Bluetongue

Disease Monograph Series – 20

Virus | Orbivirus | Sheep | Cattle | Goats | Buffalo

IDRC | Bartay

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Acronyms

AGID	Agar Gel Immunodiffusion
APHIS	Animal Plant Protection Inspection Service
AU-IBAR	African Union Inter-African Bureau for Animal Resources
BHK	Baby hamster kidney
BMGF	Bill & Melinda Gates Foundation
BT	Bluetongue
BTV	Bluetongue virus
cELISA	Competitive Enzyme-linked immunoassay
CFT	Complement Fixation test
CLPs	Core-like Particles
CPE	Cytopathic effects
CVO	Chief Veterinary Officer
DG	Director General
DIVA	Differentiation between infected and vaccinated animals
DoI	Duration of immunity
DVS	Director Veterinary Services
EC	European Community
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay

EU	European Union
FAO	Food and Agriculture Organization of the United Nations
HI	Haemagglutination Inhibition
IFNAR	Interferon-α receptor
IIL	Indian Immunologicals Limited
LA	Live attenuated
LVIF	Livestock Vaccine Innovation Fund
MAb	Monoclonal antibody
MLV	Modified live virus
OIE	World Organization for Animal Health
PCR	Polymerase chain reaction
PM	Post mortem examination
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
TPP	Target Product Profile
QRT-PCT	Quantitate reverse transcription polymerase chain reaction
RT-PCR	Reverse transcription polymerase chain reaction
SC	Subcutaneous
SNT	Serum neutralisation test
SHF	Small holder farmer
TPP	Target Product Profile
VLPs	Virus like particles
VNT	Virus neutralisation test

Executive Summary

Etiology, epidemiology and impact

This monograph briefly describes Bluetongue (BT), an infectious, non-contagious vector borne disease affecting domestic and wild ruminants. Its causal agent, the Bluetongue virus (BTV) is a non-enveloped double stranded RNA, that belongs to the genus *Orbivirus.* The BTV is considerably diverse and to date 26 serotypes, likely 27, have been identified. There is marked genetic variation within the virus, as a consequence of both genetic shift and drift. The vector is an insect belonging to the genus *Culicoides*, which has a number of species that host and transmit the virus. Once infected with BTV, female midges remain persistently infected for the remainder of their lives.

Bluetongue has a worldwide distribution governed by the distribution of the vector *Culicoides* spp., wherever the vector species thrives the virus can potentially exist and cause disease. The global distribution of BTV is limited to a band between approximately 50°N and 35°S; however, *Culicoides* midges, including known BTV-vector competent species, occur beyond this global range. Although transmission is mainly by midges, some BT strains can be transmitted between ruminants in close contact, but it is thought to be of little epidemiological importance. Serotypes 25 and 26 might be the exceptions, as they do not seem to replicate readily in some *Culicoides* vectors.

Sheep tend to be the ruminant species that are most clinically affected by BT, but cattle, goats, buffaloes and a number of wild antelopes also contract the disease and may have mild to inapparent disease manifestation.

Clinical cases of BT occur mainly in sheep, while subclinical infections seem to predominate in most other species. Clinical disease in cattle is rare except in herds infected by the BTV-8 serotype. Common clinical signs include fever, depression, serous to mucopurulent nasal discharge, which may crust around the nostrils, hyperemia of the muzzle, oral and nasal mucous membranes, conjunctive and coronary band of the hooves. The muzzle, periocular region and face often become edematous. The lips and tongue might be very swollen in some animals; the tongue is occasionally cyanotic in severe cases, and may protrude from the mouth. Pregnant ewes can abort or give birth to lambs that are stillborn or have central nervous system lesions, retinal lesions and skeletal malformations. Deaths are often the result of pulmonary edema in acute cases, or secondary bacterial complications when the course is more prolonged

Morbidity is very variable. Virulence and therefore mortality varies with the strain of BT virus and also with the ruminant host species affected; with sheep it also depends on the breed infected. Morbidity rates in sheep range from <5% to 50-75% or higher, and reach its highest point when the virus is first introduced. The case fatality rate is typically <30%, but can reach 50-90% in highly susceptible populations. Once a virus has become endemic, morbidity may decrease to low levels (1-2%), with very few deaths.

After recovery from natural infection, sheep have a solid, life-long immunity to the homologous serotype but only partial or no protection immunity against heterologous types.

The economic and social impacts of the disease, are varied. In recent decades they have been particularly important in Europe, not only for the cost of the disease in terms of mortality and decreased production, but also for the impact on the cattle industry due to trade barriers. In 2007, a BTV-8 outbreak in France was estimated to cost $1.4 billion. The USA losses in trade and associated testing of cattle for BTV status has been estimated at $130 million annually. As for the LVIF target 20 countries, the economic and socio impact of bluetongue are most felt in India and South Africa, but there is very limited information, and is difficult to make definitive statements due to concerns regarding differential diagnosis with common diseases in the area.

Incidence / Prevalence

The incidence and prevalence of BT in the target countries for the LVIF differs widely. In Asia, only India has reported BT outbreaks regularly to the OIE. Indonesia and Nepal last reported BT to the OIE in 2006, however there are recent publications with prevalence data from Nepal. In Africa, only South Africa has reported it regularly to the OIE. But there are recent publications with BT seroprevalence data from Ethiopia, Madagascar and Uganda. More details are given in Section 3.

Diagnostics

This monograph also briefly outlines a number of techniques used in detecting exposure of ruminants to the virus. Definitive diagnosis of BT is based on the detection of antibody using a serological assay, or of antigen, using a virological assay, or of specific nucleic acids using molecular techniques, RT-PCR and sequence analysis, or by virus isolation in cell culture.

No specific treatment is available for BT. Treatment of BT-affected involves only nonspecific supportive and nursing care. The most effective way to control BTV is by employing measures that prevent the introduction of BTV to a susceptible herd. All efforts to control BTV are generally directed at preventing the ruminant species from contracting the disease, mainly through vaccination, and denying/reducing exposure of susceptible ruminants to the vector.

Current vaccines for BT

Currently, two types of bluetongue vaccines exist, the live (attenuated) and the inactivated (killed) vaccines. Both types of vaccines may be monovalent or polyvalent. Presently, they are all serotype specific, and there are no vaccines that allow the differentiation between vaccinated and infected animals (DIVA).

Live attenuated vaccines can be highly effective. They generate protective immunity after a single inoculation and they have proven effective in preventing clinical BT disease. They have been successfully used in places such as South Africa. When multiple serotypes exist, the situation is more complicated as it requires multivalent

vaccines because protection from BTV vaccines is serotype specific. Unfortunately, multivalent vaccines have problems resulting from interference between virus strains, varying immunogenicity and growth rates between virus strains, as well as variations in the immune responses of individual animals. Concerns have also been raised about under-attenuation (impact may vary with the different breeds of sheep), potential depressed milk production in lactating sheep and abortion/embryonic death and teratogenesis. Other concerns include the possibility tat the vaccine virus will infect vectors and will be established in the environment.

Some authorities prohibit the use of live attenuated vaccines against BTV. Nonetheless, live attenuated BT vaccines have wide usage in South Africa, Italy, Morocco, Spain and France, and to a smaller extent in the USA. To overcome some of the drawbacks of live vaccines, inactivated vaccines have been developed although their main disadvantage is poor immunogenicity, and they usually require repeated immunization. Other potential disadvantages include increased costs due to the large amount of antigen required, and there are some concerns over the reliability of inactivation for each vaccine batch. Inactivated vaccines are generally safe.

Commercial manufacturing of BT vaccines

Some of the multinational large pharmaceutical companies are producing the BT vaccine such as Merial, Zoetis and MSD. Their focus is the developed countries. In Africa, OBP produces a live vaccine, and in India, IIL has recently launched the Raksha-Blu, an inactivated pentavalent vaccine.

Research and Potential new vaccines and the way forward

Despite the major advances in the understanding and prevention against BTV in trials with new generation vaccines, a commercial recombinant vaccine against BT remains elusive.

Many approaches have been followed in order to develop new generation vaccines. Promising results have been obtained with virus-like particles (VLP)s, some recombinant viral vector vaccines, and lately with the use of reverse genetics, replication deficient virus vaccines have been developed which are stable and safe. Details are included in Section 8. Some of these vaccines can be used as a mixture of serotypes, and can potentially protect for multiple serotypes. These vaccines need to be tested at large scale. It is important to consider that many of the new vaccines are aimed to the developed countries markets (for example, they are based on the strains prevalent in Europe) and that explains why big multinational pharmaceutical vaccines are already producing the vaccine. It is very possible that they have their own in-house research, and some of the progress might not be public knowledge.

Clinical disease overview

Etiology & Epidemiology

Bluetongue (BT) is an infectious, non-contagious, vector-borne viral epizootic disease that affects domestic and wild ruminants including sheep, goats, cattle, South American camelids, buffalo, bison, North American elk, bighorn sheep, antelope and deer. BT was first identified at the end of the 18th century in South Africa following importation of wool sheep from Europe, and was originally referred to a “malaria catarrhal fever of sheep” or “epizootic malignant catarrhal fever of sheep”.

The bluetongue virus (BTV) belongs to the family Reoviridae, genus *Orbivirus*, which consists of 20 known species including other orbiviruses of economic importance such as epizootic hemorrhagic disease virus (EHDV), African horse sickness virus and equine encephalosis virus. The BTV is considerably diverse and to date 26 serotypes, likely 27, have been identified [1]. Bluetongue virus serotypes 25 to 27 have been identified only recently as infections of small ruminants in Europe and the Middle East, and serotype 25 (BTV-25; also known as Toggenburg orbivirus) has yet to be isolated, although it has been sequenced [1].

Structurally, BTV is a non-enveloped double stranded RNA (dsRNA) virus consisting a triple-layered icosahedral capsid composed of 10 linear segments with a genome of approximately 19.2 kb. These segments encode 4 non-structural proteins (NS1-NS4) and 7 structural proteins referred to as viral sub-core proteins (VP1-VP7). The 10 double stranded linear segments together with the viral sub-core proteins VP1, VP4, and VP6 are enclosed in a double-layered protein shell, with an inner layer of VP3 and an outer layer of VP7 proteins. Two protein units, VP2 and VP5, consist the outer capsid and encapsidate the inner core (See Figure 1). The VP2 and VP5 proteins are the only BTV proteins with capacity to induce neutralizing antibodies with VP2 being the major protein involved in serotype specificity [2].

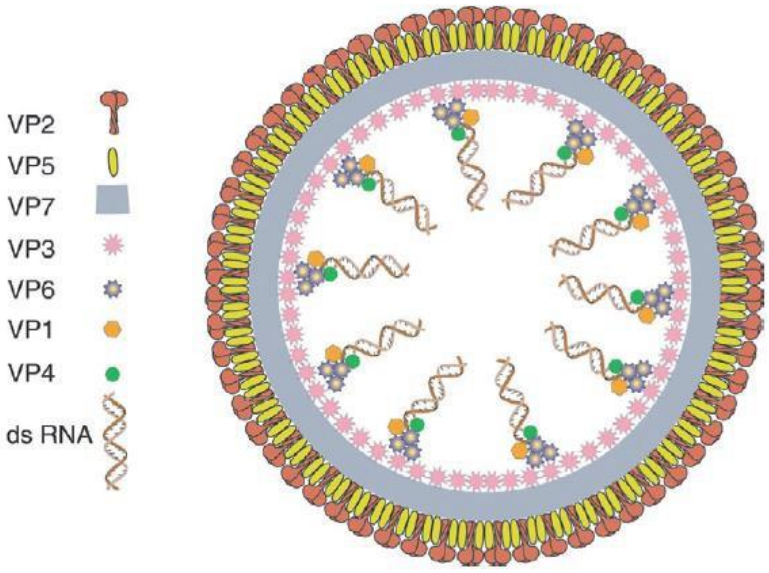

Figure 1: Representative scheme of BTV structural proteins and dsRNA segments. Source: Schwartz-Cornil et al., 2008 [3].

Bluetongue virus infects its insect and mammalian hosts in alternating cycles, which gives the virus the opportunity to genetically diversify. Consequently, there is marked genetic variation among field strains of BTV in historically endemic regions, even among viruses of the same serotype from the same region. The genetic diversity and heterogeneity of field strains of BTV arise as a consequence of both genetic shift and drift. Specifically, genetic shift occurs by reassortment of individual viral gene segments during infections of cells with more than one virus serotype or strain or by intragenic recombination. In contrast, individual genes evolve by genetic drift as a consequence of quasispecies (a swarm of genetic viral variants all related to a common consensus sequence) evolution and founder effect during alternating cycles of virus replication in insect and mammalian hosts. Importantly, however, there is currently some uncertainty about the genetic basis of virulence and other important biological characteristics of individual BTV strains, e.g. the potential role of quasispecies (population) diversity in determining these characteristics is not yet known [1].

The BTV is known to be inactivated by 50°C and 60°C within 3 hours and 15 minutes respectively and by ß-propiolactone; iodophores and phenolic compounds. The virus is easily damaged by exposure to pH below 6.0 and above 8.0; and reported as very stable in the presence of proteins, noted to have survived in blood stored at 20°C for years (Anonymous, 2011).

