BLUETONGUE
STANDARD OPERATING PROCEDURES:
1. OVERVIEW OF ETIOLOGY AND ECOLOGY

FAD PReP
Foreign Animal Disease
Preparedness & Response Plan

United States
Department of Agriculture

August 2016
The Foreign Animal Disease Preparedness and Response Plan (FAD PReP) Standard Operating Procedures (SOPs) provide operational guidance for responding to an animal health emergency in the United States.

These draft SOPs are under ongoing review. This document was last updated in August 2016. Please send questions or comments to:

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# Contents

1.1 Introduction                                                                                           ............................................................ 1-2
  1.1.1 Further Information ........................................................................................................ 1-2
  1.1.2 Goals ............................................................................................................................. 1-2
1.2 Purpose ............................................................................................................................................... 1-2
1.3 Etiology............................................................................................................................................... 1-3
  1.3.1 Name ................................................................................................................................ 1-3
  1.3.2 Virus Characteristics ....................................................................................................... 1-3
  1.3.3 Morphology ....................................................................................................................... 1-3
1.4 Ecology ............................................................................................................................................... 1-4
  1.4.1 Susceptible Species ......................................................................................................... 1-4
  1.4.2 Transmission and Vector ................................................................................................. 1-4
  1.4.3 Morbidity and Mortality ................................................................................................. 1-7
  1.4.4 General Clinical Signs ................................................................................................... 1-7
  1.4.5 Bluetongue Virus Serotype 8 Clinical Signs .................................................................... 1-8
  1.4.6 Differential Diagnosis ..................................................................................................... 1-9
1.5 Environmental Persistence of Bluetongue Virus ..................................................................................... 1-10
1.6 Distribution ........................................................................................................................................... 1-10
  1.6.1 European Distribution and Significance .......................................................................... 1-11
  1.6.2 Distribution in North America ....................................................................................... 1-12
1.7 Vaccines ............................................................................................................................................. 1-13
1.8 Risk of BTV-8 Introduction to the United States .................................................................................... 1-14
Attachment 1.A References and Resources ........................................................................................ 1-16
Attachment 1.B Abbreviations .............................................................................................................. 1-20
**Bluetongue**  
Etiology & Ecology Quick Summary

**Disease**  
Bluetongue, caused by bluetongue virus (BTV), is also known as sore muzzle, pseudo foot-and-mouth, malarial catarrhal fever, epizootic catarrh, Beksiekte, and muzzle disease.

**Mortality & Morbidity**  
Morbidity in sheep ranges from less than 5 percent to 50–75 percent or higher; mortality can reach up to 100 percent, although generally is less than 30 percent. Cattle and goats have reduced mortality and morbidity is rare.

**Clinical Signs**  
Edema, hemorrhage, and ulceration of mucosae throughout the body.

**Susceptible Species**  
Wild and domestic ruminants: sheep, goats, cattle, buffaloes, deer, bighorn sheep, North American elk, most species of African antelope, and other Artiodactyla, such as camels.

**Zoonotic Potential**  
Not a threat to public health.

**Reservoir**  
Possibly cattle, other ruminant species and/or biting midges of the *Culicoides* species.

**Transmission**  
Primarily vector-borne via *Culicoides* spp. (biting midges). Vertical transmission in hosts occurs; direct transmission plays minor epidemiological role.

**Persistence in the Environment**  
Is not known to persist in fomites or in animal carcasses or products; much is still unknown about environmental persistence.
1.1 Introduction

First described in 1905 among merino wool sheep in South Africa, bluetongue virus (BTV) is an arbovirus which replicates in wild and domestic ruminants causing subclinical to fatal symptoms. Bluetongue has a very wide distribution, historically between 40° North and 35° South, in subtropical and tropical climates, owing to the distribution of its vector, the biting midge (Culicoides spp.). Recently, there have been incursions beyond its normal geographic distribution, as observed in the United States and Europe, where strains have moved between geographic regions and adapted to different species of midges.¹ These adaptations, the discovery of new serotypes with unpredictable virulence, and clinical pathology make bluetongue a disease of concern.

Various serotypes of BTV are endemic in the United States. This standard operating procedure (SOP) is concerned primarily with BTV serotype 8 (BTV-8), which does not currently occur in the United States but could have epidemic potential. Incursion of BTV-8 into the United States is concerning due to the significant consequences upon livestock in Europe observed after 2006 when BTV-8 first entered Europe.

