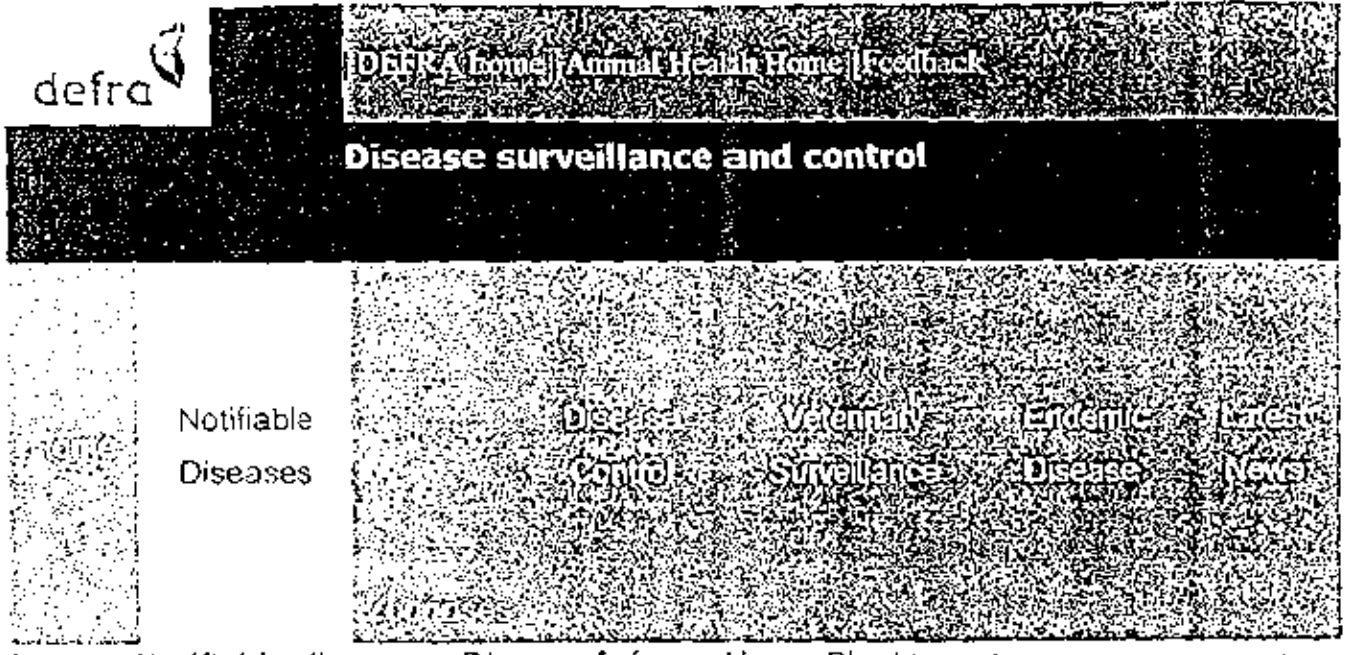
Ex. 54



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Technical review - Bluetongue

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Technical Review - Bluetongue : The Virus, Hosts and Vectors

1. Introduction

1.1 Bluetongue (BT) is an infectious, non-contagious, insect-borne virus disease of ruminants of variable clinical severity, characterised by inflammation of mucous membranes, widespread haemorrhages and oedema. Twenty-four serotypes of bluetongue virus (BTV) have been identified worldwide.

1.2 BT occurs as a clinical disease of small ruminants in many countries of Africa, the Middle East, the Indian subcontinent, China, the United States, Mexico and in recent years in the Mediterranean Basin. BTV is also present in Southeast Asia, northern Australia, Papua New Guinea and northern South America, normally without associated clinical disease.

1.3 The virus was thought to be confined to Africa but in the past 50 years BTV has increasingly been recognised wherever substantial populations of ruminants occur in the tropics and subtropics. The initial detection of virus in countries outside Africa has sometimes occurred because of spectacular outbreaks of disease. Recent outbreaks of BT in the Mediterranean Basin have followed this pattern with severe losses in sheep. It appears that at least some serotypes of BTV may now be enzootic in parts of south-eastern Europe.

1.4 BT has never occurred in Great Britain.

1.5 BTV is limited to those geographical areas where competent *Culicoides* vectors are present. Its transmission is limited to those times of the year when the climatic conditions are favourable for adult vector activity. Peak populations of vector *Culicoides* occur in the late summer and autumn and therefore this is the time when BT is most commonly seen.

1.6 Serotypes that most directly threaten the United Kingdom are BTV serotype 2 from North Africa and BTV serotypes 1, 4, 9, 16 from eastern Turkey and Mesopotamia via western Turkey, Greece, Italy and the Balkan states. It is likely that some of these serotypes are now enzootic in south-eastern Europe.

1.7 BTV 10 is also present in West Africa, and could be introduced through North Africa and the Mediterranean, similar to BTV 2.

1.8 It is not possible to be definitive about the threat, as there is insufficient detailed knowledge about the precise environmental requirements of the virus or of its vectors, particularly in the more northerly areas of Europe.

1.9 BTV does not affect or infect humans, so this disease has no public health significance.

2. The Virus

2.1 BTV belongs to the *Orbivirus* genus of the Reoviridae family. Thus far 24 serotypes are recognised. There are numerous strains - each isolate is a different strain based on molecular analysis, regardless of serotype. The virulence of BTV strains varies considerably. However, other factors also influence the severity of the disease in sheep, including breed, age, exposure of animals to sunlight, walking on rough ground and stress.

2.5 The serotypes are differentiated by serum neutralisation tests, but there are cross-reactions between some serotypes. All BTV's share group antigens, which can be demonstrated by agar gel diffusion tests, fluorescent antibody tests and the group reactive ELISA.

