic integration in host

DNA and virus persistence. They can also accommodate large fragments of foreign DNA. Several members of *Poxviridae* family such as canarypox, capripox and vaccinia viruses have been used for expression of BTV antigens.

6.5.3.1 Capripox

The capripox virus has been used as carrier for BTV gene for vaccine preparation. The VP7 gene of BTV1 strain (BTV-1SA strain) from South Africa was expressed in a recombinant capripox virus (rCPV.BTV1.VP7). This recombinant vaccine elicits partial cross-protection against a lethal dose of BTV3. The partial cross-protection was probably observed due to cell-mediated immunity against VP7 (Wade-Evans *et al.*, 1996). Other attempts for expression of BTV2 proteins such as VP2, VP7, NS1 and NS3 in recombinant capripox viruses were also made (Perrin *et al.*, 2007). However, vaccination of animals with VP2, VP7 or NS3 recombinant vaccine only developed humoral immune response. Thus, this vaccine only partially elicited the immune response against homologous BTV challenge.

6.5.3.2 Canary pox virus

Canary pox virus is a dsDNA enveloped virus of the *Poxviridae* family. The large genome size (365 kbp) of canary pox virus (CNPV) can easily accommodate foreign genes. The CNPV expression system is advantageous because it undergoes abortive infection. However, it expresses the necessary antigens to elicit sufficient immune response in hosts system (Tulman *et al.*, 2004). The recombinant canary pox virus was also used as an expression system for VP2 and VP5 proteins of BTV17. Upon challenge infection, none of the vaccinated animals showed viremia or clinical signs of BT (Boone *et al.*, 2007).

6.5.3.3 *Vaccinia virus*

The WR strain of vaccinia virus was used for expression of either VP2 alone (VV-VP2) or both VP2 and VP5 (VV-VP2-VP5) of Australian BTV1 for recombinant vaccine preparation (Lobato *et al.*, 1997). The vaccination induced only partial protection against homologous virus in sheep. The viremia was observed in 66% of sheep vaccinated with VV-VP2-VP5 construct. However, VV-VP2 construct-vaccinated sheep did not show any viremia (Lobato *et al.*, 1997).

6.5.3.4 *Modified vaccinia virus Ankara*

Modified vaccinia virus Ankara (MVA) is also used as vaccine vector for high level of expression of foreign proteins (Carroll *et al.*, 1997). The recombinant MVAs (rMVAs) are used as vectors to generate vaccines against several viral pathogens. Originally this virus was obtained from Chorioallantoic vaccinia virus Ankara and passaged over 570 times in cell culture. Thus, it lost 15% of its parental genome and became replication-deficient in most mammalian as well as human cells (Esteban, 2009). The recombinant MVA (rMVA) induces both humoral and cell-mediated immunity in host (Ramirez *et al.*, 2000). The rMVA has been used to construct several vaccines expressing different BTV-specific proteins from several BTV serotypes. The recombinant vector vaccine against VP2 and VP5 protein of BTV4 was developed using rMVA. The IFNAR (-/-) mice vaccinated with rMVA-VP2-VP5 elicit high level of protective immunity against homologous serotype (Calvo-Pinilla *et al.*, 2009; 2012). Similarly, rMVAs were also generated to express the VP2, VP7 and NS1 proteins of BTV4 (Marín-López and Ortego, 2016).

Recently, a new recombinant BTV vaccine candidate was engineered using VP2, VP7 and NS1 protein of BTV serotype 4 (Marín-López *et al.*, 2017). These proteins were incorporated into recombinant modified vaccinia virus Ankara (rMVA) and avian reovirus muNS-Mi microspheres (MS-VP2/VP7/NS1). The combined effect of these two antigens elicited adequate levels of neutralizing antibodies in IFNAR(-/-) mice and increased the ratio of IgG2a/IgG1 in sera, which indicates the induction of a Th1 and CD8 T cell response (Marín-López *et al.*, 2017). The post immunization challenge with homologous BTV serotype 4 and heterologous BTV serotype 1 showed adequate protection.

6.6 DNA vaccines

DNA vaccines consist of microbial DNA that is directly administered into host body. They are administered to host body through a needle and syringe or using high pressure to shoot gold particles coated with DNA directly into cells. These vaccines have advantages of easy manufacturing, safety, cost effectiveness and biological stability. The VP2, VP7 and NS1 proteins from BTV4 expressed in plasmid vector showed only partial protection in IFNAR(-/-) mice dur-

ing homologous virus challenge (Calvo-Pinilla *et al.*, 2014). Although this vaccine did not show any clinical protection, viremia was delayed in DNA-BTV-vaccinated animals. The DNA vaccines often showed lower immunogenicity, thus they can be used as a boosting agent for immune system along with heterologous vaccination regimes (Calvo-Pinilla *et al.*, 2009, 2012; Jabbar *et al.*, 2013).