1.1.1 Further Information


1.1.2 Goals

As a preparedness goal, APHIS will provide etiology and ecology summaries for bluetongue, and update these summaries at regular intervals.

As a response goal, animal health officials and stakeholders will have a common set of etiology and ecology definitions and descriptions, to ensure proper understanding of bluetongue when establishing or revising goals, objectives, strategies, and procedures.

1.2 Purpose

This document provides responders and stakeholders with a common understanding of the disease agent.

1.3 Etiology

1.3.1 Name

Bluetongue originally derived its name because the cell injury and necrosis it causes leads to vascular thrombosis, edema, and hemorrhage that may result in a cyanotic or blue tongue. However, the clinical sign of a blue tongue is often not present. Bluetongue is also known as sore muzzle, malarial catarrhal fever, epizootic catarrh, Bek sickness, and muzzle disease.4

1.3.2 Virus Characteristics

According to the International Committee on Taxonomy of Viruses,5 BTV is categorized as follows:

- **Family: Reoviridae**
- **Subfamily: Sedoreovirinae**
- **Genera: Orbivirus.**

There are currently 27 different serotypes,6 of which, all but one can be genetically traced to their original geographic origin;7 the exception is BTV-27 due to its recent (2014) discovery. Serotypes differ in virulence, hence the extent of clinical signs may vary. The numerous serotypes are the result of genetic shift (reassortment) and drift (mutation)8 from alternating passage of BTV through ruminant and insect hosts.

1.3.3 Morphology

BTV is a non-enveloped, linear, and segmented double-stranded ribonucleic acid (dsRNA) virus. There are 10 segments that code for 10 proteins, 7 structural proteins (VP1–VP7) and 3 non-structural (NS1, NS2, NS3/NS3a) proteins. Two of the structural proteins (VP2 and VP5) make up the icosahedral capsid of the virus. Serotype is primarily determined by VP2, the most variable of the BTV proteins, which interacts with neutralizing antibodies. The geographic origin of the serotypes is reflected in the variable sequence of the segments that make up a specific serotype’s genome, allowing further classification of serotypes into topotypes.9

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1.4 Ecology

1.4.1 Susceptible Species

Most wild and domestic ruminants are susceptible to BTV infection, including, sheep, goats, cattle, water buffalo, African buffalo (*Syncerus caffer*), bison, deer, bighorn sheep, elk, most species of African antelope, and other Artiodactyla such as camels. Recent studies have also suggested that BTV could be infecting carnivorous species such as dogs, cats, cheetahs, lions, etc.\(^{10,11}\) However, BTV infection does not always result in disease: cattle, goats, dromedaries, and some wild ruminants often have subclinical infections\(^{12}\) and may be reservoirs of the disease (especially cattle). Sheep most commonly show clinical signs. The severity of the disease may be influenced by breed, immunological conditions, strain, and environmental factors. In North America, bluetongue (of various serotypes) have been detected in wild species including: white-tailed deer, pronghorn, bighorn sheep, as well as captive Reeve’s muntjac and captive greater kudu.

The serotype of concern, BTV-8, infects both domestic and wild ruminants; however, to-date, cattle and sheep are most frequently affected. BTV-8 has also been found in deer, goats, zoo animals, and a Eurasian lynx (carnivore).\(^{13}\) In addition, it is believed that South American camelids are susceptible to BTV-8 based on seroprevalence, though other research notes negligible epidemiological significance of camelid infection.\(^{14,15}\)

1.4.2 Transmission and Vector

BTV can be transmitted both mechanically and biologically. Surgical equipment and needles can also transfer the virus between ruminants, however this is thought to be insignificant compared to biological means. The biting midge (*Culcoides* spp.), commonly referred to as gnats, are the primary vector transmitting BTV. These insects become infected by feeding on viremic animals and remain infective for the duration of their lives, up to 3 months,\(^{16}\) replicating the virus in their...
salivary glands every 6–8 days. Vector activity is directly influenced by temperature—increasing with the rising temperature maximizing at 28 to 30°C and decreasing or ceasing at cooler temperatures.

After a bite by a BTV infected Culicoides midge, it has been demonstrated that the virus can persist in animal blood for 5–9 weeks, as demonstrated in cattle, and in viral ribonucleic acid (RNA) much longer. Prolonged viremia may have been seen in other species as well, though the existence and characteristics of the carrier state in any species is not yet well understood.