Transmission

BTV is mainly transmitted between its ruminant hosts by biting midges (flies) of the genus *Culicoides* in the family ceratopoginidae in the order Diptera (See Figure 2). To date, up to 1400 species of *Culicoides* are known,

although only 30 of these are competent BTV vectors [4]. *Culicoides* spp are small (<3 mm) blood-sucking insects that occur worldwide. These flies breed in a wide variety of semi-aquatic sites and those that cause veterinary disease prefer soil that is organically enriched with dung and which is found in close proximity with their ruminant hosts. The most competent BTV vectors are *C. imicola* (Africa, the Middle East, southeast Asia and parts of Southern Europe), *C. sonorensis* (North America) and *C. brevitarsis* (Australia). A comprehensive summary of major vector species for ruminants and their distribution is shown in Figure 3. Transmission of BTV is mainly through female *Culicoides*, which, like mosquitoes, require a blood meal for egg production. *Culicoides* have a wide range of hosts including birds, amphibians and mammals, although this monograph will focus on susceptible ruminants only. Occurrence of bluetongue tends to be seasonal as it largely corresponds with the distribution of the infecting *Culicoides* spp (see Figure 3), which also displays a seasonal pattern [5]. Evidence from cattle and sheep indicates that BTV can also be transmitted transplacentally although whether this route of transmission results in persistent carrier status in animals has not been demonstrated [6][7].

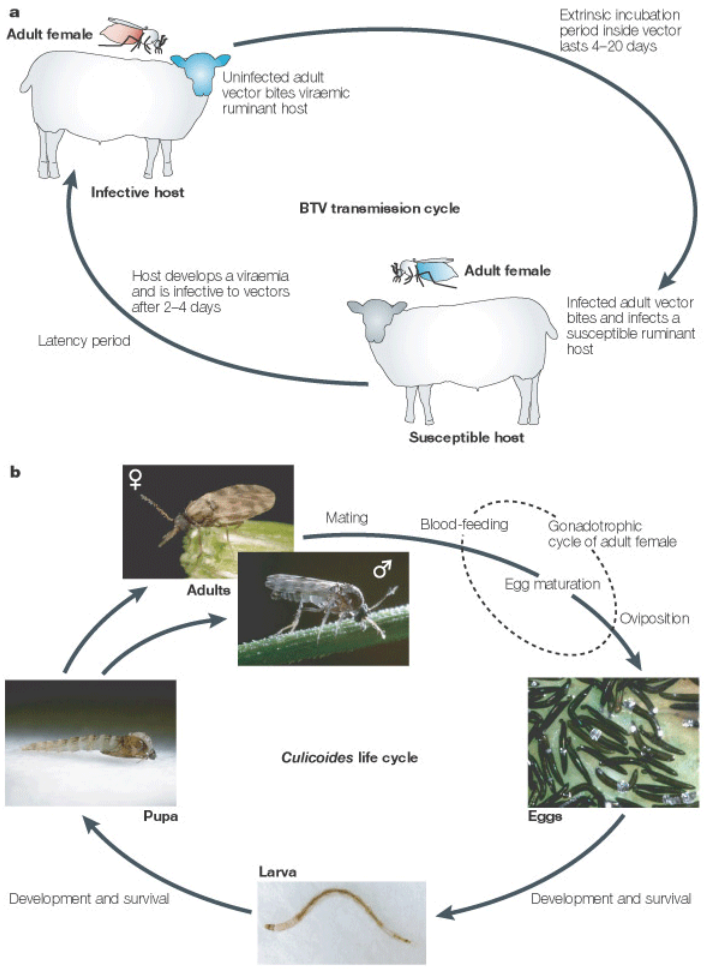

Figure 2: The transmission cycle of bluetongue virus. Source: Purse et al, **2005** [8]

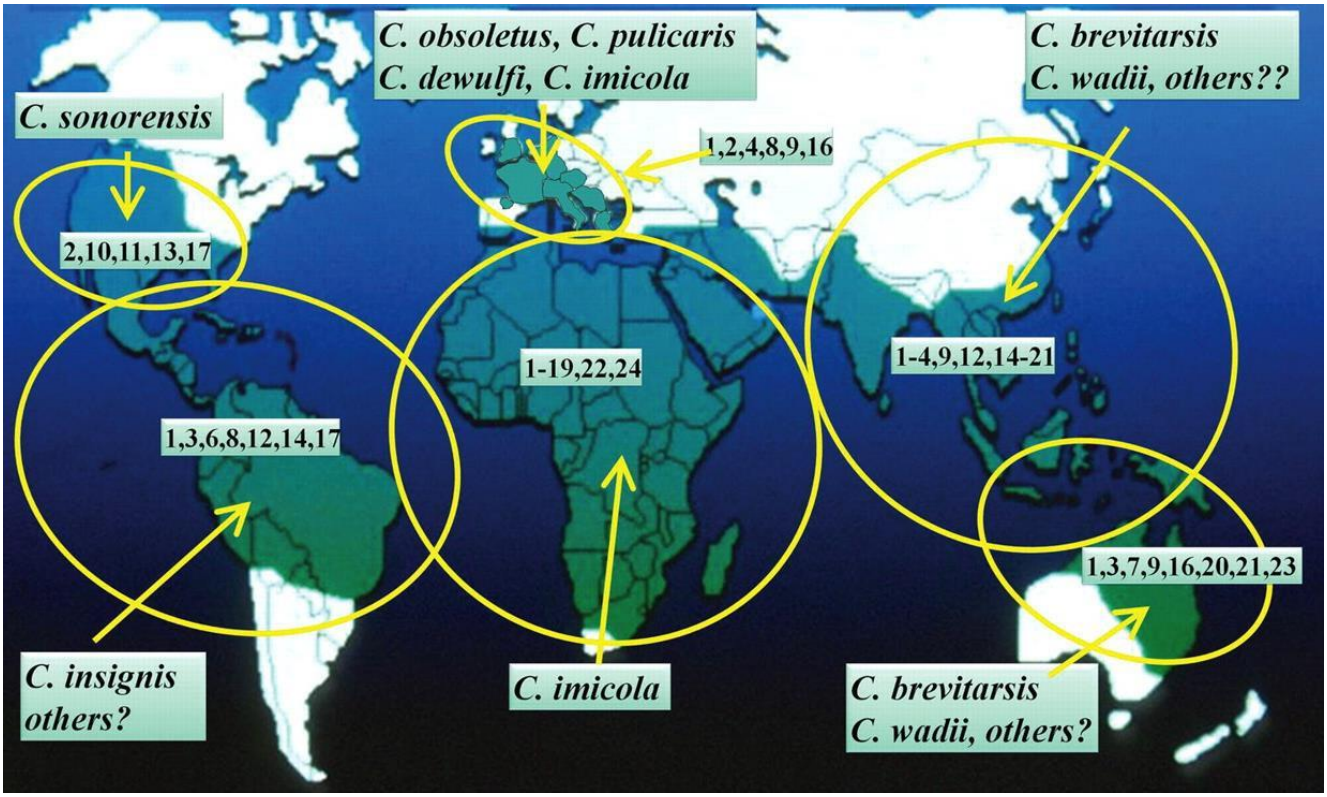

Figure 3: The worldwide distribution of Bluetongue virus (BTV) serotypes and the primary Culicoides vectors in different geographical regions denoting six predominant BTV episystems. Source: Tabachnick 2010. [9].

There is uncertainty regarding the exclusive role of *Culicoides* midges in the transmission of BTV-25 and BTV-26 [1]. Vector-independent transmission of BTV clearly can occur, although its significance is largely unknown. The epidemiology of BTV-25 infection of goats in Europe appears to be different from that of the other serotypes (BTV 1 to 24) and may not involve *Culicoides* midges. Recent studies also suggest direct contact transmission of BTV-26, likely by aerosol, between livestock.

Epidemiology

Bluetongue has been observed in Africa, the Middle East, Asia, Australia, the Americas and recently in Europe (Figure 3). Upon ingestion of the BTV from a viraemic host, the extrinsic incubation period of BTV involves the entry of the virus into the *Culicoides* vector, dissemination through the haemocoel and subsequent infection of the salivary glands. The replication period in the insect's salivary gland is 6–8 days and infected midges remain infective for their entire lives.

Temperature affects most stages of the *Culicoides* life cycle, including the survival of adults and larvae through the winter months (enhanced by high winter temperatures), recruitment to the adult population and activity rates of adult *Culicoides*. Most stages of the *Culicoides* life cycle are also affected by the availability of moisture. Breeding habitats are semi-aquatic; larvae and pupae require moist habitats and adults are prone to desiccation. The incubation period for bluetongue is typically 7 days although experimental inoculation has demonstrated a wider range of incubation of between 2 and 15 days.

Although adult *Culicoides* are killed by cold temperatures, in temperate regions BTV infections do not persist beyond 60 days. One notable aspect of BTV is its ability to survive between two midge seasons in temperate climates. This phenomenon is called “overwintering” and the mechanisms by which this happens are not well understood [10]. More recently, researchers at the University of California, Davis have demonstrated that the BTV virus is able to replicate and survive in a proportion of long-lived female midges which survive winter [11].

Mortality, morbidity and virulence depend on the infecting BTV strain and ruminant host species. Typically, sheep, yak, llamas, and alpacas are most susceptible, while cattle and other wild ruminants display some resistance to disease, although are fully susceptible to infection [12]. Cattle are considered reservoirs of infection as they experience longer durations of viremia. Goats appear resistant to disease from BTV although conflicting reports in literature due to varying experimental conditions make it difficult to compare the data [6][13]. Susceptibility of sheep to BTV is determined by the breed, age and immune status of the animal. For instance, North European breeds are very susceptible, while African or South-East Asian breeds are less susceptible. BTV infects animals of all ages and sexes, but older animals experience more severe disease [14]. BTV infection is also more acute in susceptible lambs that have not previously been exposed or those with declining maternal antibodies, while animals with previous exposure or higher maternal antibodies display a favourable course of disease. This in part, could explain the occurrence of outbreaks when susceptible animal species are introduced into BTV is endemic areas or when BTV virulent strains enter previously unexposed ruminant populations [15].

In sheep and wild deer and antelopes, the mortality rate can reach 70% and 90% respectively. In recent outbreaks in Europe, the BTV serotype infected high numbers of cattle but mortality remained relatively low at 1%. Following initial entry of BTV into a flock of sheep, between 50-70% may show clinical signs. In a number of European, Asian and South American ruminant species kept in European zoos, morbidity rates varied but reached up to 40%, depending on the species [5].

Clinical Signs

In sheep, BT occurs in three forms: acute, chronic and subclinical. Following incubation, animals develop fever, apathy, laboured breath, nostril and lip hyperaemia and excessive saliva and serous nasal discharges. The nasal discharge is initially clear, but becomes mucopurulent and forms a crust around the nostrils after drying. The tongue, lips and sub-mandibulum develop oedema, petechial haemorrhage is observed on the conjunctiva and ulceration of the oral mucosa is seen. The tongue may swell and develop a purple colour (hence the name "bluetongue") due to cyanosis, although this is not common. A proportion of animals may suffer from dyspnea, haemorrhagic diarrhoea or vomiting, resulting in aspiration pneumonia. At the end of the pyrexia stage, affected sheep may stand with an arched back and avoid movement due to coronitis, laminitis or paresis and necrosis of striated muscles. Affected sheep may also develop torticollis and dermatitis resulting in breaks in the wool. Pregnant ewes abort, the fetus may become mummified or ewes may give birth to lambs with congenital defects like hydrocephalus, cerebral cysts o retinal dysplasia. Sheep that are chronically infected may succumb to secondary infections such bacterial pneumonia [15].

Clinical disease in cattle is rare except in herds infected by the BTV-8 serotype [16]. Clinical signs typically resemble those of sheep and are largely thought to be a consequence of type I hypersensitivity mediated by IgE. The skin around teats becomes inflamed may crack and peel. Cattle display reduced milk production and during early pregnancy, embryos may die, undergo resorption, abort or if they survive, could be born with malformities [16][17]. Foetuses infected between 2 and 4 months of gestation develop serious central nervous system (CNS) defects while infections that occur few weeks before birth, could result in mild encephalitis.

As mentioned above, goats are rarely infected with BTV, and do not typically display signs of clinical disease. However, those that do, display similar signs to those of sheep but with less severity.

Diagnosis

Definitive diagnosis of BT is based on the detection of antibody using a serological assay, or of antigen, using a virological assay, or of specific nucleic acids using molecular techniques, RT-PCR and sequence analysis, or by virus isolation in cell culture. The main clinical pathology sample is blood for both serology and detection of the virus or viral antigen.

OIE recognized tests:

The techniques briefly highlighted in the Table below are the ones acknowledge by the OIE for diagnosis of bluetongue and the purpose for using them. Some more details are given on a number of the tests noted in Table 1.

Table 1: Tests recommended by the OIE for BT (OIE Terrestrial Manual of Diagnostic tests and vaccines for terrestrial animals 2015) and their purpose.