2.6 Several other Orbiviruses have been loosely termed 'bluetongue-related' viruses because of serological and other relationships to BTV. The only such viruses known to be pathogenic for livestock are some members of the epizootic haemorrhagic disease of deer (EHD) serogroup and the Palyam serogroup of Reoviridae.

3. Vectors

3.1 Vector species of *Culicoides* biting midge tend to breed in damp or wet soil enriched with fresh or composted dung and blood-feed opportunistically on large vertebrate hosts. Since appropriate breeding sites are very common around livestock holdings *Culicoides* are particularly abundant at such sites and therefore feed predominantly upon domestic livestock (cattle, horses, sheep). They rapidly become much less abundant as distances from livestock holdings increase. *Culicoides* tend to be most active from about 1 hour before sunset until 1 hour after sunrise. They are most active in the evening until about midnight, then ease off with another peak of activity around sunrise. However, on dull days or in shady areas vectors may be active during the day. On windy days, they tend to be less active. Measures to protect susceptible animals from infection should particularly target these active periods. Most *Culicoides* species, including the British species, are averse to entering confined spaces such as buildings or vehicles, although small numbers might be brought inside while biting a host and/or while host seeking.

3.2 *Culicoides* species have a normal insect complete metamorphosis life cycle – egg, four larval instars, pupa and adult. In temperate and cool regions they "overwinter" at the fourth larval instar stage. Some species enter diapause when the number of daylight hours declines below a threshold level but others are more influenced by temperature which affects their activity levels. The adult populations in Britain tend to fall dramatically from mid to late October. From December adults are usually either not at all detectable or only in very small numbers, depending upon the prevailing temperature, until April-May. These periods may be even longer in northern Britain. The life span of adults is usually about 10 days, but in cooler conditions their metabolism slows and they may survive for periods of more than a month.

Most species require a blood meal before laying eggs, although *C. impunctatus* (the Scottish highland biting midge), a member of the *C. pulicaris* group, does not require a blood meal for maturation of the first egg batch. This enables this midge to persist in areas with few mammalian hosts.

3.3 Different *Culicoides* species have different preferences for breeding sites ranging from damp dung, damp soil to tree holes, streamsides and the edges of ponds. The *C. obsoletus* group breeds in damp soil and composted organic material such as old manure heaps common around stables and animal housing. The *C. pulicaris* group prefers to breed in wet soil, sphagnum marsh and bogs.

3.4 *Culicoides imicola* is the major vector of BTV in the Old World. It is one of the most widely distributed of *Culicoides* species. It occurs throughout most of Africa, the Middle East, southern Asia, much of Portugal, south-west Spain and the Balearics, many Greek Islands, substantial parts of the Greek mainland, Corsica, Sardinia, Sicily and wide areas of southern and central mainland Italy.

C. imicola appears to be expanding its range both northwards and westwards but is still restricted in Europe to southern parts. *C. imicola* has not been recorded in Great Britain.

3.5 *Culicoides obsoletus* is probably one of the commonest *Culicoides* species across the whole of central and northern Europe. Similarly, *C. pulicaris* is also common throughout central and northern Europe. Both of these species are widespread throughout most of the British Isles.

3.6 In practice, the usual reference to *C. obsoletus* really relates to a complex of closely related species (*C. obsoletus, C. dewulfi, C. scoticus, C. chiopterus, C. montanus*) the females of which are difficult or impossible to separate. In Bulgaria, *C. obsoletus* and *C. scoticus*, at least, co-exist. Since it is the females that are the vectors of BTV, it is not always possible to determine the identity of the vector when undertaking virus isolation from midges. In the UK, *C. obsoletus, C. dewulfi, C. scoticus and C. chiopterus* occur. These *C. obsoletus* group species belong to a larger grouping (subgenus Avaritia) that includes *C. imicola* (the major European and African BTV vector) and *C. brevitarsis* (the major Australian BTV vector). A similar taxonomic situation exists with *C. pulicaris* which is a complex of morphologically similar species, eight of which occur in the UK and with *C. nubeculosus*, a European species which is closely related to the North American BTV vector *C. sonorensis* (= *C. variipennis*).

3.7 The distributions of *C. obsoletus* and *C. pulicaris* group midges in UK are not well understood. Observations of both have been made in many parts of the British Isles. When observations have not been recorded in certain areas it usually means that efforts have not been made to collect rather than the species is absent there. Generally, the insects congregate where there are breeding sites and hosts upon which to feed.

Thus, the highest concentrations of *C. obsoletus* and/or *C. pulicaris* group midges are found where cattle, horses, pigs and, to a lesser extent, sheep populations are highest. If domestic animals are removed from a site, over several months the midge population reduces significantly, by a factor of ten to twenty times, but will usually persist at the lower level if other ecological factors are favourable, by feeding on wild hosts and/or humans.Vector numbers are likely to be low in hill sites where sheep are at low densities and where the climatic conditions are likely to be more extreme.

3.8 Studies of *Culicoides spp.* in Britain are being expanded under a DEFRA-funded project out of the Institute of Animal Health, Pirbright. Monitoring is being expanded to twenty-five or more sites, one of the aims being to determine the species list, species distribution, seasonal incidence and vector competency of the various species.

Vector competency

3.9 The *C. obsoletus* group has long been suspected of being a vector, mainly on the basis of BTV isolations from this species made in Cyprus, and African horse sickness virus (AHSV) isolations made from mixed pools of *C. obsoletus* and *C. pulicaris* in Spain. In this context it should be borne in mind that BTV and AHSV tend to utilise the same *Culicoides* species as vectors.

3.10 It is strongly suspected that *C. obsoletus* and/or *C. pulicaris* group midges acted as BTV vectors in northern Greece and southern Bulgaria during the 1999 BTV epizootic, as they were by far the most abundant and most prevalent detected. It is similarly suspected that these species may also have mediated the BT outbreaks in Serbia, western and southern Bulgaria, FYR Macedonia, Croatia and Bosnia during the period 2001-2002. *C. imicola* has not been recorded in these regions.