Some serotypes, including BTV-8, appear to have the ability to survive over winter, though it appears to be uncommon. This overwintering may be the result of midge survival in warmer environments during colder months, such as indoors. A recent example of overwintering occurred during the 2015–2016 European outbreak where BTV-8 resurfaced in France: genetic testing resulted in 95 percent homology to European strains that circulated in 2006–2008, but full typing is still being performed. The exact method(s) in which overwintering can occur remain unknown: research has presented evidence that latent virus found in ruminants may play a role, others have posited that it may be possible for Culicoides larvae to extend development through the winter, though lengthy cold temperatures will still kill the pupae. There are many theories about overwintering but little confirmed evidence from field conditions.

Additionally, field observations and experimental studies have shown that BTV-8 is capable of transplacental (vertical) transmission and oral transmission in cattle and sheep. BTV-8 is found in BTV-8 infected animals.

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known to be shed in semen from cattle\textsuperscript{27} and also has been detected in rams.\textsuperscript{28} The method of carnivore infection is unknown, but it is speculated carnivores become infected by eating infected carcasses or being bitten by \textit{Culicoides}.'\textsuperscript{29} Like overwintering, additional evidence remains needed on these transmission methods; the complete scope of close-contact transmission for BTV-8 is not well understood, though it is presumed to be of minor epidemiological significance. BTV-25, also known as Toggenburg orbivirus, along with BTV-26, lack the typical replication patterns seen in Culicoid vectors, suggesting that alternative or additional transmission pathways may exist for these new viruses.\textsuperscript{30}

Figure 1 depicts the normal BTV transmission pathway and some of the suspected overwintering mechanisms for the virus. Midges and sheep are colored based on infection—those in black are non-infected and those in red are infected.

\textbf{Figure 1. Bluetongue Virus Transmission in Summer (Left) and Winter (Right)}\textsuperscript{31}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{bluetongueTransmissionDiagram.png}
\caption{Bluetongue Virus Transmission in Summer (Left) and Winter (Right)}
\end{figure}

Note: This figure expresses an example of BTV transmission in sheep including secondary routes that may be taken by some serotypes suggestive of overwintering pathways. Please note that other species are affected; sheep were chosen as a representative species. This figure does not show mechanical, sexual, or direct transmission.


\textsuperscript{31} Wilson, A., et al. (2008). Where does bluetongue virus sleep in the winter. \textit{PLoS Biology, 6}(8), 1612–1617. \url{http://dx.doi.org/10.1371/journal.pbio.0060210}. 

SOP Manual

Bluetongue Etiology and Ecology
1.4.2.1 Incubation Period

The incubation period of BTV is approximately 5–10 days and may vary with the atmospheric temperature. Cattle with subclinical infections have been found to be viremic as early as 4 days after initial infection. It is suspected that during the BTV-8 European outbreak, cattle and sheep had similar incubation times consistent with normal BTV incubation.

1.4.3 Morbidity and Mortality

Morbidity and mortality vary widely by species (even by breed in sheep), serotype of virus, prior exposure, and uncharacterized environmental factors. Most species usually experience subclinical infections; ruminants, primarily sheep, do show clinical signs. Morbidity ranges from less than 5 percent to 50–75 percent or higher and mortality can reach up to 90 percent although generally less than 30 percent. Cattle and goats typically present with reduced morbidity and mortality is rare. In endemic regions, morbidity is as low as 1–2 percent or even absent.

1.4.3.1 Bluetongue Virus Serotype 8 Morbidity and Mortality

BTV-8, as seen during the 2006–2008 European outbreak, resulted in atypical morbidity and mortality. Cattle, normally asymptomatic, presented clinical signs including reproductive disorders. During 2007 in Germany, case fatality rates were calculated to be as high as 13 percent; however this was not indicative of the entire outbreak. Overall, it was reported that there was approximately a mortality rate of less than 1 percent mortality rate in Europe. On the other hand, wild whitetail deer and pronghorn antelope had morbidity rates as high as 100 percent and case fatality rates up to 80–90 percent. In Germany, one of the few countries where detailed data was available, the case fatality rate during the BTV-8 outbreak was 37 percent for sheep and 26 percent for goats.