Method	Purpose					
	Population freedom from infection	Individual animal freedom from infection prior to movement	Contribute to eradication policies	Confirmation of clinical cases	Prevalence of infection – surveillance	Immune status in individual animals or populations post-vaccination
Agent identification[1]						
Real-time RT-PCR	–	+++	–	+++	++	–
RT-PCR	–	+++	–	+++	++	–
Classical virus isolation	–	+++	–	+++	–	–
Detection of immune response[2]						
C-ELISA (serogroup specific)	++	+++	++	–	++	++
VN (serotype specific)	++	+++	++	–	++	++
AGID	+	–	+	–	+	+
CFT	+	–	+	–	+	+

Key: +++ = recommended method; ++ = suitable method; + = may be used in some situations, but cost, reliability, or other factors severely limits its application; – = not appropriate for this purpose.
Although not all of the tests listed as category +++ or ++ have undergone formal validation, their routine nature and the fact that they have been used widely without dubious results, makes them acceptable.

Virus isolation:

It is performed in embryonated chicken eggs, cell culture or sheep. BTV can be isolated from a variety of tissues including spleen, lung, bone marrow, liver, kidney, lymph node, tongue, thoracic fluid, semen, brain, mucosal epithelium, post mortem blood and urine, and from foetuses. Blood collected into EDTA or heparin tubes can be used for virus isolation and for extraction of viral RNA for molecular assays. Blood for virus detection should be collected while the animal is pyrexic, as this is the height of the viraemic period. Blood for virus isolation should be stored at 4 °C, not frozen. Sterile blood samples can be transported at room temperature for a few days and virus isolation should still be possible. Tissues obtained at necropsy can be stored in tissue culture medium containing antibiotics. This allows both virus isolation and PCR to be carried out on the samples.

Immunological methods:

These methods can be used to serogroup BT viruses by i) immunofluorescence and/or ii) antigen capture enzyme-linked immunosorbent assay and iii) immunospot test.

Antigen detection ELISA can be used for the detection of BTV antigens. Polyclonal antibodies or serogroup-specific MAb adsorbed to the ELISA plate is used to capture virus-derived proteins from embryonated chicken eggs, cell culture, infected insects or sheep blood, and the bound antigen is then detected using a second antibody.

Also immunological methods can be employed for serotyping by virus neutralisation via i) plaque reduction, ii) plaque inhibition, iii) microtitre neutralisation, and iv) fluorescence inhibition test

- *Virus neutralisation tests (VNTs)* are used to serotype virus isolates. They are based on the inactivation of the infectivity of the test virus by standardised preparations of polyclonal neutralising antibodies to each of the known BTV serotypes. They depend on the antigenic specificity of VP2 and to a lesser extent VP5. Different types of VNT include the plaque reduction neutralization test, plaque inhibition test, microtitre neutralization test and fluorescence inhibition test. These assays can only be carried out if the laboratory has high-quality standardised antisera for each of the BTV serotypes available. These assays are slow.

First, time is required to adapt the isolated virus to grow in cell culture; this can take several weeks. Once this has been done, a week is required to carry out the test.

- *Serum neutralisation test* is a serotype-specific test used to differentiate between antibodies produced against different BTV serotypes. The sera to be tested are each reacted separately with a constant amount of BTV of each serotype, after which the amount of neutralisation of the virus, compared to virus not treated with serum, is measured by infection of mammalian cell cultures. This test is highly specific and sensitive; it does not cross-react with other Orbivirus. However, it is time-consuming, and uses expensive reagents. If two or more serotypes of BTV are involved in an infection, interpretation of results can be difficult as sheep can develop a broad, heterotypic antibody response against multiple

serotypes of BTV following infection with more than one serotype. SNTs require the availability of reference strains of BTV of all serotypes, are time consuming and labour intensive, therefore are not widely used.

Serological tests (to detect BTV antibodies)

- *Complement fixation test (CFT)* - largely replaced by the AGID test.
- *Agar gel immunodiffusion test (AGID)* serves as an alternative test for international trade. It is simple to perform and the antigen used is relatively easy to generate. However, its lack of specificity due to detection of other Orbiviruses, including those in the EHD group can render it less usable and AGID positive sera may have to be re-tested using a B serogroup specific assay. Those disadvantages have led to this test being largely superseded by the competitive ELISA (c-ELISA).
- *Competitive enzyme-linked immunosorbent assay* (ELISA). A prescribed test for international trade. BT competitive or blocking ELISA was developed to measure BTV-specific antibody without detecting cross-reacting antibody to other Orbiviruses; its specificity depends on using one of a number of BT serogroup- reactive MAbs. The Indirect ELISA has been shown to be reliable and useful for surveillance purposes for bulk milk samples. Both the indirect and competitive ELISA are good tests for rapid diagnosis. These have high sensitivity and specificity. Serogroup-specific ELISAs primarily identify antibodies to VP7, which is highly conserved across the known BTV serotypes. Competitive ELISA has higher sensitivity than indirect ELISA and has been extensively validated in the field. It is a prescribed test for international trade. The 50% inhibition value established for sheep and cattle sera also appears to be applicable for wild ruminants. Standard cELISA does not distinguish between natural infection and animals vaccinated with live attenuated vaccine. However, it can be used to detect the presence of circulating virus (even in the absence of clinical disease) by testing young or otherwise unvaccinated individuals. An indirect ELISA has been developed and used with both individual and bulk milk samples, but should be validated for the relevant serotype(s) before use. An indirect ELISA has been developed to antibodies against NS3. This could be used in DIVA alongside inactivated vaccines, since individuals vaccinated with an inactivated vaccine do not develop antibodies against NS3.
- *Haemagglutination inhibition (HI) techniques. C*an be used for the detection of BTV type-specific antibodies.

Detection of BTV and/or its components. A number of techniques can be used to detect the BTV or some of its components. These include i) animal inoculation, ii) cell culture, PCR and immunochemistry techniques.

- *Animal inoculation techniques.* Inoculation of susceptible sheep is a sensitive method for detection of infective BTV. Inoculation of chicken egg embryos has been a standard method for isolation of BTV for some time. Intracerebral inoculation into suckling mice (2-3 days old) has been used.

- *Cell culture techniques.* Mammalian cell lines (e.g. Vero cells, BHK-21 cells) cell cultures can be used for isolation of BTV and similarly insect cells such as *Aedes albopictus* clone C6/36; however, cell cultures were reported as less sensitive for detection than are embryonated chicken eggs.
- *Reverse transcription-polymerase chain reaction* (RT-PCR) can be used as a sensitive test for the presence of BTV RNA in clinical samples and in cell culture. Serogroup-specific RT-PCR targets RNA segments which are highly conserved within the BTV but different from other orbiviruses. Serotype specific RT-PCR is also possible, by targeting segment 2 of the BTV genome. PCR is highly sensitive, but is subject to false- positives from cross-contamination. Real-time RT-PCR is extremely sensitive and it has a reduced risk of cross-contamination in the laboratory (single-tube reaction) but its sensitivity means that even low-level contamination may produce false-positive results. It is also more expensive than conventional RT-PCR.
- *Immunofluorescence (Immunohistochemistry) techniques.* They can be used for the detection of BTV antigen in cell culture or infected tissues.

Immunity

After recovery from natural infection, sheep have a solid, life-long immunity to the homologous serotype but only partial or no protection against heterologous types. Protective immunity is generally associated with the presence of type-specific neutralizing antibodies which may persist for years, but is not associated with the group specific antibodies which usually disappear after 6 to 18 months. However, infection or immunization with more than one virus type usually results in protection against a wider range of serotypes, even types against which no neutralizing antibodies are present. This suggests that cell-mediated immunity plays a role as it is less type-specific than is the humoral response [5].

Incidence and Prevalence in Selected Countries

Global

Bluetongue virus infection occurs throughout tropical and temperate regions of the world, coincident with the distribution of competent vector *Culicoides* midges. The global distribution of BTV is limited to a band between approximately 50°N and 35°S; however, *Culicoides* midges, including known BTV-vector competent species, occur beyond this global range. Therefore, climate and other environmental factors potentially limit the global distribution of BTV, even in the presence of appropriate vectors. The global range of BTV has expanded recently, especially in the Northern Hemisphere.

In 2015, active BTV disease was observed in pockets of southern Europe (Italy and Greece), Northern Africa (Tunisia) Southern Africa (South Africa) and south Asia (Afghanistan). New strains (not traditionally reported in this countries) were reported in Australia, Portugal, Spain, Morocco and in the United States including Alaska (OIE, 2015). In Brazil, BTV was limited to specific zones and the disease was suspected to be present in Canada and the southern American countries of Venezuela and Ecuador but this was not confirmed. Figure 4 shows the map of reported BTV globally, although only the regions coloured green are considered free of the disease.

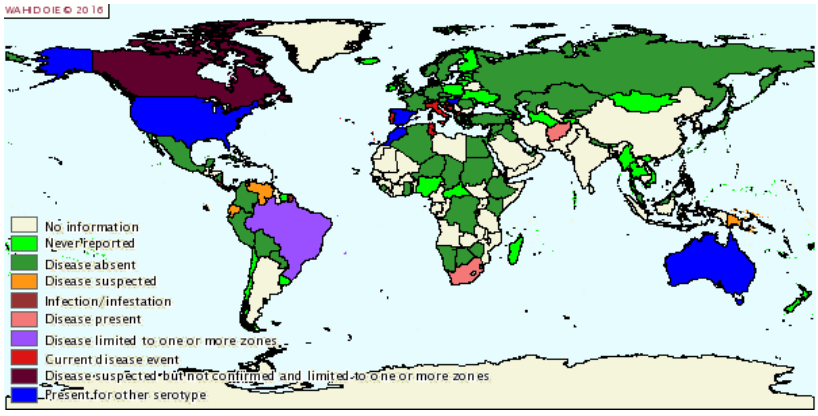

Figure 4: Global status of Blue tongue during the first half of 2015.
Source: OIE. http://www.oie.int/wahis_2/public/wahid.php/Countryinformation/Countrytimelines

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Regional

Incidence data by country

There are two main sources of information, OIE and AU-IBAR (which includes only Africa), but data are not always similar.

1- Source: OIE.

Data of outbreaks reported to the World Animal Health Organization (OIE) are shown in Tables 2 and 3. Data are not always reliable, as many countries do not seem to report, or to be reporting consistently over time.
http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail

Similar information but presented in a different manner can be seen in Annex 1.

Number of cases reported to the OIE by disease and by country:

- No information,+ Present but quantitative data not known, ? Disease suspected

Table 2: ASIA – BTV outbreaks notified to OIE from the Asian countries of interest.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Bangladesh	-	-	0	0	0	-	-	-	-	-	-
India	1,182	154	302	132	73	41	38	5	13	14	-
Indonesia	+	+	-	0	-	-	-	-	-	-	-
Myanmar	0	0	0	0	0	0	0	0	0	0	-
Nepal	0	+	0	0	0	0	0	0	0	0	-
Vietnam	0	0	0	0	0	0	0	0	0	0	-

Table 3: AFRICA – BTV outbreaks notified to OIE from the Asian countries of interest.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Burkina Faso	-	-	-	-	-	-	-	-	-	-	-
Ethiopia	0	-	-	-	?	-	-	0	0	0	-
Ivory Coast	-	-	-	-	-	-	-	-	-	-	-
Kenya	0	0	0	0	0	0	0	0	0	0	0
Madagascar	0	0	0	0	0	0	0	0	0	0	-
Malawi	-	?	?	?	?	?	?	?	?	-	-
Mali	0	0	-	-	0	-	-	-	-	-	-
Mozambique	0	0	0	0	0	0	0	0	0	0	-
Rwanda	-	-	-	0	-	-	-	-	-	-	-
Senegal	0	-	0	0	0	-	-	-	-	-	-
South Africa	21	32	6	50	104	15	>62	23	31	86	-
Tanzania	-	-	-	-	-	-	-	-	-	-	-
Uganda	0	0	0	0	+?	+?	+?	+?	+?	+?	+?
Zambia	-	0	0	-	0	0	0	0	0	0	0

2- Source: AU-IBAR.

Number of outbreaks per year as reported to AU-IBAR and published in the Pan African Animal Resources Yearbook. (**http://www.au-ibar.org/pan-african-animal-resources-yearbook?showall=&limitstart**=). Table 4 shows the number of BTV outbreaks reported to AU-IBAR from 2000 – 2005, and table 5 from 2006-2015. Table 6 shows the number of BTV outbreaks in LVIF countries of interested between 2005 and 2015.

Table 4: BTV outbreaks in African countries for the period 2000 - 2005, as reported to AU-IBAR.

Country	2000	2001	2002	2003	2004	2005
South Africa	98	23	75	64	31	23
Namibia	4	2	1	0	1	0
Uganda	*	*	2	1	0	0
Lesotho	0	0	0	0	0	3
TOTAL	102	25	78	65	32	26

* = Data Not Available

Table 5: Number of BTV outbreaks in African countries for the period 2006 – 2015, as reported to AU-IBAR.

Country	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Algeria	28			16	48	12				
Botswana	1		1						1	
Namibia	1			7	6	2	1	1		
Kenya									1	
Lesotho	5	6	2	6	9	11	10	7	9	
Tunisia	4	16		5	8	99	4	2		
South Africa	30	1	50	3	15	41	20		83	
Uganda										
Comoros			3	NS						
Zimbabwe					1		1			

Table 6: Number of BTV outbreaks per year as reported to AU-IBAR and published in the Pan African Animal Resources YearBook.