3.11 Vector competence studies on a British population of *C. obsoletus* have recorded oral susceptibility rates of less than 2% in comparison with a known major vector *C. sonorensis* (19.5%). This initially suggested that *C. obsoletus* is likely to be only a minor or inefficient vector of BTV. Nevertheless, the high abundance and survival rates of *C. obsoletus* as exhibited in Bulgaria in 1999, and as seen on farms and around stables in South East England, could compensate for its low levels of vector competence. Observations of cattle exposed to midges have shown up to ten thousand bites per hour. It should be noted that *C. brevitarsis*, the major vector of BTV in Australia, has an experimental competency of only 0.3 percent when feeding on sheep although it is quite an effective vector in the field.

3.12 Vector competence for a particular virus is a hereditary trait and populations of a vector species with high, low or intermediate levels of competence can be derived by selective breeding.

Potential impact of global warming

3.13 Vector competence of *Culicoides* vectors for Orbiviruses is partly influenced by temperature. Orbivirus development in *Culicoides* vectors is unable to occur at temperatures below about 10°C to 15°C depending on the Orbivirus species and serotype. Furthermore, there needs to be a minimum amount of time at suitable temperatures (expressed as "day degrees or hour degrees") for completion of the development cycle in the *Culicoides* vector before virus transmission can occur. This "physiological" time is the cumulative product of virus development time multiplied by the temperature in degrees above the threshold for virus replication. Increasing environmental temperature (climate change) will also extend the vector season. Combined, these conditions may result in Orbivirus development within *Culicoides* being able to take place over a greater proportion of the year and over a wider geographical area. In addition, within the range of temperatures over which Orbivirus development can occur, the levels of vector competence of a *Culicoides* vector population for some Orbivirus serotypes increases linearly with temperature and so the impact of warmer temperatures may be even greater.

3.14 Temperature can also affect the competence of 'non-vector' *Culicoides* species. For example, *C. nubeculosus* generally is considered to be incapable of transmitting BTV due to a midgut infection barrier. However, exposure of the immatures to rearing temperatures close to their upper lethal limit (33-35°C) can result in >10% of adults becoming competent to transmit BTV. It is likely that the integrity of the gut wall of some adults is damaged by the extreme rearing temperatures, thereby allowing virus particles to bypass the midgut barriers, enter the haemocoel and develop as in a normal vector. The increase in frequency and intensity of extremely warm days predicted to occur with climate change will enhance the chances of this phenomenon occurring in non-vector *Culicoides* species and hence could increase the number of BTV competent adults within populations.

3.15 The vectorial capacity of a *Culicoides* population (and hence the potential for virus transmission) is affected by (a) the number of adult midges in the population and (b) the proportion of adults capable of transmitting the virus, and is greatest when these factors are at a peak.

3.16 Within favourable limits, the development rate of *Culicoides* from egg to adult is directly related to temperature. Thus increasing temperatures coupled with an extension in the developmental season may result in a greater number of generations (and therefore adults) per year. In addition, the overwintering ability of adult *Culicoides* is likely to improve, as winters become both warmer and shorter. Improved overwintering success is also likely to increase the spring population input, which in turn could result in even larger populations during the summer

3.17 The proportion of adult *Culicoides* capable of transmitting virus is dependent on (a) vector competence (the capacity for the virus to develop in and be transmitted by the vector), (b) adult survival, (c) the blood-feeding interval and (d) the extrinsic incubation period (EIP; development time of the virus in the vector). In order to transmit virus *Culicoides* must not only be vector competent, but also survive long enough to blood-feed after the completion of the viral EIP. *Culicoides* vectors are more likely to satisfy these criteria at high temperatures (e.g. 27-30°C), because, although adult survival is reduced at high temperatures, this is more than compensated for by the accompanying decrease in duration of the EIP and blood-feeding interval. Consequently, it is likely that warmer temperatures as a result of climate change will increase the likelihood that *Culicoides* will survive long enough to transmit virus.

3.18 Changes in weather (temperature, precipitation, humidity and wind) and climate from global warming could produce both wider distribution of vectors towards the poles or upwards in elevation and increased vectorial capacity (the ability of a vector population to transmit virus to a vertebrate population) of *Culicoides* vector populations, resulting in increased prevalence of BTV in Europe. The present BT outbreak in the Mediterranean Basin is already the most serious epizootic on record.

3.19 An expansion in the range of *C. imicola* will increase the areas of Europe at risk from BTV. Also, the extended distribution of *C. imicola* could bring BTV into the range of *C. obsoletus* group and *C. pulicaris* group midges much more frequently and this could result in even greater areas of Europe being affected by BTV.

3.20 The impact of climate change on the vectorial capacity of *Culicoides* populations will have three main effects on BTV transmission in the Mediterranean basin:

- the greater abundance of adult *Culicoides* combined with the increased proportion of adults capable of transmitting the virus will increase the likelihood and severity of an epizootic, following the introduction of BTV into an area. The greatest risk will be at times of the year when temperatures reach approximately 25-30°C (i.e. when conditions are optimal for *Culicoides* development and virus transmission
- as temperatures will be conducive for both viral and *Culicoides* development for a greater proportion of the year, the length of the viral transmission season will increase.
- the enhanced overwintering success of adult *Culicoides* combined with the extension in the *Culicoides* development season will prolong the seasonal occurrence of adult midges and hence improve the overwintering chances of BTV.

3.21. Studies are needed to correlate the day degrees required for BTV development in the potential vectors against British climate data to establish the risk of establishment of a BTV infection under present climatic conditions and with global warming.

Vector monitoring

3.22 Collections should aim to give:

- A list of all the potential vectors present;
- The relative abundance of those species
- The age structure of those populations, and
- The seasonal incidence of each species.