1.4.4 General Clinical Signs

For most strains of the 27 serotypes known to cause disease, serious clinical signs are rarely seen outside of sheep and a few cervid species. The predominant mechanism of disease is vascular injury resulting in edema, hemorrhage, and ulceration of mucosa throughout the body. Infected

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animals, such as sheep and deer, can experience death within 8–10 days or have an extended recovery period with subsequent alopecia, sterility, and growth delay.\textsuperscript{41} In general, the severity of infection and clinical signs expressed are dependent on the serotype, which can result in rapid fatality to mild infection and quick recovery.

1.4.4.1 Sheep Clinical Signs

Sheep are the most susceptible to BTV infection, which may be subclinical or result in mild to severe clinical signs. Signs of illness can include fever, excessive salivation, depression; dyspnea and panting; nasal discharge that becomes mucopurulent and crusts around the nostrils; hyperemia, congestion, and edema of the head and facial tissues; and ulceration and necrosis of the oral mucosa. The coronary bands of the hooves may become hyperemic and can result in lameness due to the pain. Pregnant ewes infected with BTV may abort their fetuses or give birth to malformed lambs. Torticollis, pneumonia, emaciation, and conjunctivitis may be additional clinical signs.\textsuperscript{42}

1.4.4.2 Cattle and Goat Clinical Signs

Typically cattle and goats have subclinical infections, but in clinical cases, the presentation is similar to that in sheep. If present, signs of illness may include facial edema, oral inflammation with vesicles or ulcers in the mouth, excess salivation, nasal discharge, crusting around the muzzle, edema of the distal limbs, hyperemia of the coronary band with lameness, udder and teat lesions, decreased milk production, and abortions or births with Central Nervous System lesions resulting in “dummy” lambs. Specifically, cattle can have skin lesions varying in severity, including photodermatitis that leads to thickening, cracking, and sloughing.\textsuperscript{43}

1.4.4.3 Clinical Signs in Wildlife and Other Species

Species of cervids can present with clinical signs depending on the serotype; however, subclinical infections are most common. White-tailed deer tend to have the following signs in addition to those presented in sheep: severe fever, depression, anorexia, and loss of normal fear responses. Pronghorn may suddenly die or have prolonged sickness. There is little information on other cervid species.\textsuperscript{44}

There have been only a few incidents where carnivore species have been infected by BTV. They are suspected to remain predominately asymptomatic. Known infections resulted in clinical signs including, but not limited to, abortions, unspecified illness, and dyspnea.\textsuperscript{45}

1.4.5 Bluetongue Virus Serotype 8 Clinical Signs

Until 2006, infection of cattle was largely subclinical; clinical signs were believed to be linked to confounding factors. The 2006–2008 BTV-8 epidemic in Northern and Central Europe was


notable because cattle were more frequently infected than sheep, although it is unclear if this is attributed to herd populations or virulence. These cattle were considered a naïve population, which could have added to the multitude of factors that gave way to such a large and significant outbreak.

Signs of illness caused by BTV-8 in cattle are similar to those observed in sheep and cervids infected with various other serotypes. In both cattle and sheep, the type and frequency of signs that can be seen are highly variable, affecting every organ system. Commonly observed signs included anorexia, lethargy, ulcerated and necrotic muzzle tissue, rash on the muzzle, edema localized to the distal limbs, lesions on the udder skin, decreased milk production, and an increased rate of reproductive disorders (abortion, stillbirth, and congenital abnormalities). Cattle predominately experienced a reduced milk yield or reproductive disorders, whereas sheep experienced hyperthermia, in addition to general signs associated with BTV-8. The high rate of reproductive disorders observed is believed to be correlated with the virus’ affinity for trophoblast cells or its ability to cross the placenta.

1.4.6 Differential Diagnosis
Bluetongue presents with clinical signs similar to the following conditions:

- Contagious ecthyma
- Foot-and-mouth disease
- Vesicular stomatitis
- Malignant catarrhal fever
- Bovine virus diarrhea
- Infectious bovine rhinotracheitis
- Parainfluenza-3 infection
- Sheep pox
- Photosensitization
- Pneumonia
- Polyarthritis, footrot, foot abscesses
- Plant poisonings (photosensitization)
- Peste de petits ruminants

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• Coenurosis (*Oestrus ovis* infestation)
• Epizootic hemorrhagic disease of deer.