Country	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
Burkina Faso	-	-	-	-	-	-	-	-	-	-	N/A
Ethiopia	-	-	-	-	-	-	-	-	-	-	N/A
Ivory Coast	-	-	-	-	-	-	-	-	-	-	N/A
Kenya	-	-	-	-	-	-	-	-	-	1	N/A
Madagascar	-	-	-	-	-	-	-	-	-	-	N/A
Malawi	-	-	-	-	-	-	-	-	-	-	N/A
Mali	-	-	-	-	-	-	-	-	-	-	N/A
Mozambique	-	-	-	-	-	-	-	-	-	-	N/A
Rwanda	-	-	-	-	-	-	-	-	-	-	N/A
Senegal	-	-	-	-	-	-	-	-	-	-	N/A
South Africa	23	30	1	50	3	15	41	20	-	83	N/A
Tanzania	-	-	-	-	-	-	-	-	-	-	N/A
Uganda	-	-	-	-	-	-	-	-	-	-	N/A
Zambia	-	-	-	-	-	-	-	-	-	-	N/A

In summary, the bluetongue situation based on the number of outbreaks per year as reported to AU-IBAR and published in the Pan African Animal Resources Yearbook, only 3 out of the 14 African LVIF target countries appear to have reported the disease between 2000 and 2014; these were Uganda in 2002 & 2003, Kenya in 2014 and South Africa virtually throughout the entire period under review except in 2013.

Prevalence data by country

- Sources: PubMed, and internet engine searches (English and French when applicable).
- Efforts have been made to include the year of the study, and not the year of the publication. If they are known to be different, the year of publication is included in the reference.
- Note that not all papers have been read in full. In many cases, only the abstracts have been read. Critical evaluation of the papers for inclusion has not been conducted. If a review paper included some references, the source of the review is mentioned.

Table 7: Number of BTV outbreaks per year as reported to AU-IBAR and published in the Pan African Animal Resources YearBook.

Region/Country	Apparent Prevalence (%)	Study Design	Time Period	References
Bangladesh	Never reported to OIE			No publications found.
India (Jharkhand)	Sheep: 43.68 Goat: 43.44 Cattle: 57.50	Sheep: 190 Goats: 210 Cattle: 80	2015	Tigga et al, 2015
India (North Kerala)	Cattle: 6.9 Sheep: 16 Goat: 7.5	Cattle: 82 Goat: 40 Sheep: 50	2014	Arun et al, 2014
India (Orissa)	Sheep: 26.66 Goats: 31.25 Cattle: 52.27	Sheep: 120 Goats: 112 Cattle: 132	2015	Joardar et al, 2015
India (Assam)	Sheep: 58.82 Goat: 31.79 Cattle: 70	Sheep: 68 Goat: 195 Cattle: 50	2013	Siddharta et al, 2013
India (Uttar Pradesh)	28.6	91 sheep and goats	2012	Bitew et al, 2013
India (Andhra Pradesh)	60.6	Sheep: 1,299	2005-2009	Sairaju et al, 2013

India (West Bengal)	47	Goats		De et al, 2007
India (Kerala)	5.1	Sheep and goats: 1,010	2005	Ravishankar et al, 2005
Indonesia	Last reported to OIE in 2006.			No recent publications available
Myanmar	No reported to the OIE, at least since 2005.			Gard et al, 1995 suspected subclinical circulation. No recent publications found.
Nepal	Seroprevalence: 25.0% sheep and 31.3% goats. Estimated that 25% of all small ruminants are positive and positivity associated with exotic breeds	Cross-sectional survey of 318 184 sheep and 134 goats	2012-2013	Gaire et al, 2014
Vietnam	No reported to OIE, at least since 2005.			Gard et al, 1995 suspected subclinical circulation. No recent publications found.

Prevalence of the different BTV serotypes in India. The data for BTV-4, 6, 17 and 18 are not shown in figure, as they are not available/not known. Source: **Chand et al, 2015.** Bluetongue in India: A review. *Advances in Animal and Veterinary Sciences*, 2015, 3 (11) 605-612.

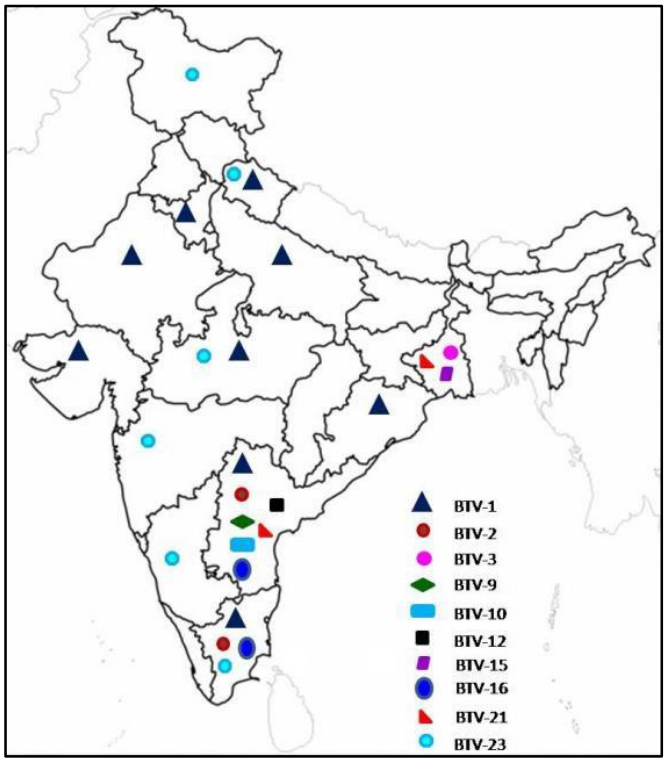

Table 8: BTV prevalence in LVIV focus countries – AFRICA.
No recent information (since 2000) has been found for Burkina Faso, Ivory Coast, Malawi, Mali, Mozambique, Rwanda, Senegal, Tanzania and Zambia.

Region/Country	Apparent Prevalence	Study Design	Time Period	References
Ethiopia (Wolyita, Southern Ethiopia)	41.17	Goat: 211 Sheep: 265	2014?	Yilma and Mekonnen, 2015
Ethiopia (Central Ethiopia)	46.67 Highland: 9.67 Lowland: 92.85	90 serum samples from sheep.	2000?	Woldemeskel et al, 2000
Kenya (Western Kenya)	Calves: 0.94	455 calves	2013	Toye et al, 2013
Madagascar	Cattle: 95.9%	Random sampling of 4,393	2014	Andriamandimby et al,

	Small ruminants: 83.7%	ruminants in 30 districts, 175 cattle longitudinally followed for 11 months		2015
South Africa (Mpumalanga)	96%	1,260 cattle using commercial C-ELISA. Isolation of BTV RNA observed in 51% of midges in autumn and 76% in winter	2013	Steyn et al., 2015
Uganda (5 regions of Karamoja District)	90% in goats by ELISA and 84% by BTV RT-PCR	300 goats. Testing was done by commercial ELISA and RT-PCR	2013	Mulabbi et al, 2013

Economic and Social Impacts at Global and Regional Levels, and in Selected Countries

Although mortality due to bluetongue is often low, it is not uncommon to witness mortality rates approaching 50% - 100% in susceptible flocks. There are also losses due to morbidity and the need to provide care for the sick animals. Costs associated with morbidity of sick animals include weight loss, reduced milk yield, abortion and associated veterinary costs.

During a BTV-2 epidemic in Italy in 2000-2001, approximately 263,000 diseased sheep and goats were reported (18% morbidity) and 48,000 sheep and goats died (3% mortality). During a second epidemic in 2001-2002, approximately 251,000 diseased sheep and goats were reported (18% morbidity) and 73,000 sheep and goats died (5% mortality). In 2007, BTV-8 outbreaks occurred on over 20,000 farms in Germany with disease in approximately 35,000 cattle, sheep or goats.

Additional costs for bluetongue come from the required testing for the virus in animals being considered for movement. The impact on cattle industries is through the effects of trade barriers. Bluetongue positive cattle are not allowed to be moved from outbreak areas because of the prolonged viremias. Cattle are capable of infecting *Culicoides* and spreading the virus for as long as a month after infection.

In 2007, a BTV-8 outbreak in France was estimated to cost $1.4 billion. Losses were largely due to the inability to trade cattle, a very substantial industry in France, on the international market. In 2007 a BTV-8 outbreak in the Netherlands cost approximately $85 million. The southern regions of the U. S. have been endemic for certain BTV serotypes for many years though animal disease has been rare. Nevertheless, the impact on the U. S. has been losses due to restrictions on the international cattle trade in animals and animal products including semen and embryos to regions considered bluetongue-free like some countries in the European Union. The U. S. losses in trade and associated testing of cattle for BTV status has been estimated at $130 million annually **(www.cabi.org/isc/datasheet/).**

In regard to the LVIF target 20 countries, the economic and socio impact of bluetongue are most felt in India and South Africa where seasonal occurrence of the disease persists, and numbers of outbreaks are reported to the OIE. It is difficult to make definitive statements on the impact of bluetongue on the remaining LVIF target

countries, because in some cases it might be poor differential diagnosis that BT could have been mistaken for one of the other similar haemorrhagic diseases.

For most of the 14 target countries for the LIVF in Africa, BT economic impact in comparison to currently other prevalent major livestock diseases may not be so obvious. However, of the target countries probably South Africa is the most economically affected by bluetongue. For the other countries, its impact socially and economically is probably masked by diseases such as FMD, CBPP, PPR and tick-borne diseases.

Analysis by the World Bank

The World Livestock Disease Atlas – a quantitative analysis of global animal health data [18], published by the World Bank (with cooperation of OIE and FAO) in 2011, is an attempt to understand which livestock diseases cause the heaviest losses, which countries suffers the worst disease-related losses and which livestock species are most affected. **http://www-wds.worldbank.org/external/default/WDSContentServer/WDSP/IB/2012/02/17/000356161_20120217030841/Rendered/PDF/668590WP00PUBL00Livestock0Atlas0web.pdf**

The World Livestock Disease Atlas bases its analysis on the Livestock Units (LSU). Each species has a LSU value, and the losses of LSU have been given a value. See Figure 5. For more information on the methodology description, please refer to the World Bank Atlas itself (pages 6 & 7). BT is one of the top 10 diseases causing losses for small ruminants, as shown in Figure 6. However, looking at the data in detail, there are few data from sub-Saharan Africa and Asia. Their analysis shows that India and South Africa belonged to the bluetongue most affected countries globally, and as a result of BT they each were assessed to be losing 34 and 25 LSUs respectively (Figure 7).

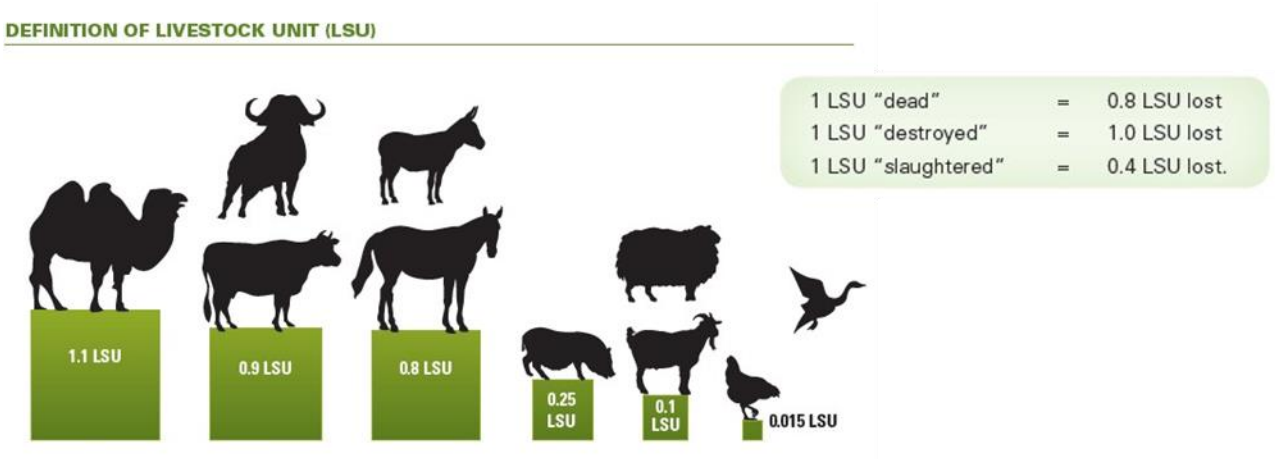

Figure 5: Livestock Units. Source: World Livestock Disease Atlas – The World Bank, 2011 [18].

