Chapter 15
Bluetongue Disease

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Abstract Bluetongue (BT) is a noncontagious OIE-listed disease of domestic and wild ruminants caused by a virus (Bluetongue virus—BTV) of the *Orbivirus* genus within the family *Reoviridae* and transmitted by biting midges of the genus *Culicoides*. BT is a considerable socioeconomic concern and of major importance for the international trade of animals and animal products. In the past, BT endemicareas were considered those between latitudes 40 °N and 35 °S; however, BT has spread far beyond this traditional range. BTV has multiple serotypes and these serotypes exist in a complex network of serological cross-relationships, varying from partial to no protection between heterologous strains. This chapter summarizes several aspects of BT and BTV with particular emphasis for BTV epidemiology inSahelian Africa.

Keywords Bluetongue · Bluetongue virus · Epidemiology · Sahel · Vectors

Economic Impact, Etiology, and History

Bluetongue (BT) is a vector-borne disease of domestic and wild ruminants listed as a notifiable disease by the World Organisation for Animal Health (OIE). BT is a disease of considerable socioeconomic concern and of major importance in the

international trade of animals and animal products. Losses due to any livestock disease may be classified as losses in production (direct losses), expenditure, and lost revenue (indirect losses) (Rushton 2009). The former may be visible, such as reduced milk yield or increased mortality, weight loss, reduced fertility rate, abortion, reduced meat production, efficiency, and death (Sperlova and Zendulkova 2011). Indirect losses include costs of vaccines or lost revenue, such as through trade restrictions limiting access to higher value markets. Multiple outbreaks of BTV from 1998 in North Africa and in southern Europe caused the death of hundreds of thousand animals causing enormous economic losses.

With regard to BTV-8, the epidemic in northern Europe has probably caused greater economic damage than any previous single-serotype BT outbreak (Wilson and Mellor 2009). A study commissioned by the Scottish Government assessed the impact on production as direct costs estimated an amount of £30 million per year (Scottish Government 2008). The deterministic economic model used to evaluate the cost of the BTV-8 epidemic in the Netherlands estimated an overall cost as 32.4 million euros and 164−175 million euros in 2006 and 2007, respectively (Velthuis et al. 2010). In 2012, an economic evaluation of the surveillance and control program applied in 2008−2009 in Switzerland estimated an amount of the disease costs as 12.2 million euros and 3.6 million euros, respectively (Häsler et al. 2012).

BT is caused by Bluetongue virus (BTV), the prototype species of the genus *Orbivirus* within the family *Reoviridae*, which includes 30 genera divided into two subfamilies infecting a wide variety of plants, vertebrates, and invertebrates, including crustaceans, fish, insects, reptiles, and mammals (Mertens et al. 2005; Attoui et al. 2009; ICTV 2018, https://talk.ictvonline.org/taxonomy/). The genus *Orbivirus* contains 22 recognized viral species. As the most widespread and of economic importance, BTV has been studied more extensively than the other orbiviruses. BTV is a non-enveloped virus, 90 nm in diameter, structurally organized into three concentric shells enclosing the viral genome consisting of ten linear dsRNA segments (Seg-1 to Seg-10) encoding for 7 structural (VP1 to VP7) and 5 nonstructural proteins (NS1, NS2, NS3/NS3A, NS4, and S10-ORF2) (Belhouchet et al. 2011; Ratinier et al. 2011; Stewart et al. 2015). The outer capsid is composed by two major proteins, namely VP2 and VP5 (coded by Seg-2 and Seg-5, respectively), which are essential in host receptor binding and virus entry. VP2 is responsible for the generation of neutralizing antibodies and determines, mainly, serotype specificity. Up to 2008, only 24 classical serotypes of BTV were officially recognized (Maan et al. 2008). These 24 serotypes exist in a complex network of serological cross relationships, varying from partial to no protection between heterologous strains. Any of these classical BTV serotypes has potential to cause BT. Except for serotype diversity, the localized circulation of the virus in different ecosystems throughout the world has also led to the evolution of distinct geographical variants or topotypes. BTVs are, indeed, broadly divided into western (w) and eastern (e) topotypes, based on phylogenetic analysis of nucleotide sequences from the majority of the genome segments. Viruses from the western topotypes circulate in Africa, Europe, the Caribbean, and the Americas, whereas those from eastern topotypes are endemic in Asia, Indonesia, and Australia (Gould and Hyatt 1994; Carpi et al. 2010). In the