3.23 The most commonly used traps for collecting biting midges are light traps. Carbon dioxide and octenol may be useful additional attractants for biting midges when used in conjunction with light traps.

A vehicle-mounted trap, sometimes called a truck trap, is particularly useful where evening temperatures are low enough to reduce insect activity before it is sufficiently dark for light traps to become attractive.

3.24 Studies are also needed to determine the correlation between *Culicoides* light trap collection data and biting rates on animals so that these data can be used to accurately estimate biting rates.

3.25 Larval sampling is considerably more time-consuming than adult sampling, and may not be as reliable an indicator of presence or prevalence as adult trapping.

3.26 If adult *Culicoides* collections are to be processed for virus isolation, insects will need to be collected live for immediate processing, or holding in suitable storage such as liquid nitrogen or at temperatures of <-70°C. Collections for population analysis should be stored in 70% ethanol. The technology that would allow detection of virus from insects preserved in alcohol (polymerase chain reaction) is currently being refined. Virus isolation from vectors is not a recommended method for BTV monitoring and surveillance.

4. Hosts

4.1 All ruminants, including sheep, goats, cattle, buffaloes, camels, antelopes and deer, are susceptible to BTV infection. Of the domestic species, sheep are clinically the most severely affected. Sickness is sometimes reported in goats and severe disease and mortalities occur in white-tailed deer in the United States. Although the infection of cattle is of great epidemiological significance, it is generally sub-clinical. Horses and pigs are not infected by BTV but *Culicoides* may feed upon them and the premises where they are kept may provide suitable vector breeding sites.

4.2 After infection via the saliva of a biting midge, BTV multiplies in the regional lymph nodes and then spreads in the blood. This systemic multiplication and spread allows ample opportunity for humoral and cell-mediated immune responses to develop. As BTV is associated with the cellular fraction of the blood where it is protected from the effects of humoral antibody, extended viraemia may occur and virus and antibody may circulate in the system at the same time.

4.3 The duration of viraemia (i.e. time over which a vector can be infected) depends on several factors, including the strain of the virus and the longevity of the mammalian host's cells with which virus is associated. The sensitivity of the system used to detect the virus also influences the period viraemia is detectable. Although virus may be detected in the blood of cattle in the experimental situation for several months (and in sheep for several weeks), infected animals usually only transmit virus to a competent biting vector for several weeks after infection. Some authorities now consider that the maximum duration of effective viraemia is about 50 days in cattle and 20 days in sheep, although most animals are infectious to vectors for a much shorter period. However, other authorities consider that the period of effective viraemia may be longer for both species and no consensus has yet been arrived at. The OIE International Animal Health Code specifies an infective period of 100 days. The OIE infective period should be used for contingency planning while being mindful of the shorter periods of high risk of viraemia.

4.4 Sheep indigenous to tropical countries in Africa, the Middle East, Asia and the New World can be infected with BTV, but do not usually exhibit disease. The lambs of immune ewes of susceptible breeds will be partially protected by colostral immunoglobulins when challenged several weeks after birth. This protection is short-lived, is serotype specific, and may be dependent on the amount of colostral IgG transferred. It appears to have little value as a disease control mechanism.

4.5 Systemic antibody is first detected around 1–2 weeks after infection and humoral immunity. Detectable antibody is considered to be life long and the most important protective mechanism against reinfection. After a single infection, group and type-specific antibodies can be detected. Neutralising antibodies are usually monotypic, although cross-reactions have been noted.

Consecutive infections with a second and especially a third serotype frequently give rise to a comparatively short-lived, broad-reacting neutralising antibody response, besides the long-lasting monotypic responses.

4.6 The immune response can sometimes be harmful. For example, the occasional disease in cattle is thought to be allergic, and although dual (concurrent) infections of sheep are uncommon, when they do occur the disease can be unusually severe, possibly via an antibody-dependent enhancement mechanism.

4.7 The virus is usually present in highest concentration in the blood of sheep during the early stage of the fever. Viraemia persists after the temperature subsides, but at a lower concentration.

4.8 There has been recrudescence of clinical BT on a number of occasions in geographical locations which cannot be explained by the normal "overwintering" processes of uninterrupted cycling between vector and host or by re-introduction of BTV from an endemic area by infected animals or vectors. Recent research at IAH-Pirbright provides experimental evidence for the existence of a novel overwintering mechanism in the host. The research demonstrates that BTV can persistently infect ovine γδ T-cells (gamma delta T-cells) and that interaction of these cells with skin fibroblasts can induce conversion of this persistent infection to a lytic infection with increased virus release. Furthermore, feeding of *Culicoides* midges has been shown to stimulate an inflammatory reaction causing recruitment of lymphocytes, including infected ovine γδ T-cells, to the bite site in the skin. Thus, latent, overwintering BTV infections may be re-activated as a result of the migration of infected γδ T-cells into the skin in response to the bites of a new generation of midges.

4.9 Infection of γδ T-cells occurs early in BTV infection of the host, before antibody production. However, infected γδ T-cells have been identified several weeks after overt viraemia was no longer detectable. Sheep or cattle with these silent infections would not be viraemic but would be antibody positive. Currently such animals are considered neither infectious nor infected.

4.10 This mechanism has not yet been fully proven. If it does occur in the field, the amount of BTV released from the infected γδ T-cells is likely to be very low and high numbers of *Culicoides* midges would be required to re-establish the infection cycle. It may be an uncommon occurrence but would explain otherwise inexplicable overwintering observations.

4.11 Virus rarely may be excreted in the semen when males are viraemic. Excretion is more likely if there is inflammation of the genital tract, if the animal is aged or if the virus has been laboratory adapted (as in live vaccines or experimental infection). Contaminated semen may infect recipient cows, but these will not initiate a cycle of transmission unless competent insect vectors are abundant. Infection of other ruminant species presumably occurs under similar circumstances.