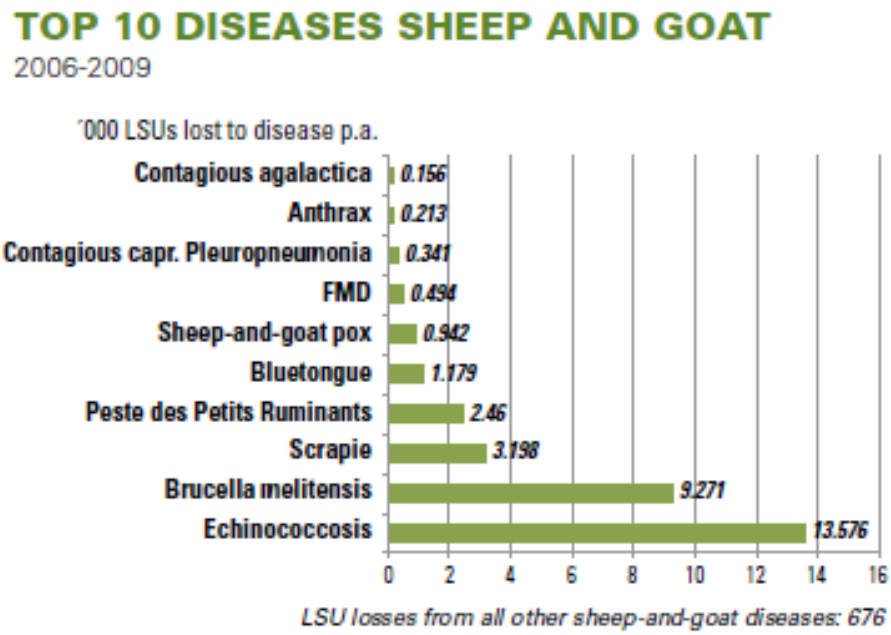

Figure 6: Top 10 diseases in terms of LSU losses for cattle, buffalo, and sheep & goats. Source: World Livestock Disease Atlas – The World Bank, 2011 [18].

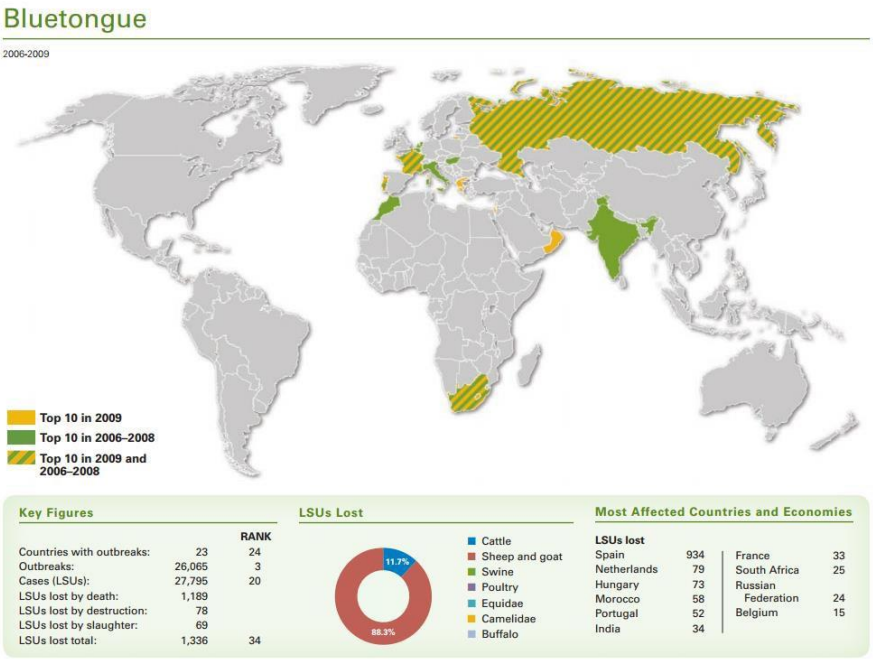

Figure 7: BT most affected countries and LSU lost for the period 2006-2009. Source: World Livestock Disease Atlas – The World Bank, 2011 [18].

Disease Prevention and Control Methods

Treatment (Control)

No specific treatment is available for BT. Treatment of BT-affected ruminants is often unrewarding and logistically challenging during outbreaks, as it involves only nonspecific supportive and nursing care.

Prophylaxis (Prevention)

The most effective way to control BTV is by employing measures that prevent the introduction of BTV to a susceptible herd. All efforts to control BTV generally fall into measures to prevent infection in disease free zones (see section c below) and a different set of distinct measures for areas that are already affected and where the focus is on avoiding further transmission within the affected area (see section d below).

Prevention of new BTV infections in BTV-free areas

Prevention of entry of bluetongue into a bluetongue-free area hinges on:

i) serological surveillance for BT

ii) vector surveillance and control followed by definition of bluetongue-free zones

Guidelines and recommendations for ensuring BTV-free zones are presented by the OIE (**http://www.oie.int/doc/ged/D12367.PDF)**. There are sanitary measures, and mechanical and biological control methods. The most important sanitary measure to avoid introduction of BTV in a free country is testing and safe importation of live animals including semen and embryos. The OIE has developed clear guidelines on the type of diagnostic tests that should be carried out prior to importation of animals or animal materials from BTV endemic areas. Additional measures include vector surveillance and control. This approach involves use of use of insecticides in the animal premises and in the areas where these insects live, insect repellents onto animals, mosquito nets, etc. especially in areas that are under threat of infection.

Control of BTV in Endemic Areas

For areas that are already affected, the approaches used are:

iii) quarantine of BT affected animal populations

iv) vaccination of potentially susceptible populations with BT vaccines

v) vector surveillance and control.

Measures to control the disease and the spread of infection include prompt reporting of BT outbreaks and implementation of appropriate serological and entomological surveillance. Vaccination of naïve animals is critical.

Simple husbandry changes and practical midge control measures may help break the BT livestock infection cycle. Housing livestock during times of maximum midge activity (from dusk to dawn) may lead to significantly reduced biting rates. Similarly, protecting livestock shelters with fine mesh netting or coarser material impregnated with insecticide will reduce contact with the midges. The *Culicoides* midges that carry the virus usually breed on animal dung and moist soils, either bare or covered in short grass. Identifying breeding grounds and breaking the breeding cycle will significantly reduce the local midge population [19]. Turning off taps, mending leaks and filling in or draining damp areas will also help dry up breeding sites. Control by trapping midges and removing their breeding grounds may reduce vector numbers. Dung heaps or slurry pits should be covered or removed, and their perimeters (where most larvae are found) regularly scraped. If cattle are treated with Ivermectin, *Culicoides* feeding on the cattle have high mortality and faeces passed for the following 28 days may be larvicidal.

Ultimately, effective control of BT can only be achieved through restriction of quarantine of infected animals and the use of mass vaccination in areas under threat of outbreaks.

Vaccination as a method for control of Bluetongue

Mass vaccination has successfully been used to control BT. The vaccines (see Section 6) used against BT must correspond to the serotype under circulation. For vaccination to be effective, the experience of vaccination against serotype BTV-4 in the Balearic Islands of Spain, has demonstrated that coverage must attain 80% of animals over a prolonged period of time to successfully stop an outbreak (**http://www.discontools.eu/Diseases/Detail/38**).

Vaccination with inactivated vaccines in the recent northern European outbreaks has been extremely successful, for example the UK completely prevented re-emergence of the BTV outbreak in 2008. The number of infected

farms in France was also reduced from 29,000 to <100 between 2008 and 2009. This was achieved through high levels of vaccine coverage (>80%) using a compulsory vaccination programme. Similar reductions in the incidence of disease have been achieved in Holland, Germany, Belgium and the UK through vaccination.

Different vaccines have been applied since the disease started in the EU. During the outbreaks of 2000-2005, sheep in France were vaccinated with live attenuated vaccines against serotypes 2, 4 and 16; and sheep in Spain were vaccinated against serotypes 2 and 4. In Italy from 2002-2005 domestic ruminants (cattle, goats and sheep) were vaccinated with live attenuated vaccine against serotypes 2, 4, 9 and 16.

It is important to note that the use of vaccines to control BTV can only be successful with cross-border agreements and uniform international or regional policies, as has been demonstrated in Europe under the joint action plan against BTV during the outbreaks of the last decade (see **http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_en.htm**). It is difficult to foresee how the same approach can succeed in developing countries with the existing weak legislative and policy frameworks against livestock diseases.

The use of these live vaccines does have some drawbacks. They may revert to virulence or may already be virulent in naïve populations; they may induce abortion when given to pregnant females, they cause viraemia, can circulate in the field in *Culicoides* midge populations and the vaccine virus may undergo reassortment with circulating field strains of another serotype or topotype. However, despite these drawbacks, live attenuated vaccines have been used successfully for many years to protect animals and control the disease in endemic areas such as Southern Africa, and in some circumstances (Balearic Islands) eradicating the infection.

Disease situation and government policies by country

Tables 9 and 10 below have been completed with the information received from the questionnaires sent to the Director Generals and Directors of the Veterinary Services for BT.

Table 9 covers the disease situation (if it is notifiable or not), the presence of official surveillance and/or control programs, and the treatment situation. Table 10 refers to the vaccination situation.

The definitions that were given to the respondents are:

1Surveillance: is the systematic ongoing collection, collation and analysis of data and the timely dissemination of information to those who need to know so that action can be taken.

2Control: a program which is approved, and managed or supervised by the Veterinary Authority of a country for the purpose of controlling a vector, pathogen or disease by specific measures applied throughout that country, or within a zone or compartment of that country.

Table 9: Official status, official programs and treatment for BT in the countries of interest. Information provided by the questionnaire sent to the DG/DVS as part of this monograph. Replies were not received from India, Indonesia, Burkina Faso, Ethiopia, Madagascar, Mozambique, Senegal and South Africa

Country	Notifiable (yes/no)	Official surveillance[1] program (yes/no) (if yes, active or passive)	Official control[2] program (yes/no)	Treatment (Chemotherapy)	
				Treatment authorised (yes/no)	Frequently practiced (yes/no)
ASIA					
Bangladesh	N/A	-	-	-	-
Myanmar	No	No	No	No	Yes
Nepal	Yes	Yes, passive	No	No	No
Vietnam	Yes	Yes, passive	No	-	-
AFRICA					
Côte d'Ivoire (Ivory Coast)	Yes	Yes, passive but active if outbreak	No	-	-
Kenya	Yes	Yes, passive	No	No	No
Malawi	Yes	Yes, passive	Yes	N/A	N/A
Mali	N/A	N/A	N/A	N/A	N/A
Rwanda	-	-	-	-	-
Tanzania	Yes	Yes, passive	No	No	No
Uganda	Yes	No	No	N/A	N/A
Zambia	Yes	Yes, passive	No	No	No

- Left blank in the questionnaire by the respondent.

Table 10: Vaccination for BT in the countries of interest.
Information provided by the questionnaire sent to the DG/DVS as part of this monograph. Replies were not received from India, Indonesia, Burkina Faso, Ethiopia, Madagascar, Mozambique, Senegal and South Africa.

Country	Vaccination			
	Compulsory vaccination (yes/no)	Who pays for the vaccine (Government, farmers, combination, others-specify)	Who delivers the vaccine (official, private vaccinators or both)	Species vaccinated (cattle, sheep, goats, pigs, poultry)
ASIA				
Bangladesh	-	-	-	-
Myanmar	No	-	-	-
Nepal	No	N/A	N/A	N/A
Vietnam	No	-	-	-
AFRICA				
Ivory Coast	No	-	-	-
Kenya	No	Combination	Both	Cattle, sheep, goats
Malawi	No	N/A	N/A	N/A
Mali	N/A	N/A	N/A	N/A
Rwanda	-	-	-	-
Tanzania	No	Not done, disease has never been reported		
Uganda	No	Never vaccinated		
Zambia	No	N/A	N/A	N/A

- Left blank in the questionnaire by the respondent.

Vaccines Available

Broadly, the bluetongue vaccines fall into three groups, namely i) Live modified/attenuated, ii) Inactivated (killed) vaccines, and iii) Recombinant vaccines. Live attenuated vaccines and inactivated vaccines may be monovalent or polyvalent. Presently, there are no recombinant BTV vaccines that are licensed or on the market. There are no vaccines (live or inactivated) that allow the differentiation between vaccinated and infected animals (DIVA).

Live attenuated BTV vaccines

Live attenuated vaccines are developed by the multiple passage of virulent strains in culture or in embryonated chicken eggs (ECE). Production time is estimated in about 8 – 10 weeks, including production and quality control [20]. These vaccines can be highly effective in epidemic situations where only one serotype of BT virus is involved. They generate protective immunity after a single inoculation and they have proven effective in preventing clinical BT disease.

In endemic areas where multiple serotypes exist, the situation is more complicated as it requires multivalent vaccines because protection from BTV vaccines is serotype specific. Unfortunately, multivalent vaccines have problems resulting from interference between virus strains, varying immunogenicity and growth rates between virus strains, as well as variations in the immune responses of individual animals to the components of such vaccines [5]. In addition, concerns have been raised about the drawbacks of using live attenuated BTV vaccines. These include under-attenuation, although impact may vary with sheep of different breeds, potential depressed milk production in lactating sheep and abortion/embryonic death and teratogenesis in offspring if used in pregnant animals. The risk is increased when the live vaccines are injected during the first third of pregnancy. Other concerns include the presence of vaccine virus in semen secretions of bulls and rams, and the possibility that vaccine virus will infect vectors and establish in the environment. Furthermore, there are fears of inducing recombinant progeny virus, with novel genetic and biological properties following re-assortment of genes from wild and vaccine virus in the vaccinated animal or the vector.