Another approach of prime DNA vaccination along with booster recombinant vaccine was also practiced in sheep. The DNA vaccine and recombinant fowl pox virus (rFPV) vaccine co-expressing VP2 and VP5 proteins or VP2 in combination with VP5 or VP2 alone of BTV1 were evaluated for successful vaccination (Li *et al.*, 2015). The best strategy for sheep was validated as combined prime DNA vaccine consisting of a plasmid pCAG-(VP2+VP5), co-expressing VP2 and VP5 followed by an rFPV-(VP2+VP5) vaccine boost (co-expressing VP2 and VP5). This combination elicits high titer of neutralizing antibodies against BTV1 in sheep (Li *et al.*, 2015).

6.7 DISC (disabled infectious single cycle) vaccines

DISC vaccines of BTV allow the virus to infect the vaccinated host animal without completing the replication cycle. This vaccine approach is based on production of modified virus using gene deletion technique, which selectively deletes BTV replication-specific gene(s). It results in expression of BTV antigenic proteins at natural sites of infection without the production of infectious viral particle or disease in host animal. Thus, it is a form of extreme level of attenuation. The DISC vaccines possess several safety features of inactivated vaccines. However, they also act like live attenuated vaccine because they express the viral antigenic proteins at sites of infection. DISC vaccines have exciting future potential as safe BTV vaccine (Roner and Joklik, 2001; Coleman *et al.*, 2008). The replication-deficient DISC BTV has been recently prepared using reverse genetics technology (Boyce *et al.*, 2008). The replication-deficient nature of the virus was introduced through insertion of a lethal mutation in gene encoding viral helicase VP6 protein (Matsuo *et al.*, 2011). The replication-defective BTV1 DISC virus was allowed for reassortment with vp2 and vp5 gene of BTV8, creating a new replication-deficient reassortant virus BTV1/8D1. The vaccination of sheep using these BTV1 and BTV1/8D1 DISC viruses showed absence of viremia after challenge with homologous BTV1 or BTV8, respectively. The further study showed that combination of DISC viruses using antigenic genes from BTV2, 4 and 8 elicit protective immune response against homologous virus without any viremia and clinical signs (Celma *et al.*, 2013). For BTV control, DISC vaccine can be used as next-generation vaccination strategy. The Multi-DISC vaccine trial containing cocktail of six BTV serotypes 1, 2, 4, 8, 13 and 21 was successfully made in sheep (Celma

et al., 2017). The MultiDISC vaccine elicited the complete protective immune response both in early virulent virus challenge, as well as 5 months post-vaccination challenge, against individual BTV serotypes. However, triDISC vaccine (BTV2, 4 and 8) elicited somewhat lesser protection in cattle than in sheep (Celma *et al.*, 2017).

6.8 DISA (*disabled infectious single animal*) vaccines

DISA vaccines are replicating, highly protective and non-pathogenic vaccines, which do not cause viremia in host animal. The NS3/NS3a protein is required for release of BTV from infected host cells. However, it is essentially not required for *in vitro* virus replication. The deletions in NS3/NS3a gene allow minimal multiplication of BTV in vaccinated animal. The deletion of NS3/NS3a leads to hindrance in BTV release from infected mammalian cells and also reduces the virus uptake by *Culicoides* vector and further release from insect cells (Feenstra *et al.*, 2015a). However, the minimal multiplication allows the expression of BTV antigenic protein, which is essential for immune response. BTV knockout without NS3/NS3a segment can elicit immune response in sheep without causing viremia. A next generation vaccine having NS3/NS3a deletion in avirulent BTV6 or virulent BTV8 and laboratory-adapted strain of BTV1 was used for vaccination in sheep (Feenstra *et al.*, 2014b). A challenge infection with respective serotypes at 3 weeks post vaccination showed protective immunity. However, no viremia was detected using sensitive molecular diagnosis. Moreover, lack of NS3/NS3a expression also enables the differentiation of BT-infected from vaccinated animals. Thus, DISA vaccine can also be used in DIVA strategy for monitoring and surveillance of virus spread in livestock.