last few years, novel and generally asymptomatic BTV serotypes have been discovered by researchers in the field. These include so far BTV-25 (TOV strain) from Switzerland, BTV-26 from Kuwait, BTV-27 (variants 01, 02 and 03) from Corsica (France), BTV-XJ1407 from China, a BTV strain isolated from a sheep pox vaccine (SP vaccine derived BTV) from the Middle East, BTV-X ITL2015, BTV-Z ITA2017 from Italy, and BTV-Y TUN2017 from Tunisia (Hofmann et al. 2008a; Maan et al. 2011; Zientara et al. 2014; Schulz et al. 2016; Sun et al. 2016; Bumbarov et al. 2016; Savini et al. 2017; Marcacci et al. 2018; Lorusso et al. 2018). A putative novel BTV serotype has also been described from an Alpaca in South Africa (Belbis et al. 2017).

BT was historically considered as an African disease and it was first described in the late eighteenth century, following the importation of European Merinos sheep in Cape Colony in South Africa. Initially, the illness was thought to be caused by a parasite and was initially referred as fever, malarial catarrhal fever of sheep, or epizootic malignant catarrhal fever of sheep (Hutcheon 1902). In 1902, Spreull provided the first scientific detailed description of infected sheep, including details of typical lesions, such as the presence of a dark-colored (blue) tongue. As a consequence of this observation, the scientist introduced the name of "bluetongue," which is the English translation of the name for the disease of "Blaauwtong," which was named by South African farmers who noticed tongue cyanosis in seriously diseased animals (Spreull 1902; MacLachlan et al. 2009). In 1905, by performing experimental infections in goats and cattle, Spreull discovered that the disease was transmissible to other ruminants but without production of clear clinical signs. The viral cause of the disease was determined in the same year by Sir Arnold Theiler who demonstrated that the etiological agent of Bluetongue was filterable. The scientist, by serial passages of the virus on susceptible sheep, formulated the first vaccine for BTV that was used in South Africa for several years (Theiler 1906). The fact that BTV might be transmitted by vectors was initially supposed by Spreull (1902) and Hutcheon (1902) which observed the seasonal incidence of the disease and the protection of sheep to the infection stabling them during summer nights. The role of insects in disease transmission was first confirmed by Du Toit in 1944, who indicated the hematophagous *Culicoides* midges as the biological competent vectors of BTV. Subsequently, Foster in 1963 definitively demonstrated the trans- mission of BTV by *Culicoides*. Information regarding the morphology of BTV wasobtained by using the first electron microscopy images of the virus suggesting a Reovirus-like structure (Owen and Munz 1966; Studdert et al. 1966). Following thefirst report of BTV purification from infected cell lines (Vervoerd 1969), extensiveand more detailed studies on the structure of BTV were performed in the 1970s providing essential information regarding the identification of viral structural pro- teins, their organization in two concentric capsids, and their possible role in virus infection (Vervoerd 1970). The development of molecular biology techniques, as well as the use of cell culture systems as alternative to inoculation of susceptible animals, greatly improved the knowledge on BTV etiology. The nonstructural pro-teins NS1 and NS2 were identified in 1979 (Huismans 1979), NS3 in 1981 (Gormanet al. 1981). Only recently a fourth and a fifth viral protein have been characterized

(Belhouchet et al. 2011; Ratinier et al. 2011; Stewart et al. 2015). Since the beginning of this century, several discoveries have been made to elucidate BTV biology, replication and evolution. These discoveries had a tremendous impact on the development of diagnostic tools and control strategies and improved the understanding of the complex epidemiology and pathogenesis of the virus.