Several monovalent live attenuated vaccine combinations have been used in the field with side effects evident mainly in sheep. In this species, following vaccination, some animals developed fever, oedema in the facial region and lameness. In most cases these symptoms appeared during the second week of vaccination and disappeared in 7-10 days. Symptoms were generally more critical when animals suffered from other concurrent diseases. More severe clinical signs involving a higher number of sheep also occurred when BTV-16 were included in the vaccine combination. Because of these drawbacks, the use of BTV-16 monovalent vaccine was banned from the BTV vaccination campaigns in Europe.

These concerns have been considered serious enough that some authorities prohibit the use of live attenuated vaccines against BTV. Nonetheless, live attenuated BT vaccines have wide usage in South Africa, Italy, Morocco, Spain and France, and to a smaller extent in the USA. It must be noted that the attenuation is not characterized.

Inactivated BTV Vaccines

To overcome some of the drawbacks of live vaccines, inactivated vaccines have been developed although their main disadvantage is poor immunogenicity, so they usually require repeated immunization. Other potential disadvantages include increased costs due to the large amount of antigen required, and there are some concerns over the reliability of inactivation for each vaccine batch [as quoted in [21]].

Inactivated BTV vaccines are produced in large-scale suspension cell systems, in cell lines that are free from contamination and which are adapted for large scale commercial use. After growth, inactivation is carried by the use of chemicals like binary ethyleneimine (BEI) or other manufacturer specific methods. Typically, inactivation should not interfere with immunogenicity of the inactivated virus, and adjuvants are added. The production time frame for inactivated vaccines is estimated to be 6-8 months depending on the vaccine needs. (**http://www.oie.int/fileadmin/Home/fr/Health_standards/tahm/2.01.03_BLUETONGUE.pdf**).

Inactivated vaccines are generally safe although on a few occasions, local reactions occurred. Of particular interest are the cases of anaphylactic shock reported in areas where live vaccination was previously applied [20].

In the BT review done by the EU Scientific Panel on animal health and welfare [20], they noted that when BT inactivated vaccines where administered in two doses, all BTV inactivated vaccines were able to fully protect the animals from clinical signs and viraemia for a long period. Conversely, a single shot of a BTV-4 inactivated vaccine gave only partial reduction of viraemia in cattle when challenged 7 months after vaccination. It has to be mentioned that each of these studies on the efficacy of the inactivated vaccine followed its own challenge protocol and used different age, breed and number of animals, dose and type of challenge, route of inoculation. Differences were also on the methods employed to evaluate the efficacy of the vaccine. To detect viraemia, some trials utilized quantitative RT-PCR, some others classical virus isolation. The immunogenicity was assessed by serum neutralisazion (SN) assay and discrepancy existed also on the way the SN test was interpreted. Some considered a serum as positive for BT when titers were ≥ 1/4, others when they were ≥ 1/10.

Recombinant BTV vaccines

A number of approaches have been used to develop recombinant or sub-unit BTV vaccines and are covered under Section 8, describing current research for BTV vaccines.

Commercial vaccines manufactured in Africa and Asia

As indicated above, bluetongue virus is characterised by several serotypes that do not necessarily cross-protect. Consequently, there are several bluetongue vaccines to take into account the different BTV serotypes.

In the literature reviewed, the 8 monovalent BT vaccines comprised i) BT-1, ii) BT-2, iii) BT-4, iv) BT-8, v) BT-9, vi) BT-10, vii) BT-11,and viii) BT-17 while the 4 polyvalent BT vaccines were i) BT-1,4, ii) BT-1,8, iii) BT-2,4, and iv) the South African polyvalent that consists of (bottle A - BT-1, 4, 6, 12, 14; bottle B – BT-3, 8, 9, 10, 11; and bottle C – BT-2, 5, 7, 13, 19).

Below, Tables 11 and 12 list the BT vaccine manufacturers with the information sourced from The Center for Food Security and Public health, Iowa State University (**www.cfsph.iastate.edu/vaccines/index.php**) and Vetvac (**www.vetvac.org**). Inserts of some of the commercial vaccines are included in Annex 2.

Table 11: Manufacturers of BT monovalent vaccines

BTV Serotype	Vaccine trade name	Vaccine type	Manufacturer
1	BLUVAC® 1	Inactivated	CZ Veterinaria S.A., Spain
1	BTVPUR Alsap™ 1	Inactivated	Merial SAS (France)
1	Syvazul 1	Inactivated	SYVA Laboratorios, Spain
1	Zulvac 1 Bovis	Inactivated	Zoetis Spain
1	Zulvac 1 Ovis	Inactivated	Zoetis Spain
2	BTVPUR Alsap™ 2		Merial SAS (France)
4	Freeze Dried Monovalent Bluetongue vaccine for sheep	Live	Central Veterinary Control and Research Institute, Turkey
4	BLUEVAC® 4	Inactivated	CZ Veterinaria S.A., Spain

4	BTVPUR Alsap™ 4	Inactivated	Merial SAS (France)
4	Syvazul 4	Inactivated	SYVA Laboratorios, Spain
8	BLUEVAC® 8		CZ Veterinaria S.A., Spain
8	BOVILIS® BTV8		MSD Animal Health (Merck)
8	Syvazul 8		SYVA Laboratorios, Spain
8	Zulvac 8 Bovis		Zoetis Spain
8	Zulvac 8 Ovis		Zoetis Spain
9	BTVPUR Alsap™ 9	Inactivated	Merial SAS (France)
10	Bluetongue vaccine	Live	Colorado Serum Company, USA
10	BlueVac-10	Live	Poultry Health Laboratories, USA
11	BlueVac-11	Live	Poultry Health Laboratories, USA
17	BlueVac-17	Live	Poultry Health Laboratories, USA
?	Bluetongue vaccine	?	Institute of Animal Health and Veterinary Biologicals*

NB. All CZ Veterinaria S.A., Spain BT vaccines are "aqueous" while all Merial SAS (France) & Zoetis Spain BT vaccines have Aluminium hydroxide, saponin adjuvant but all the SYVA Laboratorios, Spain BT vaccines have oil adjuvant. The rest are not adjuvanted.
* According to the website, it is in experimental stage: http://www.kvafsu.kar.nic.in/IAHVB.htm

Table 12: Manufacturers of BT monovalent vaccines

BTV Serotype	Vaccine trade name	Vaccine type	Manufacturer
Serotype 1, 4	BLUEVAC® 1+4	Inactivated	CZ Veterinaria S.A., Spain
Serotype 1, 8	BLUEVAC® 1+8	Inactivated	CZ Veterinaria S.A., Spain
Serotype 1, 8	BTVPUR Alsap™ 1-8	Inactivated	Merial SAS, France
Serotype 1, 8	Syvazul 1+8	Inactivated	SYVA Laboratorios, Spain

Serotype 2, 4	BTVPUR Alsap™ 2-4	Inactivated	Merial SAS, France
1, 2, 3, 4, 8, 12 and 13	Bluvax TM	Live	KEVEVAPI, Kenya
Polyvalent as below A [1, 4, 6, 12 and 14] B [3, 8, 9, 10 and 11] C [2, 5, 7, 13 and 19]	Bluetongue Vaccine	Live	Onderstepoort Biological Products Ltd, South Africa
1, 2, 10, 16 & 23	Raksha-Blu	Inactivated	Indian Immunologicals Ltd, India

With respect to the 20 target countries for the Livestock Vaccine Innovation Fund, it is only South Africa (Onderstepoort Biological Products Ltd), Kenya (KEVEVAPI) and India (IIL) that manufacture vaccines against bluetongue. For the polyvalent vaccine used in South Africa, the three bottles of vaccine, each containing five serotypes of BTV, should be given in the correct order (A, B then C), at intervals of three weeks. Sheep should be vaccinated with live attenuated vaccines yearly. It is noted that in most of the LVIF target countries in Africa, the most likely source of vaccine for protection of their domesticated animals is OBP. However, the OBP Bluetongue vaccine carries with it the undesirable feature of introducing some BTV serotypes that might not be present in the vaccine importing country. The OBP product accompanying leaflet is scanned and included in Annex 2 of this monograph.

In January 2015, Indian researchers and Indian Immunologicals Ltd (IIL) were reported to have launched its bluetongue vaccine, named 'Raksha Blu', expected to protect the animals against unspecified five strains of the 'bluetongue' virus prevalent in the country. It was publicised in various media including **The Hindu – BusinessLine** and **Pharmabiz.com.** Following direct contact with the responsible of the Research & Development at ILL, a product vaccine information leaflet was provided and the scanned copy of it is included in Annex 2 of the monograph. It is also interesting to note that despite the many BT outbreaks, vaccination did not feature as a prominent method of control of the disease in India; this could be probably because they did not have their own nationally produced vaccine. The other control measures, which they have adopted, particularly since 2008 seem to be quite effective as evidenced by the sharp decline in the number of outbreaks. On the other hand, South Africa was clearly using vaccination as one of the main control measures of BT.
http://www.oie.int/wahis_2/public/wahid.php/Diseaseinformation/statusdetail.

Other manufacturers, particularly the European ones, are likely to supply West and North Africa and probably some of Asian countries where there are no BT manufacturing facilities.

Commercial vaccines imported into Africa and Asia

Based on the questionnaire sent to the Directors of Veterinary Services office and regulators of the countries of interest, only Zambia seems to have been importing the vaccine (see Table 13). Note that replies were not received from India, Indonesia, Burkina Faso, Ethiopia, Madagascar, Mozambique, Senegal and South Africa.

Table 13: Commercial BT vaccines imported into the countries of interest

Country	Vaccine name	Strain or type	Country of origin	Doses imported 2015	Doses imported 2014	Doses imported 2013	Doses imported 2012
Zambia			Ireland	0	10,000	0	-
		Polyvalent	South Africa	16,000	10,000	0	-

Characteristics of Ideal Vaccine Candidates for Smallholders

The Target Product Profiles (TPPs) reflect the availability and utility of current agents and incorporate features that will be necessary to improve on the current products and to address unmet needs, taking into account the particular requirements of the poorest livestock keepers.

The TPPs are more robust when they include the opinions and consider the needs of the different stakeholders. While efforts have been made to encompass them, the TPP showed in Table 14 below, should be considered a proposal, a live document subject to improvements.

Table 14: Target Product Profile (TPP) BT vaccine – Proposal:

	Attribute	Minimum (current available vaccine)	Ideal
1	Antigen	Immunogen with protective antigens against a specific serotype of BTV. There are some polyvalent vaccines, but none protects against all serotypes.	Immunogen with protective antigens against all 27 serotypes of BTV
2	Indication for use	For active immunization of sheep, goat & cattle to prevent incidence of BT	For active immunization of ruminants, to prevent infection with BTV
3	Recommended species	Sheep, cattle, goats	All susceptible domesticated and wild ruminants
4	Recommended dose	1-2 ml SC	1 ml
5	Pharmaceutical form	Reconstituted injectable solution	Ready to use solution
6	Route of administration	Subcutaneous	Either subcutaneous or intramuscular

7	Regimen - primary vaccination	Primary vaccination - some have to be repeated with either same or different serotypes 3 weeks apart	Preferably single inoculation
8	Regimen - booster	Annual injection is recommended	Lifelong immunity after primary vaccination
9	Epidemiological relevance	Protects against specific serotypes used in vaccination	Protects against all field BTV serotypes
10	Recommended age at first vaccination	1 month old in naïve sheep & cattle; 2.5 to 3 months in young born to immune sheep/cattle	Preferably within first 2 months of age
11	Onset of immunity	3 weeks post vaccination	One week following vaccination
12	Duration of immunity	Generally about 1 year	Lifelong
13	Expected efficacy	Prevents viraemia and reduce clinical signs	Prevent BT clinical disease & BTV transmission in all vaccinated animals
14	Expected safety	Inoculation may be followed by a small local swelling at the injection site for a short period (at most 14 days). A transient increase in body temperature may also occur.	Not cause any clinical disease; incapable of replicating in the inoculated host; no reversion to virulence; no re-assortment with field strains
15	Withdrawal period	Depending on manufacturer could be 0 to 21 days	None
16	Special requirements for animals	Only vaccinate healthy animals.	Vaccinate all animals
17	Special requirements for persons	None	None
18	Package size	50, 80, 100, 250 ml	Multiple pack size from 10 doses
19	Price to end user		
20	Storage condition and shelf-life as packaged for sale	2 to 8 °C, 12 months	20° C for at least 12 months

`

21	In-use stability	Within few hours of Puncturing the vial	Preferably 8 to 12 hours
22	Other: DIVA capabilities	No	Yes
23	Other: reassortment possibility	Yes (live attenuated vaccines)	No

Limitations

Scientific quality: The publications and data from the different research groups, should be carefully evaluated. The use of good science and good experimental design with use of proper controls, adequate numbers, suitable challenge model, reproduction of results by them and by independent groups, and appropriate analysis has not been verified for this monograph. If any of these projects were to be pursued, a detailed peer review taking into account the above considerations is strongly recommended.