Further advancement in DISA vaccine was made using exchange of VP2 of laboratory strain of BTV1 with VP2 of European viruses BTV2, 4, 8, 9 and 16 using reverse genetics. The VP2 exchange was combined with NS3/NS3a-negative phenotype in BTV1. This strategy did not make much effect on virus growth. The DISA vaccine having chimeric BTV1/16 VP2 elicits immune response against BTV1 and BTV16 in sheep. Thus, DISA vaccine may elicit protective immune response for both the parental serotypes in chimeric VP2 (Feenstra *et al.*, 2015b). Recently, DISA vaccine was developed for BTV serotype 8 using live attenuated virus devoid of non-structural NS3/NS3a protein. The intramuscular administration of this vaccine elicited the optimum immune response (IgG antibodies) against VP7, which persisted for longer time (van Rijn *et al.*, 2017).

6.9 Use of reverse genetics for vaccine development

Traditional live attenuated or inactivated vaccines have several limitations, which may cause adverse effect in host

animals. The recent development in molecular biology has opened new insight for development of live attenuated vaccines through reverse genetics approach. Each genome segment of BTV can be cloned individually and several transcripts of each segment can be prepared. The infectious virion particle can be produced from these clones using one transcript for each segment *in vitro* to transfect permissive cells (Harper *et al.*, 2006). This approach also allows the introduction of any mutation into BTV genome, as long as the resulting virus is viable. The study of virulence of BTV mutants and their pathogenicity in ruminant host allow the design of newer vaccine strains having multiple attenuating mutations. Moreover, BTV inclusion particle and reverse genetics study also revealed that phylogenetically distinct outer capsid proteins (VP2 and VP5) from different serotypes can be assembled on the conserved core proteins of other serotype to create viable BTV particle (Boyce *et al.*, 2008). A synthetic chimera BTV was generated using VP2 protein having regions derived from both BTV1 and BTV8. This chimera virus showed neutralization by both BTV1 and BTV8 antiserum. Thus, chimera viruses can also be used as BT vaccines (Nunes *et al.*, 2014). This approach suggests that a new vaccine virus can be prepared using outer capsid antigen-specific gene of serotypes of interest.

6.10 Gene segment reassortant vaccines

Reverse genetics can also be used for exchange of vp2 and vp5 gene of live attenuated BTV6 vaccine with those of BTV1 and 8. It results in generation of reassortant BTVac1 and BTVac8 virus, respectively (van Gennip *et al.*, 2012). Sheep vaccinated with a single dose of these reassortant viruses elicit neutralizing antibody response against homologous serotype and they showed nearly no clinical sign after challenge with virulent strain of BTV8. Thus, these studies suggested the tremendous potential of reverse genetics in vaccine development. However, further work is needed to explore the future potential of this strategy and also the bio safety risk assessment related to exchange of gene segments between field and vaccine viruses.

7. Conclusion and future perspective

The epidemiological study of BTV worldwide shows the risk of BTV to entire world as emerging infectious disease. BT also hinders the trade of livestock and its products because import of livestock and its product from BT-endemic to BT-free countries are banned. However, control of BT through relevant and timely vaccination is feasible. Although the conventional vaccines such as live attenuated and inactivated vaccines are effective, they have some serious limitations. To overcome the limitations of conventional

vaccines, new generation (modern) vaccine approaches such as recombinant, subunits, VLPs/CLPs, virus-vectored vaccines, etc. can be utilised for controlling the BT infection. The new generation vaccines have inherent safety features in comparison to conventional vaccines. Some of the recombinant vaccines have been designed to express the conserved protective antigens of BTV. Such approach may generate an effective multivalent vaccine against several BTV serotypes that would reduce the requirement of vaccination against individual serotypes or the number of multiserotype vaccinations. Thus, cross-protecting recombinant vaccines against several BTV serotypes are of particular interest in BT vaccination. The new generation vaccines may also allow the BT control along with its surveillance. The experimental new generation vaccine may also be used as a molecular tool to understand the immune system mechanism during BT infection. Despite several approaches of production of new-generation vaccines, they are mostly effective under *in house* conditions. Their large-scale field testing for efficacy and safety is required. Several new generation vaccines possess the possibility to serologically differentiate the vaccinated animals from infected ones.

Acknowledgment. Authors are thankful to college of veterinary and Animal Sciences, SVP University of Agriculture and Technology, Meerut for providing infrastructure facility to prepare the manuscript.

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