Distribution of BTV in Sahel Region, Africa

In the 1940s, it was thought that BT was restricted to southern Africa and that BT outbreaks in other regions of the world reflected the emergence of the virus from the African continent (Gibbs and Greiner 1994). This assumption was soon challenged by the recognition of the disease in geographically widespread regions outside Africa. The first recognized outbreak of BT outside Africa occurred in Cyprus in 1943 (Gambles 1949). Since 1998, BTV has moved considerably northward reaching the 50th parallel in some parts of the world such as regions of Asia, North America, and North Europe where the virus was never reported before (Clavijo et al. 2000; Lundervold et al. 2003; Tabachnick 2004; Jafari-Shoorijeh et al. 2010; MacLachlan 2010). BTV circulation has been described in several North Africa countries including Morocco, Algeria, Tunisia, Libya, and Egypt. Remarkably, BTV emergence in Europe is related to the wind-driven dissemination of infected midges from northern African countries (Calistri et al. 2004; Lorusso et al. 2013, 2014, 2017, 2018; Sghaier et al. 2017; Mahmoud et al. 2018; Cappai et al. 2019). BTV has also been described from Sahelian Africa countries, such as Senegal, Sudan, Mauritania, Cameroon, Nigeria, and Gambia. Generally in African countries BTV outbreaks occur during the wet seasons and, in these regions, BTV can be considered endemic (Sellers 1984; Bakhoum et al. 2013; Fall et al. 2015a, b). In Sudan, BT was first reported in 1953, when samples from the Blue Nile province were confirmed by the Veterinary Research Laboratory at Onderstepoort, South Africa, to contain BTV (Anon 1953). Several studies have lately been performed; they confirmed that different BTV serotypes were endemic in Sudan in domestic and wild animals (Eisa et al. 1979, 1980; Mellor et al. 1984; Abu Elzein and Tag Eldin 1985). BT seroprevalence in Sudan was 61% during the 1980s (Abu Elzein 1983) and 50–60% in recent years (Saeed 2017; Adam et al. 2014). In Nigeria, no BT outbreaks were reported until 1966, when the first BT epidemic started (Bida and Eid 1974). BTV serotypes 6, 7, 10, 12, and 16 were identified in Nigeria between 1966 and 1974 by virus isolation (Moore and Kemp 1974). During the same period, BTV outbreaks were recorded in Egypt sustained by serotypes 1, 4, 10, 12, and 16 (Hafez and Ozawa 1973; Ismail et al. 1987). BTV was also diagnosed by the Animal Research Institute of Mankon in Cameroon from samples collected from five sheep succumbed after clinical disease between June and October 1982. BTV 1, 4, 5, 12, 14, and 16 were also isolated in the same period (Ekue et al. 1985). The first official BTV outbreak in Senegal was notified in 1982 and a serological survey revealed specific BTV-10 antibodies in goats nearby the border with Guinea.

The overall seroprevalence in Senegal ranged, at that time, between 35 and 48% (Lefevre and Taylor 1983). The first overview upon BTV epidemiology in Africa and the Mediterranean region has been published by Sellers in 1984, which described the BTV serotypes that were circulating in African countries and the several cases which could have remained unnoticed as clinical signs were mild. A survey performed in Gambia in 1998 revealed an overall prevalence of BTV antibodies of 62–66% in goats and 55–58% in sheep (Goossens et al. 1998). Specific antibodies to BTV-26 or to a BTV-26-like virus have been recently described in camelids and cattle from Mauritania (Lorusso et al. 2016).

Vectors

Disease spread is affected by different factors that interplay and regulate the presence of insect vectors (De Liberato et al. 2003). The availability of susceptible hosts, along with climatic conditions, may play a decisive role in spatial and temporal distribution of BTV (Purse et al. 2004a, b, 2005, 2008). Cattle play an important rolein the epidemiology of BT, since they generally are infected (viremic) without showing clinical symptoms. However, BTV-8 in Northern Europe in 2006–2008 recapitulated this assumption. The infectious period for cattle was considered to be equal to 60 days post-infection, or more (Sellers and Taylor 1980). Also wild ruminants can play an important role to maintain the infection. This role is proved in Africa and in North America, where animals such as the American deer (*Odocoileus virginianus*), the American antelogoat (*American antelogoat*), and the desert bighorn sheep (*Ovis canadensis*) can also show clinical symptoms (Ruiz-Fons et al. 2008).