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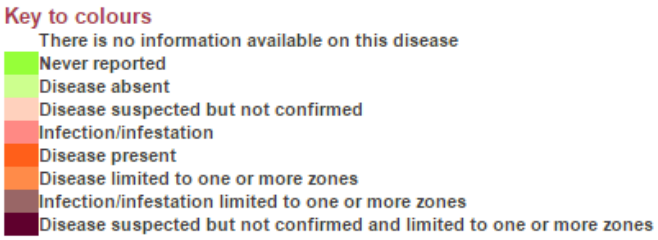
ANNEX 1: Additional data on disease presence and incidence

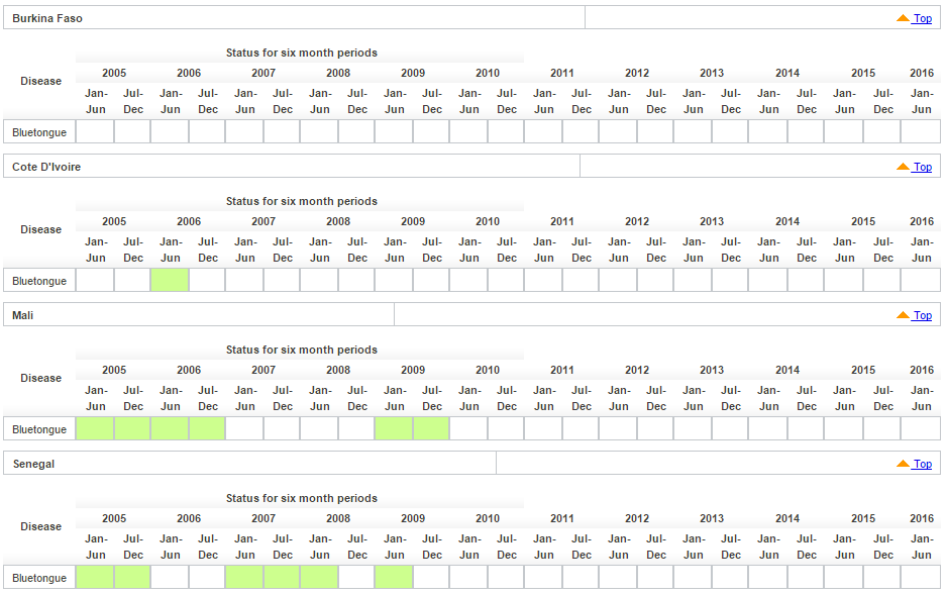
Reports to OIE on BT:



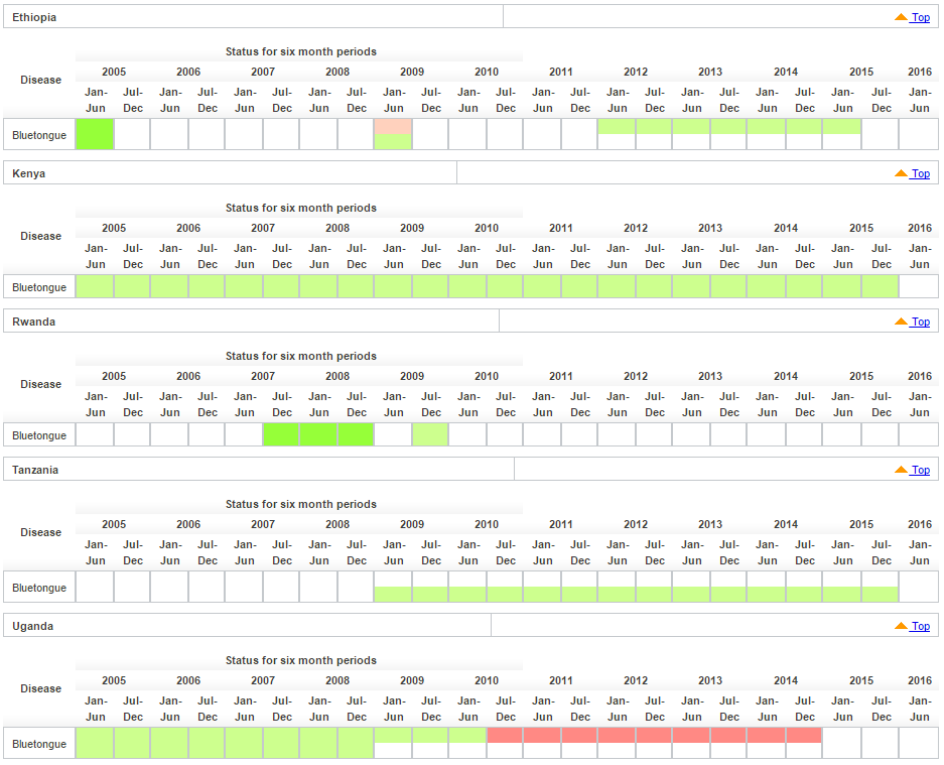
When different animal health statuses between domestic and wild animal population are provided, the box is split in two: the upper part for domestic animals, and the lower part for wild animals.

BT in Asia: Bangladesh, India, Indonesia, Myanmar, Nepal and Vietnam

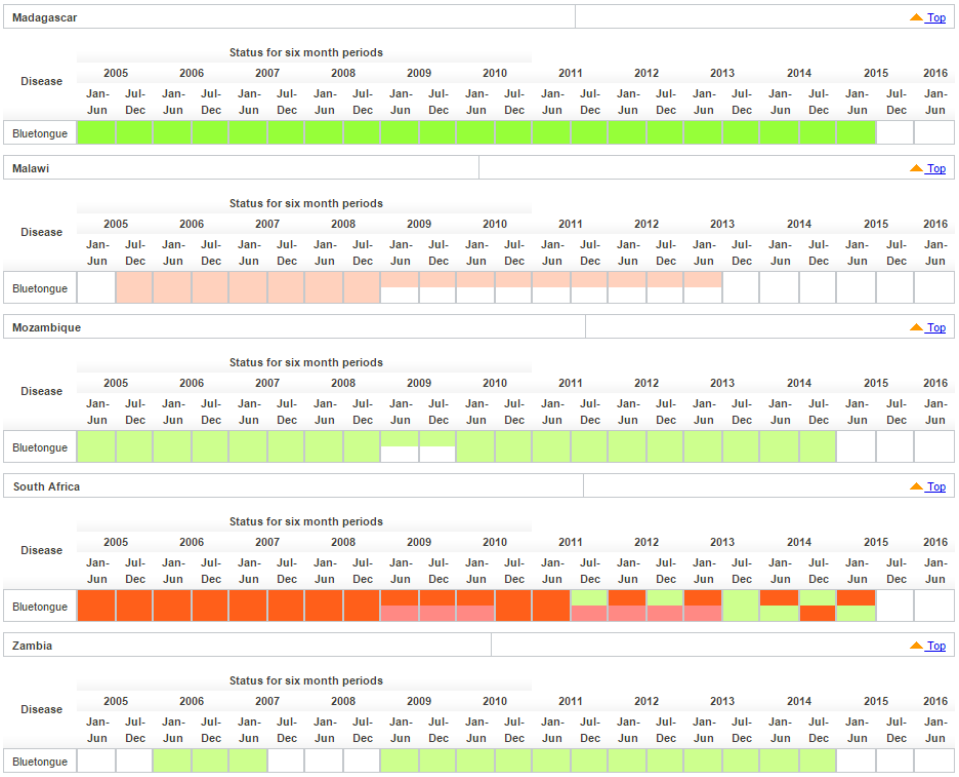
BT in Western Africa: Burkina Faso, Ivory Coast, Mali and Senegal



BT in Eastern Africa: Ethiopia, Kenya, Rwanda, Tanzania and Uganda



BT in Southern Africa: Madagascar, Malawi, Mozambique, South Africa and Zambia



ANNEX 2: Labels from different BT commercially available vaccines

ONDERSTEPOORT
BIOLOGICAL PRODUCTS

For animal use only

BLUETONGUE
VACCINE FOR SHEEP

Reg. No. G 0358 (Act 36/1947) Namibia: NSR 0566

A freeze-dried polyvalent vaccine containing live attenuated bluetongue virus strains for the prophylactic immunisation of sheep against bluetongue.

The vaccine is presented as a series of three separate injections with different serotypes of bluetongue virus in each bottle. The bottles are marked A, B and C and must be injected in the following sequence: A, first, B, three weeks later, and the bottle marked C three weeks after B. If necessary, this interval can be longer but never shorter than three weeks. **It is necessary to inject the full series, A, B and C in order to get the widest possible protection.**

Store the vaccine in a refrigerator at 4 °C to 8 °C. Do not use after the expiry date printed on the bottle.

RECOMMENDATIONS FOR USE
Vaccinate sheep from August to October. Immunisation of ewes should commence 9–12 weeks before mating. It is not advisable to inject pregnant ewes during the first half of pregnancy. Rams should be inoculated after the mating season. Inject lambs from immunised ewes at the age of six months and older. If done at an earlier age in heavily infected areas, lambs must be revaccinated at the age of six months. Sheep must be vaccinated annually.

N.B.: The vaccine will only stimulate immunity to all serotypes after a number of inoculations. Additional measures must necessarily be taken to ensure protection of sheep against bluetongue during the time of the year when the risk of transmission of infection by biting insects is greatest. Animals should be kept away from low-lying areas in vleis and next to rivers, dams and pans and valuable animals should be stabled during late afternoon, night and early morning.

WARNINGS
Do not slaughter animals for human consumption within 7 days of vaccination. Vaccinate healthy animals only. Keep out of reach of children, uninformed persons and animals. Although this product has been extensively tested under a large variety of conditions, failure thereof may ensue as a result of a wide range of reasons. If this is suspected, seek veterinary advice and notify the registration holder.

DIRECTIONS FOR USE
Use only as directed. Sterilise syringes and needles by boiling in water for at least 15 minutes. Do not use disinfectants or methylated spirits for sterilising either needles or syringes. Use a separate needle for each animal. The active ingredient of the vaccine is in the form of a powder or pellet in a small bottle. By means of a sterile syringe transfer approximately 1 ml of the 100 ml sterile diluent to the bottle marked A containing the freeze-dried vaccine. Mix thoroughly until the powder is dissolved and then transfer this suspension back to the remaining sterile diluent and again mix well by means of the sterile syringe. The vaccine is now ready for use and must be injected without delay. Three weeks later use the bottle marked B. Reconstitute in the same way as bottle A and inject into 100 sheep that have been injected with vaccine A. After another three weeks use bottle C. Follow the same directions as for A and B and inject the same sheep that have previously been injected with vaccine A and B. Shake the bottle well before use. Avoid exposure to high temperatures and direct sunlight during vaccination.

DOSAGE: 1 ml subcutaneously. Inject the vaccine behind the shoulder or on the inside of the thigh but not under the tail.

EFFECTS OF THE VACCINE
A fever reaction may follow from the seventh day after vaccination. Such animals must not be exposed to strong sunlight, adverse weather conditions and fatigue. A reasonable protection against most of the virus types is usually achieved within 3–4 weeks after the last injection but cannot be guaranteed in all animals.

PACKING
Available in packagings of 100 doses.

Registration holder:
Onderstepoort Biological Products SOC Ltd, Co. Reg. No. 2000/022686/06
Private Bag X07, Onderstepoort, 0110. Tel: +27 (0) 12 522 1500, Fax: +27 (0) 12 522 1591

Made in South Africa Edition 4 P2013

RAKSHA-BLU

Description :
Bluetongue inactivated vaccine contains Bluetongue virus (BTV) antigens of polyvalent serotype (1, 2, 10, 16 & 23), grown on BHK 21 suspension cells. BEI and formaldehyde are used as inactivating agents.

Composition :
Each dose (2 ml) contains polyvalent Bluetongue inactivated antigens of five virus serotypes (1, 2, 10, 16 & 23), adsorbed onto Aluminium hydroxide gel, Saponin added as adjuvant, Thiomersal ≤ 0.02% w/v as preservative and phosphate Buffer Diluent q.s.

Dosage and Administration :
Sheep & Goats 2 ml by subcutaneous route. Do not freeze. Discard if contents are frozen.

Vaccination Regimen :
Primary vaccination (PV): 3 months (12-13 weeks) and above
Booster dose : 1 month (4 weeks) after primary vaccination
Revaccination : Annual

Storage and Transport :
Store and transport the vaccine between 2°C and 8°C

Presentation :
Bluetongue polyvalent vaccine, inactivated is available as 50 Doses (100 mL)

INDIAN IMMUNOLOGICALS LIMITED
A Wholly Owned Subsidiary of the National Dairy Development Board
Road No. 44, Jubilee Hills, Hyderabad - 500 033, India
Toll Free No. 1800-425-5363
www.indimmune.com

INDIAN
IMMUNOLOGICALS
LIMITED

1st Bluetongue vaccine in INDIA

RAKSHA-BLU

BLUE TONGUE

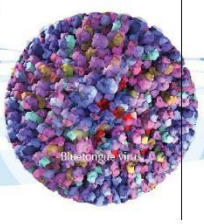
(Sore muzzle, Pseudo FMD, Ovine cattarhal fever)

Bluetongue is an acute, insect-transmitted, non-contagious disease of domestic and wild ruminants caused by Bluetongue virus (BTV). The outcome of BTV infection of ruminants is highly variable and is severe in sheep & goat. Bluetongue is most commonly encountered in sheep & goat and characterized by vascular injury that causes hemorrhage and edema in a variety of tissues. In India[1] 33.3% goats, 50% sheep and 3.7-18% cattle were found to be positive for Bluetongue. Presence in buffalo varies from state to state. Crossbred cattle are more prone to Bluetongue infection.