4.12 There is much evidence from *in vitro* and *in vivo* work that embryos from infected donors washed to International Embryo Transfer Society (IETS) protocols do not transmit the virus.

4.13 Antibodies have been detected in wild carnivores in Africa. Cross-contamination of canine vaccines with BTV during manufacture has resulted in the death of some vaccinated dogs in the United States.

5. Epidemiology

5.1 BTV is non-contagious. BTV is transmitted biologically by *Culicoides* insects (biting midges), but only a limited number of species are efficient vectors. Cattle are the main amplifying hosts for BTV. They are also probably important maintenance hosts. The competent *Culicoides* vector species feed more abundantly on cattle.

5.2 BTV is limited to those geographical areas where competent *Culicoides* vectors are present. Its transmission is limited to those times of the year when the climatic conditions are favourable for adult vector activity. Peak populations of vector *Culicoides* occur in the late summer and autumn and therefore this is the time when BT is most commonly seen.

5.3 Persistence of BTV within a particular geographical area does not mean "static". Once a vertebrate host is infected with BTV it either dies or mounts an enduring antibody response and so becomes resistant to further infection. This means that within any small geographical area (a farm or village) most or all of the initially susceptible hosts are likely to be infected and thus become "unavailable" to the virus within a fairly short space of time. BTV can only survive under such constraints by continually moving to new locations occupied by naïve vertebrate hosts. These movements are via the agency of viraemic hosts or infected vectors. BTV is therefore a peripatetic virus and even within its enzootic zones its activity may be envisaged as a pattern of endlessly shifting viral "hot spots".

5.4 The incidence and geographical distribution of BTV infections are determined largely by the distribution of insect vectors and this can vary from year to year. Infection in sheep will usually be preceded by widespread infection of cattle and an increase in vector density.

5.5 Where annual bouts of BT occur, they may represent new introductions (from adjacent infected areas) or may be the visible evidence of low-level persistence from year to year. Annual re-introduction is possible if enzootic foci of the virus are geographically close by, as infected *Culicoides* can reputedly be transported on the wind over distances of 100 kilometres or more. *Culicoides* do not normally fly far if there is a source of food (large mammals) and breeding sites.
Since BTV is not transmitted transovarially through its vectors (and is rarely transmitted directly from vertebrate to vertebrate) long term persistence (i.e. an enzootic zone) is currently thought to be possible only in areas where active adult vectors are present throughout the year. In such situations if vector-free periods do occur then they must be of shorter duration than the maximum period of viraemia in the local susceptible vertebrate population (up to about 50 days in sheep and 100 days in cattle). Otherwise the last infected vertebrate host will have died or recovered before new vectors arrive on the scene.

5.6 In summary, the major considerations when considering the development of BTV control strategy include:

- Vectors competent to transmit the virus are present but are more likely to feed on large mammals (cattle or horses) than sheep
- The most effective vector in southern Europe, *Culicoides imicola*, is not present in Great Britain, but its distribution seems to be expanding north and westwards from the Mediterranean and areas of southern France may become suitable for *C. imicola* to establish
- Cattle have an important epidemiological role as primary and amplifying hosts, and as ongoing sources of infection for vectors
- Animal carcasses and products such as meat, milk and wool are not a method of spread
- The disease in sheep is most likely to occur in late summer or autumn, following build-up of vector numbers and virus in cattle populations and an expansion in infected vector distribution, and
- Infected vectors may possibly be dispersed by winds from an outbreak in France.

5.7 The risk of establishment of BTV infection in particular areas of Britain will be influenced by

- The population density of animals, particularly cattle
- The level of susceptibility of the animal population to BTV infection
- The abundance of local competent *Culicoides* vectors.

5.8 Data indicates the highest risk of infection will be in cattle and mixed cattle-sheep areas. Seroconversion rate observations suggest infection risks could be in the order of:

- Cattle 80%
- Sheep near cattle 20-40%
- Sheep away from cattle 10-20%
- Sheep remote from cattle <10%.

5.9 It is unlikely that BTV would establish on hill sites due to the absence of cattle, low density of sheep and more severe climate that would result in a relatively low vector population. The low levels of vector competency considered to apply in the UK would militate against establishment of an infection cycle in such situations.

6. The Disease

Clinical signs

6.1 BT is primarily a disease of **sheep** but when these animals have positive BTV serology, care must be taken to avoid confusing clinical BT and diseases with similar clinical signs.

6.2 The clinical signs in sheep can be very variable, ranging from acute to sub-clinical. The acute signs begin with fever, which may last about a week. The incubation period, generally 4–8 days, is possibly influenced by the dose of virus received. Within 24–36 hours of the onset of fever the lining of the mouth and nose becomes hyperaemic. Excess salivation and a clear nasal discharge accompany this. Over the next few days the discharge becomes thick with mucus and pus and may be blood stained. It eventually dries to form a crust around the nostrils.
In acute cases, the lips and tongue become very swollen and oedema may extend over the face to include the ears and intermandibular space. The hyperaemia becomes more intense and tiny, flat, red or purple (petechial) haemorrhages appear on the mouth, nose and conjunctival linings. The clinical feature that gives the disease its name, a deeply cyanotic (blue) tongue, occurs in only a small percentage of cases.
Necrotic lesions develop on the gums, cheeks and tongue 5–8 days after the onset of fever. These heal slowly under a membrane of pus and serum (diphtheritic membrane). Breathing becomes difficult. Profuse bloody diarrhoea occurs in some cases. Vomiting may also occur and lead to inhalation pneumonia.
Foot lesions, on one to four feet, may appear towards the end of the fever period. There is acute reddening and petechial haemorrhages on the coronary band at the top of the hoof. Affected sheep stand with arched backs and are reluctant to move.
There is rapid weight loss and weakness due to loss of appetite and specific muscular necrosis. Spasmodic twisting of the head and neck to one side (torticollis) is sometimes a late sign.