The climatic conditions and the presence of geographical barriers may influence the vector and host presence. Furthermore, BT prevalence is seasonal and strongly correlated with the abundance of insect vectors and other environmental conditions involved in their life cycle (Conte et al. 2007). The increasing of the temperature, rainfall and climate changes, and wind patterns variability are also predicted to have effect on vector abundance as discussed in many studies (Wittmann and Baylis 2000; Sellers and Mellor 1993; Rawlings et al. 1998; Walton 2004; Staubach et al. 2007; De Koeijer and Elbers 2007; Purse et al. 2008). Distribution of BTV throughout the world is strictly linked to the spatial and temporal distribution of species of *Culicoides* (Diptera: Ceratopogonidae) biting midges, which are the biological vector of the virus (Tatem et al. 2003; Purse et al. 2005; Mellor et al. 2000; Carpenter et al. 2015; Foxi et al. 2016). Adult midges can only become infected by ingestion ofa blood meal from an infected mammalian host and are only capable of transmitting the virus to a new host when they have a subsequent blood meal. Although more than 1400 *Culicoides* species have been identified worldwide (Mellor et al. 2000; Borkent 2005), currently nearly 30 of them have been reported as vectors of BTV (Meiswinkel et al. 2004). Adult *Culicoides* fly during the night (from sunset to sunrise) and females bite animals feeding on their blood. However, studies

conducted in 2006 during the BTV-8 epidemic in central Europe indicated that a certain level of vector activity can be detected also during daylight (Meiswinkel et al. 2008). The insects become infected biting viremic animals and they remain infected along all their life. Humid areas, little water pods drastically favor the adult *Culicoides* reproduction (Conte et al. 2007), and it is also supposed that adults individuals remain around the place where they were born for all their life. The effect of temperature on the abundance of *C. imicola*, the main vector of BT in the Mediterranean basin, has been discussed in many studies (Sellers and Mellor 1993; De Koeijer and Elbers 2007; Purse et al. 2008). In particular, Wittmann in 2001 showed that high mean monthly temperatures (>12.5°) in winter favor the survival of *Culicoides* larvae and temperatures between 25 and 30 °C constitute their ideal habitat. In each geographic region, different *Culicoides* species may be involved in BTV transmission, and specific links between the *Culicoides* species distribution and BTV serotypes prevalence have been demonstrated (Tabachnick 2004). In the USA, the main vectors are *C. sonorensis* and *C. insignis*. In northern and eastern Australia, the main BTV vector is *C. brevitarsis*, whereas in Africa, southern Europe, and the Middle East is *C. imicola*. In northern Europe where *C. imicola* is absent, the transmission of BTV-8 was therefore assigned to Palearctic species of *Culicoides* midges including *C. obsoletus*, *C. pulicaris*, *C. dewulfi*, and *C. chipterus* (Conte et al. 2003; Purse et al. 2004b).

Although biting midges are ubiquitous (Mellor et al. 2000), they are most frequently present in warm, damp, and muddy areas. Temperature conditions are essential not only for the survival of the vector but they can also have a role in the replication cycle of the virus (Sellers et al. 1979). Another essential factor promoting vector density is represented by the level of humidity (Cappai et al. 2018). Precipitation may promote the presence of moisture of the soil generating microhabitat that can enhance vector life cycle and avoid desiccation (Purse et al. 2004a, b, 2005). In addition, the wind also affects vector survival (Mellor et al. 2000). Cold winters should halt vector-borne diseases by limiting the presence of competent vectors and, as a result, the transmission of the involved virus. In Europe, BTV outbreaks are usually reported during summer-autumn when the vector is available. The mechanism of overwintering (the way the virus survives in a given area between vectors seasons) is not entirely clear. Competent insect vectors have a complex life cycle: after the blood meal, the eggs mature inside the female in a variable time, depending on the temperature and *Culicoides* species. For example, if the temperature is 27 °C the eggs maturation time for *C. imicola* is about 2 days, but could be 3 or 4 days if the temperature is low than 22 °C. For each blood meal, a group of eggs is deposited. Insects must survive about five days in order to lay their eggs (EFSA 2007). The vital circle includes four larval stages, the pupa stage, and the adult stage. The transition to the pupa stage usually occurs in a period ranging from 10 to 30 days, depending on the *Culicoides* species, the environmental temperature, and the amount of nutrients present in the environment (EFSA 2007). The pupae remain in this stage from 2 days up to 4 weeks; furthermore, they do not need nourishment and in most species they perform very limited movements (Meiswinkel et al. 1994).