Etiology :
The BTV is a member of the genus Orbivirus, family Reoviridae. As many as 24 serotypes[1] (1-24) of Bluetongue virus have been reported worldwide. Published data reveals that 18 serotypes have been isolated in India.

Host range :
The BTV replicates in several species of Culicoides insects. Bluetongue is most exclusively a disease of sheep and goats, although the disease in cattle is also reported.

Epidemiology :
The distribution of BTV coincides with species of Culicoides insects. Approximately 1000 species[2] of Culicoides insects have been confirmed to be competent vectors of BTV. The species of Culicoides that serve as principal vectors of the virus differ between regions. *Culicoides sonorensis* is the principal vector of BTV serotypes 10, 11, 13 and 17. *Culicoides insignis* is the vector of numerous BTV serotypes (1, 2, 3, 4, 6, 8, 11, 12, 13, 14 and 17). Onset of monsoon spreads the disease.



1. Bluetongue virus infection in India: a review by G. PRASAD, N.C. JAIN and Y GUPTA www.oie.int/doc/ged/D8650
2. Current Science, Vol.90 No.2, 25 January 2006

Incubation Period :
The Incubation period in BTV-infected ruminants ranges from 2 to 10 days.

Morbidity & Mortality :
Morbidity can be as high as 100% in susceptible ruminants. Mortality is highly variable, even in susceptible breeds of sheep & goat.

Clinical Signs :
Sheep infected with Bluetongue infection exhibit fever, abortion, hyperemia, mucopurulent nasal discharge and hemorrhage on coronary band. Affected sheep are reluctant to move and their gait is stiff. The cyanotic tongue is common manifestation. Mortality is very high in new born and young sheep and goats.

Sheep recovered from Bluetongue infection exhibit abortion, marked loss of condition, with extensive muscle loss (leading to torticollis), hoof deformities, loss of wool (wool fiber break), and generalized weakness.

Diagnosis :
Diagnosis of BT is based upon the characteristic clinical signs and lesions in affected animals, especially sheep. Out breaks frequently are associated with extension of the virus into areas that were previously free from infection for substantial periods of time (years).

Prevention and control :
Vaccination is the only method for prevention and control of Bluetongue diseases among sheep, goats & cattle.

PP Packaging

Syvazul 1

Inactivated vaccine against the serotype 1 of Bluetongue Disease in injectable suspension

Composition per ml
Inactivated bluetongue virus,
strain ALG 2006/01 E1 (serotype 1) PR*≥1
* Relative potency measured in ELISA units and related to the reference vaccine, proven effective through challenge.

Indications
Active immunization of sheep and cattle for the prevention of viraemia and reduce symptomatology caused by serotype 1 of Bluetongue Disease virus.
Start of immunity:
Sheep: 39 days after vaccination (single dose application) or 21 days after second vaccination (when 2 doses applied)
Cattle: 21 days after second vaccination
Duration of immunity: 1 year
Can be used during gestation and lactation.

Administration Route
Sheep: subcutaneous injection.
Cattle: intramuscular injection.

Dosage and administration
Sheep: 2ml/animal. Starting from 3 months of age.
First vaccination: One dose if born from non-immunized mother or 2 doses separated 3 weeks if born from immunized mothers.
Revaccination: annual
Cattle: 4ml/animal. Starting from 2 months of age if born from Non-immunized mothers or 3 months from immunized mothers.
First vaccination: 2 doses separated 3 weeks.
Revaccination: annual

Shelf-life period
Two years.

Withdrawal period
0 days.

Storage
Store and transport under refrigerated conditions (between +2° C and + 8° C). Do not freeze

Supply
Vials of 80 and 200 ml.

LICENSE NUMBER: 2035-ESP (AEM SPAIN)

syva

Syvazul 1+8

Inactivated vaccine against the serotype 1+8 of the Bluetongue Disease in injectable suspension

Composition per ml
Inactivated bluetongue virus,
strain ALG2006/01E1 (serotype 1) ≥ $10^{6.9}$ $TCID_{50}$*
Inactivated bluetongue virus,
strain BEL2006/01 (serotype 8) ≥ $10^{6.7}$ $TCID_{50}$*
* Determined before inactivation.

Indications
Active immunization of sheep and cattle for the prevention of viraemia caused by serotypes 1 and 8 of the Bluetongue Disease virus.

Administration Route
Sheep: subcutaneous injection.
Cattle: intramuscular injection.

Dosage and administration
Dosage: 2 ml for sheep and 4 ml for cattle.
Initial vaccination:
Administer two doses with an interval of 3 weeks in between.
Minimum vaccination age: Sheep: 3 months.
Cattle: 2 months.
Booster:
Administer one dose before every risk season.
When used within the frame of an official control program, the vaccination schedule must follow the plan stated by the relevant sanitary authority.

Shelf-life period
One year.

Withdrawal period
Zero days.

Storage
Store and transport under refrigerated conditions (between +2° C and + 8° C) and protected from light. Do not freeze.

Supply
Vials of 80 and 200 ml.

LICENSE NUMBER: 2056 ESP (AEM SPAIN)

syva

Syvazul-4

Inactivated vaccine against the serotype 4 of the Bluetongue Disease in injectable suspension

Composition per ml
Inactivated *Bluetongue Virus*, serotype 4 0.85 Elisa Units*
* Relative potency expressed in relation to the reference vaccine.

Indications
Active immunization of sheep for the prevention and reduction of viraemina caused by the Bluetongue Disease virus.

Administration Route
Subcutaneous injection in the axillar area.

Dosage and administration
Dose: 2 ml.
Primo vaccination: administer two doses three weeks apart.
Minimal vaccination age: 3 months.
Booster: administer one dose before every risk season.
If the vaccine is used in official control programs, the vaccination schedule must follow the plan stated by the relevant sanitary authority.

Shelf-life period
One year.

Withdrawal period
Zero days.

Storage
Store and transport under refrigerated condition and + 8° C). Do not freeze.

Supply
Vials of 250 ml.

LICENSE NUMBER: 1686 ESP (AEM SPAIN)

Syvazul 8

Inactivated vaccine against the serotype 8 of the Bluetongue Disease in injectable suspension

Composition per ml
Inactivated bluetongue virus,
strain BEL2006/01 (serotype 8) $\geq 10^{6.7}$ $TCID_{50}$ *
* Determined before inactivation.

Indications
Active immunization of sheep and cattle for the prevention of viraemia caused by serotype 8 of the Bluetongue Disease virus.

Administration Route
Sheep: subcutaneous injection.
Cattle: intramuscular injection.

Dosage and administration
Dosage: 2 ml for sheep and 4 ml for cattle.
Initial vaccination:
Administer two doses with an interval of 3 weeks in between.
Minimum vaccination age: Sheep: 3 months.
Cattle: 2 months.
Booster:
Administer one dose before every risk season.
When used within the frame of an official control program, the vaccination schedule must follow the plan stated by the relevant sanitary authority.

Shelf-life period
One year.

Withdrawal period
Zero days.

Storage
Store and transport under refrigerated conditions (between +2° C and + 8° C) and protected from light. Do not freeze.

Supply
Vials of 80 and 200 ml.

LICENSE NUMBER: 2036 ESP (AEM SPAIN)

syva

Bovilis® BTV8

Merck Animal Health has developed an inactivated vaccine for the aid in the prevention of BTV 8 infection. It contains an inactivated virus strain isolated from one of the outbreaks that took place in Europe in 2006, alongside a dual adjuvant system that ensures the delivery of both a cell-mediated and a humoral response.

Independent challenge studies have shown that the vaccine can prevent viraemia in sheep following a single dose. Studies in cattle have shown that vaccination can reduce the level and the duration of the viraemia following a vaccination course comprising two doses given three weeks apart.

Vaccination schedule

It is recommended that the vaccination program is completed prior to the risk period. The risk period is associated with the time of the year during which midges are active.

Sheep

Primary vaccination:
Sheep from 1 month of age: subcutaneous injection of a single dose of 1 ml

Revaccination:
As the duration of immunity is not yet fully established, any revaccination scheme should be agreed by the Competent Authority or by the responsible veterinarian, taking into account the local epidemiological situation

Cattle

Primary vaccination:
Cattle from 6 weeks of age: subcutaneous injection of two doses of 1 ml, administered with an interval of approximately 3 weeks.

Revaccination:
As the duration of immunity is not yet fully established, any revaccination scheme should be agreed by the Competent Authority or by the responsible veterinarian, taking into account the local epidemiological situation.

Merial's bluetongue vaccine - information for veterinary professionals and farmers

BTVPUR AlSap™ 8

BTVPUR AlSap 8 is an inactivated liquid vaccine containing purified Bluetongue Virus Serotype 8 antigen in aluminium hydroxide and saponin adjuvants. It is the latest member of Merial's range of bluetongue vaccines, which have been used in different parts of Europe since 2004. BTVPUR AlSap 8 is specifically designed to immunise cows and sheep against the bluetongue serotype that is prevalent in the United Kingdom.

The vaccine is being made available in 100ml and 50ml bottles. It is administered subcutaneously with one 1 ml injection for sheep from 1 month of age in naïve animals and 2.5 months of age in young animals born to immune sheep. In cattle there are two 1 ml injections, 1 month apart, again from 1 month of age in naïve animals and from 2.5 months of age in young animals born to immune cattle. Onset of immunity is 3 weeks after completion of the primary course. It is recommended that animals are revaccinated at least 2 weeks before each risk period.

If you are a veterinary professional or farmer and require further information, please contact us.

BTVPUR AlSap 8 DATA SHEET

Presentation
Suspension for injection containing inactivated Bluetongue Virus serotype 8 antigen, at least 7.1 CCID50* per 1-ml dose, to stimulate active and specific immunity against Bluetongue Virus serotype 8 in cattle and sheep. Contains aluminium hydroxide and saponin as adjuvants.
*Equivalent to titre prior to inactivation (log 10).

Uses
For active immunisation of sheep and cattle to prevent viraemia* and to reduce clinical signs caused by Bluetongue Virus serotype 8.
*(below the level of detection by the validated RT-PCR method at 3.14log10 RNA copies/ml, indicating no infectious virus transmission).

Onset of immunity has been demonstrated 3 weeks after the primary vaccination course. The duration of immunity is not yet fully established in cattle or sheep, although interim results of ongoing studies demonstrate that the duration is at least 6 months after the primary vaccination course in sheep. Can be used during pregnancy in ewes. Safety throughout pregnancy in cows is not yet fully established, however interim results demonstrate safety in at least the last trimester of pregnancy in cows.

Dosage and administration
Apply usual aseptic procedures. Shake gently immediately before use. Avoid bubble formation, as this can be irritating at the site of injection. The entire content of the bottle should be used immediately after broaching and during the same procedure. Avoid multiple vial broaching. Administer one dose of 1 ml subcutaneously according to the following vaccination scheme:

Primary vaccination

- In sheep
  - One injection: from 1 month of age in naive animals (or from 2.5 months of age in young animals born to immune sheep).
- In cattle
  - 1st injection: from 1 month of age in naive animals (or from 2.5 months of age in young animals born to immune cattle).
  - 2nd injection: after 3-4 weeks

Revaccination
As the duration of immunity is not yet fully established in cattle or sheep, any revaccination scheme should be agreed by the Competent Authority or by the responsible veterinarian, taking into account the local epidemiological situation.

Contra-indications, warnings, etc
Keep out of the reach and sight of children.
For animal treatment only.
Vaccinate healthy animals only.
The safety and the efficacy of the vaccine has not been established in breeding males. In this category of animals the vaccine should be used only according to the benefit/risk assessment by the responsible veterinarian and/ or national Competent Authorities on the current vaccination policies against Bluetongue Virus (BTV).
If used in other domestic and wild ruminant species that are considered at risk of infection, its use in these species should be undertaken with care and it is advisable to test the vaccine on a small number of animals prior to mass vaccination. The level of efficacy for other species may differ from that observed in sheep and cattle.

No information is available on the safety and efficacy of this vaccine when used with any other veterinary medicinal product. A decision to use this vaccine before or after any other veterinary medicinal product therefore needs to be decided on a case by case basis.

Vaccination may be followed by a small local swelling at the injection site (at most 32 cm^2) for a short period (at most 14 days).A transient increase in body temperature, normally not exceeding an average of 1.1°C, may occur within 24 hours after vaccination. No adverse reactions except these were observed after the administration of a double-dose of the vaccine.

Withdrawal period: Zero days.

Pharmaceutical precautions
Do not mix with any other veterinary medicinal product.
Store and transport refrigerated (2°C – 8°C). Protect from light. Do not freeze.

Shelf life of the veterinary medicinal product as packaged for sale: 1 year.
Shelf life after first opening the immediate packaging: immediately after broaching.
Any unused veterinary medicinal product or waste materials derived from such veterinary medicinal products should be disposed of in accordance with local requirements.

Legal category
POM-V

Package quantities
Box of 1 polypropylene bottle of 50 doses (1x 50 ml)
Box of 10 polypropylene bottles of 50 doses (10x 50 ml)
Box of 1 polypropylene bottle of 100 doses (1x100 ml)
Box of 10 polypropylene bottles of 100 doses (10x100 ml)

Box of 1 glass bottle of 10 doses (1x10ml)

Not all pack sizes may be marketed.

Marketing authorisation number
(EU/2/09/094/001 – 005 (EMEA/V/C/146)).