6.3 The mortality rate is variable: in highly susceptible sheep it can be up to 70%. Deaths may occur at any stage up to a month or more after the onset of signs. Convalescence in surviving sheep is prolonged. Breaks occur in wool, which add to the production losses.

6.4 Infection of pregnant ewes may lead to abortions, mummified foetuses, or the birth of stillborn or weak lambs, which may have congenital defects.

6.5 **Goats** are less commonly, and less severely, affected than sheep. The pathogenesis is similar and the clinical signs are milder.

6.6 Infection in **cattle**, although of great epidemiological significance, is generally sub-clinical. A report from the United States suggested only 0.01% of cattle infected with BTV show clinical signs. These include inflammation and mucosal erosions in the mouth and nose, mild laminitis and a stiff gait. Infection of early pregnant animals may lead to embryonic death and resorption.

6.7 Severe disease and mortalities occur in white-tailed **deer** in the United States where the pathogenesis and clinical signs are indistinguishable from the closely related EHD virus.

6.8 Other species of farmed or wild feral deer may have BTV antibodies, but usually no disease is observed.

Pathology

Gross lesions

6.9 In sheep the basic pathological process is endothelial damage. Haemorrhages, 2–15 mm in diameter, in the tunica media at the base of the pulmonary artery are regarded as being characteristic of BT. The most prominent gross lesions in the gastrointestinal tract are found in and around the mouth. There is oedema and hyperaemia in the mucosa, which is occasionally cyanotic. Petechial or ecchymotic haemorrhages may also be present. Abrasions, which may be covered by grey necrotic material, are found on the lips, dental pad, tongue and cheeks. Hyperaemia of the ruminal pillars and reticular folds is common.
The lymph nodes and spleen are moderately enlarged and haemorrhagic. Pale areas of necrosis are scattered through the skeletal musculature. There is inflammation of the upper respiratory tract causing excess mucus secretion (catarrhal inflammation), and oedema of the lungs may result from damaged alveolar epithelium.

Microscopic lesions (histopathology)

6.10 Histologically, there is damage to the endothelium of small blood vessels. This results in vascular occlusion and clotting. In epithelial tissues this leads to lack of oxygen and sloughing of the epithelium.

Experimental Australian cases exhibited haemorrhages, inflammatory mononuclear cell infiltrations and necrosis of the heart muscle (myocardium).

Laboratory tests

6.11 Animal specimens must be sent direct to the Institute for Animal Health, Pirbright Laboratory (Pirbright) for testing.

Specimens required

6.12 It is essential for the diagnosis of BT that the most appropriate specimens are carefully collected and properly transported. The following specimens are required for the diagnosis of BT.

- Two 10 mL samples of blood from the jugular vein of each of up to six sheep with high (in excess of 40.5°C) temperatures.
  - One 10 mL of blood is run into a sterile bottle and allowed to clot to provide a serum sample for the antibody test

  - A second 10 mL is added to an anticoagulant, in vacutainers or commercially prepared disposable tubes. EDTA is the anticoagulant of choice if blood is to be tested by PCR, but heparin is suitable for most purposes.
- Sera from 10–15 convalescent sheep (if there are any). If no convalescent sheep are present, sera should be collected from in-contact sheep.
- Sera from in-contact cattle, ideally yearlings, and from other ruminants.
- Spleen and lymph nodes from all postmortem cases.
- Cardiac and skeletal muscle (especially if abnormal) in formol saline.

Transport of specimens

6.13 Sera may be transported frozen at –20°C. Other specimens should be submitted on wet ice and MUST NOT BE FROZEN. If ice blocks are used, extreme care should be taken to ensure specimens do not contact the blocks. Direct contact with ice causes freezing which lyses the blood cells thereby releasing, the predominantly cell-associated virus that will be inactivated if antibody is present. Whole blood should be held at 4°C.

6.14 A full history and identification of samples is necessary.

Laboratory diagnosis

6.15 Diagnostic tests currently available at Pirbright, what each detects and the time required to obtain the results are shown in Table 1, below:

Table 1 Diagnostic tests currently available at Pirbright for bluetongue

Test	Specimen required	Test detects	Optimum Time to obtain result
Virus isolation	whole EDTA blood	virus	1–3 weeks
Antigen detection			
Sandwich ELISA	whole heparin/EDTA blood or tissues	Antigen - group specific	4 hours - tissues 5-14 days - blood
Polymerase chain reaction (in development)	whole EDTA blood, or tissues	Viral RNA – group specific	2 days
		Viral RNA - topotype	14-21 days
Serum neutralisation	whole heparin/EDTA blood or tissues	serotype	2-4 weeks
Antibody detection			
Competition ELISA	serum	Antibody - group specific	3 hours
Serum neutralisation	serum	Antibody - serotype specific	2-4 weeks
Pathogenicity testing in sheep	virus isolate	virulence	2 weeks

Serological examination should be capable of providing results in 24–48 hours. PCR, if available, should have a result in 48 hours.
Virus isolation and serum neutralisation tests will provide "typing" results in 2–4 weeks.

Differential diagnosis

6.16 The following sheep diseases must be considered in the differential diagnosis for BT:

- Scabby mouth (contagious pustular dermatitis, Orf)
- Acute photosensitization
- Lameness due to footrot, foot abscess and other foot conditions
- Acute haemonchosis (with depression and submandibular oedema)
- Facial eczema
- *Oestrus ovis* infestation
- Pneumonia
- Plant poisoning
- Akabane disease (when deformed lambs are seen)
- Salmonellosis
- Sheep pox
- Foot-and-mouth disease
- Peste des petits ruminants/rinderpest

7. Control of Bluetongue

Treatment

7.1 There is no fully effective treatment available for clinically affected animals. Affected animals should be handled humanely, moved as little as possible and provided with soft food, shade and water. Treatment of valuable sheep with non-corticosteroid anti-inflammatory drugs could be considered, as reducing inflammation and pain can help recovery.