The average life of adults is about 3–6 weeks, but they can survive for up to 9 weeks (EFSA 2007). In order to have viral transmission from the host to the insect, it is necessary that the blood meal occurs during the viremic period of the host, which corresponds with the feverish period of the animal: up to 11 days post-infection in sheep and 49 days post-infection in cattle (Bonneau et al. 2002). Recently, other studies have shown that the viremia resulting from experimental infections can last up to 45 days in sheep, more than 31 days for goats, and for more than 78 days in cattle (EFSA 2007). Once the BTV is ingested, it passes into the posterior part of the arthropod esophagus, bypassing all intestinal diverticula. In the first two days post-infection the viral titer in *Culicoides* decreases due to inactivation and fecal excretion which are superior to viral replication (eclipse phase). After 7–9 days post-infection the viral title reaches a plateau and this concentration remains so for the whole life of the insect. Transmission to the host is possible from 10 to 14 days post-infection (Wittmann et al. 2002; Mecham and Nunamaker 1994; Venter et al. 1991). The virion dose transmitted through the puncture itself can infect a receptive host by extrusion and exocytosis, resulting in cell damage (Schwartz-Cornil et al. 2008).

Symptoms and lesions

Following the bite, the virus establishes primary infection in fibroblast and mononuclear phagocytes, dendritic cells, lymphocytes, and endothelial cells (MacLachlan et al. 2009). Virus is transported to the local lymph nodes where replication starts (Drew et al. 2010a) and then spreads to blood circulation inducing a primary viremia reaching secondary organs, such as spleen and lungs, and then diffuse to oral mucosa and the hooves (Barratt-Boyes and MacLachlan 1994; Sanchez-Cordon et al. 2010; Melzi et al. 2016). The virus replicates in vascular endothelial cells, macrophages, and lymphocytes (MacLachlan et al. 1990, 2009; MacLachlan 2004; Barratt-Boyes and MacLachlan 1994; Drew et al. 2010b). The severity of the clinical signs of BT depends on serotype involved and by immunological status of the affected animals. Interaction of specific BTV viral proteins with the host triggers the activation of humoral and cellular immune-response which in turn determines host damage (Huismans et al. 2004; Caporale et al. 2014; Coetzee et al. 2014; Janowicz et al. 2015).

BT in sheep is manifested as hemorrhagic disease; acute symptoms appear after an incubation period of 3–6 days, which include fever that may last several days, serous and then mucopurulent nasal discharge, excess salivation, lymph node swelling, erosions and ulcers of the oral and nasal cavity, edema of the ears and neck, respiratory distress, hyperemia, and coronitis. Pathological lesions such as petechiae, ecchymosis, or hemorrhages in the pulmonary artery, cardiac lesions including pericardial effusion and myocardial necrosis, particularly the left papillary muscle, coronary bands around the hooves, vascular congestion, erosion and ulceration of the mucosa of the upper gastrointestinal tract, pulmonary edema, pleural and/or pericardial effusion, and endothelial hypertrophy with associated perivascular

hemorrhage and perivascular edema are observed in severe acute BT in sheep (Spreull 1905; Moulton 1961; Erasmus 1975; Verwoerd and Erasmus 2004).

A swollen cyanotic tongue, from which originated the name Bluetongue, although characteristic is rarely detected. Mortality rate can be as high as 70%, and it is usually reached when a naïve population of sheep is introduced into an endemic area, when novel serotypes/strains emerge, or when stressful environmental factors are present. Hyperemia at the coronary bands of the feed and ulceration of the oral cavity may be extremely painful, determining reluctance to move and to eat resulting in weakness and prostration. Other common symptoms include "wool break" and pronounced torticollis. Usually sheep can die from respiratory distress and bacterial complications. Acute infection could lead to death within 14 days.