### 1.5 Environmental Persistence of Bluetongue Virus

To date, there is no evidence that BTV can persist in the environment, as it is unable to persist outside of the vector species or host species. If it is able to persist in the environment where, for example, protein is present, there is no evidence that this persistence has led to transmission or is epidemiologically relevant. Table 1 presents other environmental persistence characteristics of BTV.

#### Table 1. Resistance of Bluetongue Virus to Physical and Chemical Action

<table>
<thead>
<tr>
<th>Action</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature</td>
<td>Inactivated by 50°C/3 hours; 60°C/15 minutes</td>
</tr>
<tr>
<td>pH</td>
<td>Sensitive to pH &lt; 6.0 and &gt; 8.0</td>
</tr>
<tr>
<td>Chemicals/Disinfectants</td>
<td>Inactivated by sodium hypochlorite, 3% sodium hydroxide, 51 β-propiolactone, iodophores, and phenolic compounds</td>
</tr>
<tr>
<td>Survival</td>
<td>Very stable in the presence of protein (e.g., has survived for years in blood stored at 20°C). No evidence of persistence in the natural environment without a vector or host species.</td>
</tr>
</tbody>
</table>

### 1.6 Distribution

BTV has been found on every continent except Antarctica. Historically, the distribution has been throughout temperate and tropical climates according to the distribution of competent *Culicoides* species vectors, specifically between 40° North and 35° South. Distribution of different BTV serotypes are typically determined by the appropriate species of *Culicoides* and its habitat. There are approximately 1,400 species of *Culicoides* throughout the world; only about 30 have been shown to be competent biological vectors.52

Figure 2 illustrates the relationship between the major *Culicoides* vectors, their predominant geographic location, and the presence of BTV serotypes (indicated within the black boxes).

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1.6.1 European Distribution and Significance

In recent years, bluetongue outbreaks have occurred in North and Central Europe (Figure 2), regions of Asia, and Western North America as far as 50° North. These areas were previously believed to be not at risk for bluetongue. The means of introduction into Northern Europe has not been conclusively determined, although it is believed that climate change factored into BTV expanding beyond its traditional boundaries.54

Research has implicated the *C. obsoletus* complex, including *C. dewulifi*, as competent vectors for transmission of BTV-8. However, the recent migration of *C. imicola* into the Mediterranean region, historically only a resident of Africa, tested positive for BTV-8, attributing to serotype 8 transmission.55 The introduction of BTV-8 into Europe has resulted in significant economic losses due to animal morbidity/mortality and trade restrictions.

The distribution of serotypes 1, 2, 4, 8, and 16 are currently found within Europe. Interestingly, cases of BTV-8 in France, along with other present serotypes, unexpectedly (based on known transmission information) continued through cold winter temperatures. At this point, the cause of these continued detections is unclear: it is possible that diagnostic testing may be detecting RNA, but not infectious virus, it could signify new methods of transmission, or overwintering may be occurring. Further research is necessary.

### 1.6.2 Distribution in North America

In North America, serotypes 10, 11, 13, and 17 are endemic, coincident with *C. sonorensis* distribution. Serotype 2, also endemic, previously was restricted to the southeastern United States, corresponding to the distribution of *C. insignis*; however, recently it was detected in California indicating translocation and reassortment due to unknown viral spread. Since 1999, isolates of 11 previously unrecognized non-endemic serotypes (1, 3, 5, 6, 9, 12, 14, 18, 19, 22, and 24) have also been found in the southeastern United States—concentrating mostly in Florida. Bluetongue is seasonally absent in the central and northwestern States; the northern and northeastern regions are BTV free.

Figure 3 was created by the National Veterinary Services Laboratory (NVSL) to illustrate detections of BTV serotypes within the United States between 1992 and 2015; therefore, this map does not reflect any type of national surveillance data.

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1.7 Vaccines

Effective BTV vaccine development is largely a challenge; however, there are a limited number of modified live attenuated vaccines available for use in the United States. The only nationally approved and available vaccine is for BTV-10 produced by the Colorado Serum Company. In addition, Poultry Health Laboratories produced modified live vaccines for BTV-10, -11, and -17 for approved use in healthy California sheep. The use of attenuated vaccines pose significant health risks to livestock that include reduced milk production, abortion, and teratogenesis. Furthermore, midges feeding on attenuated vaccinate animals can become infected and disseminate the virus where reassortment can occur with wild-type BTV in the environment resulting in novel progeny.