Vaccination

7.2 Stimulation of immunity by vaccination can effectively reduce the BTV-susceptible population of hosts. BTV can then only persist by infected vectors finding a new generation of naïve hosts or moving to new locations occupied by naïve vertebrate hosts.

7.3 Three types of vaccines can be considered: inactivated (killed), attenuated ('live') and recombinant virus vaccines, each of which is discussed below.

Inactivated vaccines

7.3 Commercial inactivated vaccines have not yet been developed because of the following disadvantages:

- The antigen mass required to elicit an immune response is high compared to attenuated vaccines;
- Two doses of vaccine are required to elicit a significant response; and
- An effective inactivated vaccine has not yet been commercially produced.

Experimentally, inactivated vaccines of varying levels of efficacy have been produced, but generally these have been slightly inferior to an attenuated live vaccine. A good inactivated vaccine would be expected to prevent clinical disease in susceptible hosts and result in little or no viraemia if challenged with the homologous serotype.

IAH Pirbright currently has an EU-funded project part of whose remit is to develop inactivated BTV vaccines.

Attenuated ('live') vaccines

7.4 These are used widely and effectively in southern Africa, the United States and Israel, and to a limited extent in the Mediterranean Basin. They are generally serotype specific and should prevent infection by the homologous field "challenge" virus. Attenuated vaccine usually provides little protection against heterologous challenge (i.e. of another serotype) although, if closely related, there may be protection against clinical disease and reduced viraemia. Multivalent live vaccines as used in South Africa, if properly administered, prevent infection with all the relevant serotypes. Increasing the number of serotypes will broaden the protection, and perhaps may provide some cross protection beyond the antigenicity of vaccine components.

The disadvantages of attenuated vaccines are:

- There is a risk of reassortment of the vaccine strain with field viruses that, potentially, could give rise to new strains of virus of high virulence
- There is the potential of reversion to virulence both in the vertebrate host and in vector insects
- Attenuated BTV can cross the placenta and pregnant ruminants vaccinated with attenuated vaccines may suffer reproductive failure or produce offspring with congenital abnormalities (field virus has not been proven to cross the placenta but teratogenicity does occur with field infections, although less frequently than in vaccinated ewes)
- Attenuated vaccine virus is more likely to be excreted in the semen of vaccinated males during and soon after the viraemic period (field virus is rarely excreted in semen)
- The vaccine virus causes a viraemia in vaccinated animals and there is therefore the possibility that it may be picked up and transmitted by vector insects
- The vaccine must contain the serotype(s) responsible for the outbreak of clinical disease
- The existing vaccines are designed for sheep; there are few data on their safety and efficacy in other ruminant species.

7.5 The disadvantages have not yet been proven to be a major problem in the field with the attenuated vaccines currently in use although the jury is still out. Some circumstantial evidence from Greece suggests that reversion to virulence of BTV 4 vaccine and vector transmission may have occurred. Resortment of genes between vaccine virus and field virus has been produced experimentally in insects and animals. Also, some BTV 10 isolates from the USA have had similar genes as are present in the BTV 10 vaccine in use there.

Recombinant vaccines

7.6 Second generation non-infectious subunit vaccines (recombinants and constructs) could overcome some of the problems of attenuated vaccines. Virus-like particle (VLP) vaccines have been successfully produced and have been shown in experimental studies to protect sheep from clinical disease against a homologous serotype challenge. Core-like particle (CLP) vaccines on the other hand are not serotype specific and show promise as a generic vaccine for the future. CLP vaccines will require two doses but if efficacious would offer major advantages.

7.7 Attenuated vaccines are cheaper than inactivated vaccines to produce, and stimulate an effective immune response from a single low dose. Attenuated vaccines are the only ones currently available for use in the field. Inactivated, VLP or CLP vaccines are still several years away from commercial availability.

7.8 Attenuated vaccines have been used extensively in sheep in South Africa, Israel and USA with success at preventing disease in endemic areas and in nearby areas intermittently exposed by seasonal movement of infected vectors. In southern Europe recently, BTV appears to have been eliminated from some areas where vaccination has been used but not in others. The reasons for this variation are not yet clear.

7.9 If BTV was to establish an annually recurring, endemic infection anywhere in Britain and should vaccination be introduced, it would be essential that cattle as well as sheep be immunised for eradication to be possible since cattle are the major reservoir host. Although attenuated vaccines will reduce the susceptible population of sheep, their suitability and effectiveness in providing an immune population of cattle (or other species) has not been established. Italian workers have produced adequate antibody titres in cattle (similar levels as in sheep) after vaccination but prevention of viraemia has not been demonstrated. In 2002, the Italian authorities vaccinated cattle as well as sheep, presumably to produce a near fully immune host population to abolish BTV transmission altogether. If the mammalian population is largely immune, BTV should die out, including the vaccine virus if it were being spread. The results of this programme will be watched with great interest.

7.10 Attenuated BTV vaccine for fifteen serotypes is manufactured by the Onderstepoort Biological Products Ltd in South Africa. The European Commission maintains a BTV vaccine Community bank at Onderstepoort for use by member countries. Colorado Serum Company, Denver, Co, USA also produces a US Federal registered BTV serotype 10 attenuated vaccine.

7.11 No BTV vaccines currently have a Marketing Authority (MA) from the Veterinary Medicines Directorate (VMD). In an emergency, use of a vaccine that does not have a MA from VMD could be approved by the CVO subject to European Commission approval. However, current policy is that animals vaccinated with such a product would not be permitted to enter the food chain and would have to be killed and the carcasses disposed of safely. This would be likely to result in more animals being destroyed following vaccination that would have died due to the disease.

8. Vector Control

8.1 Reducing the population of vectors or limiting the exposure of susceptible hosts to the vectors can also assist in minimising the incidence of BTV infection and clinical disease. Several approaches are available.