BTV infection of sheep during initial stages of gestation with field, vaccine or low passaged field strains may result in abortion, stillbirth, birth of viraemic animals, fetus cerebral and skeletal malformations, or even fetus death (Gard et al. 1987; MacLachlan et al. 2009; Rasmussen et al. 2013; Savini et al. 2014; Spedicato et al. 2019).

Infection in cattle and goats is usually asymptomatic or subclinical with prolonged viremia (Tweedle and Mellor 2002; MacLachlan et al. 2009). However, outbreaks of BTV-8 in European cattle have induced clinical symptoms like those described in sheep, including decreased milk production and reproductive disorders (abortion, stillbirth, and congenital abnormalities).

Diagnosis of BTV: a rapid overview

Prompt diagnosis of BTV infection is essential for the activation of specific control and restriction measures as established by the OIE terrestrial manual (OIE 2018). In recent years, along with standard and traditional techniques, sophisticated, fast, and sensitive methods have been used for the diagnosis of BTV. Diagnosis of BTV usually involves detection and identification of specific antigens, antibodies, or RNA in diagnostic samples taken from potentially infected animals using virus isolation, serological and molecular assays able to identify and characterize the involved serotype/strain. Furthermore, recognition of the clinical signs of BT can provide an early indication of infection and forms a basis for the passive surveillance. However, none of clinical signs are pathognomonic and its severity and range may be influenced by several factors such as species, age, virus strains, and immune status of the host.

Currently, the OIE Manual of standard for diagnostic tests and vaccines (OIE 2018) cites the competitive ELISA as a prescribed test for the detection of BTV group-specific antibodies. Then, serum neutralization test (SNT) is regularly used to detect and quantify neutralizing antibodies, that are specific for each BTV serotype, in serum samples (Jeggo et al. 1986).

Molecular assay methods are widely used to identify viral RNA of BTV from biological specimens (whole blood, spleen, lymph nodes, midges) or from cell-

culture isolates by targeting specific viral segments (Wade-Evans et al. 1990; Katz et al. 1993; Jimenez-Clavero et al. 2006; Orru et al. 2006; Anthony et al. 2007; Shaw et al. 2007; Wilson and Mellor 2009; Hofmann et al. 2008b). A pan RT real-time PCR targeting Seg-10 of all known BTV serotypes is available and commonly used in reference laboratories (Hofmann et al. 2008b). Genotyping is generally performed after genogroup identification with several typing assays which are normally performed according to the epidemiological scenario of a given region. These assays, either PCR-based or microarrays, target the Seg-2 of the viral genome (Curini et al. 2019). Sequencing-based techniques of viral genes are nowadays so improved that the complete nucleotide sequence of a single gene can be achieved in few hours with a very low error rate. Sequencing of the entire Seg-2 allows the identification of BTV serotypes and the characterization of the topotype (Johnson et al. 2000; Zientara et al. 2006; Mertens et al. 2007; Maan et al. 2012; Lorusso et al. 2017). Due to the reassortment capability of BTV, whole genome sequencing has become essential to identify reassortant strains. The understanding of this mechanism is important to predict possible generation of emergent strains in area where two or more serotypes/strains normally circulate. In this perspective, next-generation sequencing technique is becoming an essential tool to obtain, in a fast manner, the complete genome constellation of a BTV isolate or directly from nucleic acids purified from biological specimens (Savini et al. 2017; Marcacci et al. 2018; Cappai et al. 2019).

Application to Prevention and Control/Adopted Surveillance and Control Strategies

The application of control measures for a vector-borne disease such as BT can be difficult to adopt and are greatly influenced by the constraints of relevant nation and international legislation and agreements. Diagnostic methods are essential to collect fundamental information about origins, distribution, and prevalence in BT monitoring and surveillance, to identify routes of incursion and movements of viruses. These data are on the basis of risk assessment and for the implementation of appropriate control strategies.