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Inactivated vaccines are not available in the United States, although considered a safer alternative than attenuated vaccines. During the incursion of BTV-8 in Europe, inactivated BTV-1, and -8 vaccines were available in two doses, but not until 2008—2 years after the outbreak began. These inactivated vaccines are no longer available for use.63

Currently, both inactivated and live-attenuated BTV vaccines do not have the capability to differentiate infected and vaccinated animals (also known as the DIVA strategy); however, virus-like particle vaccines could provide an alternative as research progresses.64

1.8 Risk of BTV-8 Introduction to the United States

In the United States, there are now a total of 16 BTV serotypes that have been isolated—starting as early as 1950, but mostly detected after 1998. Incursive serotypes are continually pressing the U.S. southern border. It is believed that the majority of these serotypes originated in the Caribbean and Central America, brought in by Culicoides spp. that were carried by prevailing winds or by large weather events such as tropical storms and hurricanes. BTV-8 has not been detected in the United States, but it has been identified in Central America.65 The initial introduction of BTV-8 into Northern and Central Europe in 2006 was also believed to be through wind-borne insects, with subsequent transmission by local insects. This recent expansion of BTV-8 and the movement of the C. imicola vector from Africa into Europe clearly indicates that vector patterns and host-ranges are rapidly changing: BTV incursions into the United States are possible, including BTV-8, particularly given its presence in Central America.

Once a novel BTV serotype or BTV-8 enters the United States, there are also significant challenges: there are both competent vectors that can transmit the virus and significant populations of wild cervids which could be a potential virus reservoir. There are 1,357 species of Culicoides midges66 and 110 species of Culicoides found in North America.67 While the exact number of competent vectors for BTV-8 in the United States is unknown, it is clear that there is the potential that at least one or more Culicoid vectors could transmit BTV-8 (or another novel serotype) if it was introduced. In addition, USDA ARS, Arthropod-Borne Animal Diseases Research Unit, and the USDA APHIS National Wildlife Research Center have conducted experiments on U.S. white-tailed deer—excellent sentinel animals for Orbivirus activity68—with European BTV-8. When experimentally infected, white-tailed deer developed clinical signs and

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exhibited viremia for as long as 28 days. These results suggest that white-tailed deer may exhibit disease and could pose as reservoirs for BTV-8 in the United States.

As with any emerging disease or re-emerging disease, it is difficult to predict transmission pathways and epidemiological patterns in advance; more field research is needed on initial introduction pathways, vector competence, transmission, disease incidence, and carriers. However, the recent BTV-8 experience in Europe clearly demonstrates the impact bluetongue disease can have on animal agriculture. Particularly for BTV-8 and any novel serotype that affects cattle as well as other ruminants, the economic impact could be serious: for example, the U.S. beef industry had a retail equivalent value of $95 billion in 2014. Trade restrictions, lost production, and costs from controlling and containing the disease may all contribute to negative economic consequences and give reason to maintain vigilance against BTV-8 and novel BTV serotypes in the United States.

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Attachment 1.A References and Resources


## Attachment 1.B Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>APHIS</td>
<td>Animal and Plant Health Inspection Service</td>
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<td>ARS</td>
<td>Agricultural Research Service</td>
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<tr>
<td>BTV</td>
<td>Bluetongue virus</td>
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<tr>
<td>BTV-8</td>
<td>Bluetongue virus serotype 8</td>
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<tr>
<td>CFSPH</td>
<td>Center for Food Security and Public Health</td>
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<tr>
<td>EHDV</td>
<td>Epizootic Hemorrhagic Disease virus</td>
</tr>
<tr>
<td>ERS</td>
<td>Economic Research Service</td>
</tr>
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<td>FAD PReP</td>
<td>Foreign Animal Disease Preparedness and Response Plan</td>
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<td>ICTV</td>
<td>International Committee on Taxonomy of Viruses</td>
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<td>NVSL</td>
<td>National Veterinary Services Laboratory</td>
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<tr>
<td>OIE</td>
<td>World Organization for Animal Health</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<td>quantitative real-time reverse transcription PCR</td>
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<td>University of Florida, Institute of Food and Agricultural Sciences</td>
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