Husbandry modification

8.2 This measure is aimed at denying or reducing vector access to susceptible animals. Affected animals and those in immediate contact with affected animals may be housed at least pending the outcome of the initial investigation. Housing of other susceptible stock during times of maximum vector activity, from dusk until dawn, will significantly reduce biting rates and hence the likelihood of infection.

8.3 In addition, obvious portals of access to the housing, such as windows and doors, should be screened with either fine mesh sand-fly netting or with coarser material impregnated with insecticide (e.g. a synthetic pyrethroid).

Vector control

8.4 It is rarely possible to completely eliminate populations of vector *Culicoides*. The aim, therefore, is to reduce the number of potentially infecting bites that animals receive to levels where maintenance of an epizootic becomes unsustainable. A combination of approaches to vector control will yield the best results. These must be applied on all premises with large mammals, not only ruminant holdings. Horse farms and stables are particularly important as *Culicoides* actively feed on horses and these facilities have ideal breeding sites in manure piles.

Habitat alteration

8.5 Breeding sites of the *Culicoides* vector species should be identified and then destroyed. The likely target *Culicoides* vectors, (*C. imicola, C. obsoletus* and *C. pulicaris)* usually breed in organically enriched (mainly animal dung), and moist but not waterlogged soils, either bare or covered with short grass. Breeding site destruction may be possible when the sites are few and small by turning off taps, mending leaks and filling in or draining damp areas. Also, stable straw and dung heaps should be removed at weekly or shorter intervals; less than the developmental period of the immature stages.

Adult vector insecticides

8.6 Targeted application of insecticides of known low mammalian toxicity [e.g. synthetic pyrethroids, such as with deltamethrin (weekly) or fenvalerate (every second day)] in and around animal housing, and directly to the target animals should be effective.

8.7 Intradermal or subcutaneous inoculation of systemic "Ivermectin" is also effective at killing biting *Culicoides*. An additional advantage with Ivermectin, and with such insecticidal food additives as tetrachlorvinphos, is that these drugs are eliminated in the faeces which, should they be deposited on breeding sites, are toxic to the immature stages of *Culicoides*.

8.9 Eartags impregnated with synthetic pyrethroid insecticide are effective in reducing the number of bites or strikes on cattle and sheep by a number of other insect species. These eartags may be useful for *Culicoides* control also but there is little information available.

Larviciding

8.10 Application of a larvicide such as "Abate" (American Cyanamid) (5% temephos granulated with gypsum) to *Culicoides* breeding sites provides a slow but sustained release of the insecticide for periods up to 30 days.

Vector repellents

8.11 Di-ethyl toluamide (DEET) is the only commercially available repellent with a significant deterrent effect against *Culicoides* for periods of up to four hours. DEET applied to target animals at dusk before the peak attack period during the first four hours of the night may have a significant but temporary effect in reducing the biting rate of vectors.

9. Reference documents

ARMCANZ (1996). Disease Strategy: Bluetongue. Australian Veterinary Emergency Plan (AUSVETPLAN) Edition 2. Department of Primary Industries and Energy, Canberra, 1996.

European Commission Health and Consumer Protection Directorate-General (2000). *Possible Use of Vaccination against Bluetongue in Europe.* Scientific Committee on Animal Health and Animal Welfare. SANCO/C3/AH/R19/2000.

European Commission. Food Safety. Animal Health/Prevention and Control of Animal diseases. *Facts about Bluetongue.*
http://europa.eu.int/comm/food/fs/ah_pcad/ah_pcad_67_en.html.

FAO (2001). EMPRES Transboundary Animal Diseases Bulletin No. 18 July-September 2001. *Update on vector-borne diseases in the Mediterranean Basin.*
http://www.fao.org/DOCREP/003/Y2283E/y2283e04.htm#i

Mellor PS and Wittman EJ (2002). *Bluetongue Virus in the Mediterranean Basin 1998-2001.* The Veterinary Journal 164 (1): 20-37.

OIE International Animal Health Code, 10th edition. (2001). Chapter 2.1.9. Bluetongue. http://www.oie.int/eng/normes/en_mcode.htm

OIE Manual of standards. Diagnostic Test and Vaccines, 4th edition (2000). Chapter 2.1.9. Bluetongue. http://www.oie.int/eng/normes/en_mmanual.htm

OIE Data on animal diseases. A090-Bluetongue.
http://www.oie.int/eng/maladies/fiches/a_A090.htm

OIE Disease Information. Index by Disease. Bluetongue.
http://www.oie.int/eng/info/hebdo/A_DSUM.htm

OIE (1998). *Conclusions of the Research Subcommittee of the International Embryo Transfer Society (IETS) Import/Export Committee.* Res. Sci.tech.Off.int.Epiz., 1998,17 (3).

Takamatsu H, Mellor PS, Mertens PPC, Kirkham PA, Burroughs JN and Parkhouse RME (2002). *A Possible Overwintering Mechanism for Bluetongue Virus in the Absence of the insect Vector.* Journal of General Virology (submitted).

Wilbur LA, Evermann JF, Levings RL, Stoll IR, Starling DE, Spillers CA, Gustafson GA and McKeiran AJ (1994). *Abortion and death in pregnant bitches associated with a canine vaccine contaminated with bluetongue virus.* Journal of the American Veterinary Medical Association 204:1762-1765.

10. Contributors

10.1 The initial document has been prepared by Neil Tweddle, Veterinary Advisor in the Veterinary Exotic Disease Division, DEFRA. Neil is on secondment from the Office of the Australian Chief Veterinary Officer, Agriculture, Fisheries and Forestry – Australia, Canberra, Australia.

10.2 The major contribution by Dr Philip Mellor, Institute for Animal Health, Pirbright Laboratory is gratefully acknowledged.

10.3 Other people within DEFRA provided helpful comments.