In 2008, the European Food Safety Authority (EFSA) published the scientific opinion of the Panel on Animal Health and Welfare (Question No EFSA-Q-2007-201), which provides strategic guidelines for the urgent strengthening of insect vector control measures, as a key approach to preventing BT disease and responding to epidemics. Since the virus is primarily transmitted by infected *Culicoides*, it is necessary, where possible, to either limit the exposition of susceptible animals during maximum vector activity or apply strictly farm management measures (i.e., store animals during the night, avoid water stagnation and night pasture, and use insecticides and repellents) aimed to reduce the number of insects in the farming area (Braverman and Chizov-Ginzburg 1997; Tweedle and Mellor 2002). Entomological

surveillance throughout vector sampling can be used to collect essential data about the presence, abundance, proportions, and seasonal variations in the numbers of adult *Culicoides* or to identify new potential vector species.

The application of restriction of animal movement and trading and the introduction of surveillance strategies in restriction zones are necessary for the monitoring and the control of disease spreading from unaffected to affected areas (Caporale and Giovannini 2010; MacLachlan and Mayo 2013). Movement restriction could be rather impossible to achieve or even to control, in areas such as sub-Saharan Africa where camelids, which have been proven to be competent host for the virus, are used by nomadic tribes as transport vehicles during long-distance travels to reach either markets of farming areas.

Since clinical signs of BT may be unrecognized or go unnoticed, particularly in cattle and wild ruminants, serological surveillance is currently in use more than clinical surveillance. BTV circulation can be monitored using "sentinel animals," selected and tested seronegative, monitored regularly to confirm the absence of virus circulation. The rapid nature of currently available assays and their potential for high-throughput automation are particularly valuable for large-scale surveillance by national and international reference laboratories. Vaccination is the main control measure for BTV. Modified live vaccines (MLV), developed in endemic areas in South Africa, have been used in several parts of the world for seasonal vaccination campaigns and have been also used during past European outbreaks (Savini et al. 2004; Roy et al. 2009; McVey and MacLachlan 2015). However, due to safety issues such as reassortment with field strains, reversion to virulence, transmission to insect vectors and by them to unvaccinated animals, side effects in inoculated animals such as development of clinical signs, reduction in milk production, semen secretion of the virus, and abortion, MLV were withdrawn and replaced by inactivated vaccines (Dungu et al. 2004; Monaco et al. 2004; Veronesi et al. 2010; Savini et al. 2007, 2008; 2014). Inactivated vaccine can be considered safer and are currently used as monovalent, bivalent, and tetravalent formula (Savini et al. 2008; Reddy et al. 2010). Their efficiency and the presence of only few side effects have induced the pharmaceutical companies to produce copious quantities of inactivated vaccines overcoming major downsides such as large cost of production.

To date, several approaches have been used for the development of new generation vaccines able to offer major protection, low-cost production, and almost none safety issues when compared to the traditional live attenuated or inactivated vaccines. Modern vaccines are designed to accomplish what is called DIVA strategy (differentiating infected from vaccinated animals) which is normally achieved by designing a serological test detecting a viral protein that is not present in the vaccine. DIVA strategy is mainly based on the production of recombinant vaccines.

The achievement of reverse genetics system for BTV is an important landmark for the research and development of new generation vaccines, which are able to offer good protection and safety and can be used in a DIVA strategy (Celma et al. 2013). Reverse genetics allow the generation of replication competent virus starting from plasmids (Boyce et al. 2008).

This approach gives the possibility to "play" with the viral segments to either understand their role in virus replication and to generate safe replication competent vaccines. By reverse genetics it is possible to engineer disabled infectious single-cycle (DISC) and disabled infectious single-animal (DISA) vaccines.

DISA vaccines do not produce viremia in sheep and there is no risk of transmission by insect vectors (Feenstra et al. 2015). Although DISC and DISA vaccines seem to be very promising, cost production for the generation of large quantities of synthetic viruses is still an issue (Feenstra and van Rijn 2017). Overall, it is important to perform correct surveillance in order to predict as much as possible outbreaks of novel BTV strains. This can be achieved only by strong collaboration between farmers, veterinarians, and researchers.

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