Bluetongue

Scientific Opinion of the Panel on Animal Health and Welfare

(Question No EFSA-Q-2007-201)

Adopted on 19 June 2008
PANEL MEMBERS

The Scientific Panel for Animal Health and Welfare (AHAW) of the European Food Safety Authority adopted the current Scientific Opinion on 19 June 2008. The Members of the AHAW Scientific Panel were:

SUMMARY

Following a request from the European Commission (DG SANCO), the Panel on Animal Health and Welfare was asked to deliver a scientific opinion on Bluetongue (BT) virus (BTV). The Commission required an update of previous EFSA scientific opinions on bluetongue2,3 as regards: i) vector ecology and criteria for the determination of the seasonally free period; ii) any new scientific information as regards the over-wintering mechanisms of the BT virus and the length of viraemia of all BTV serotypes relevant to the EU situation; iii) scientific advice on the effectiveness and suitability of insecticides and repellents for Culicoides species, and iv) scientific advice on the different measures that can be used to protect animals against attacks by vectors.

As far as vector ecology is concerned, outbreaks of BTV-8 and BTV-1 have occurred in the last two years in Europe outside the geographical range of C. imicola, demonstrating that northern Palaearctic species of Culicoides are able to transmit BTV. To date, the specific vector(s) of BTV in these areas have not been identified, although strong circumstantial evidence implicates members of the Obsoletus complex, C. dewulfi, C. chiopterus and the Pulicaris complex as the most likely candidates. Laboratory testing for vector competence should be carried out, where possible, in parallel with field-based testing. Analyses of the vector competence of particular species should be conducted with reference not only to the ability of the vector to become infected by, replicate and transmit the virus but also to its wider ecological requirements (i.e. its vector capacity), which may vary with region and season.

There is clear evidence of indoor activity (including blood feeding) of the Culicoides vector species, especially during winter time, which may extend the seasonal activity of such species when outdoor activity is absent by an, as yet, undefined period. This has been mostly suggested by comparing indoor and outdoor light trap captures. To date, no clear scientific evidence has been reported concerning the role of adult Culicoides surviving inside stables in prolonging the transmission period of BTV. Further, the period of survival of such adults inside premises is also unknown.

No single mechanism has been demonstrated to be responsible for the overwintering of BTV in northern Europe. Year-round presence of infected Culicoides remains the most likely although transplacental transmission cannot be excluded. Due to geographical and inter-annual variations in Culicoides activity, systematic and extensive entomological monitoring is advised to be implemented for detection of overwintering adults. Analysis of the parous females captured during winter time is recommended in order to detect BTV infected individuals. This analysis is recommended to be coordinated across Europe as numbers are likely to be extremely small. The survival rates of adults Culicoides at lower temperatures is recommended to be further investigated under laboratory conditions. In order to better understand the current distribution of the species included in the Obsoletus complex, it is recommended to perform co-ordinated European surveys involving molecular identification of Obsoletus complex females to species level. In addition, the routine identification of males from these complexes is also advised to develop a clearer picture of each species distribution. These data should be made available in the centralised EU database (BT-Net).

Due to the importance of defining the seasonal pattern of the Culicoides vector species, their monitoring on a year-round basis using permanent sampling sites is recommended. It is also

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important to link data obtained from entomological sampling with data obtained from the serological data of BTV surveillance programmes. A harmonisation of the above entomological monitoring programmes across Europe is recommended for a better assessment of the annual seasonal pattern, including age grading of females, the likely total absence of adults during winter and the first detection of *Culicoides* activity the following season. Further research is recommended in order to estimate the relevance of the indoor and day-light activity in either the extension or reduction of the seasonal pattern of the different vector *Culicoides* species. Indoor and day-light activities should be defined and standardized for research purposes.

The current definition of the Seasonally Vector-Free Period (SVFP) was based on the epidemiological situation of certain areas in Europe for certain serotypes and certain species of vectors. The importance of some species (i.e.: *C. dewulfi*, *C. chiopterus*, *C. pulicaris* and the Obsoletus complex) implicated in the transmission of the BTV was not specifically included in the current definition. Recent data demonstrate that some *Culicoides* species, in some geographical areas in Europe are active to a greater or lesser extent throughout the year and that an absolute vector free period may not exist. However, there are periods of the year when the abundance of the *Culicoides* vector species is very low, mainly coinciding with winter time, and long-standing practical experience demonstrates that transmission of BTV is substantially reduced or halted during these periods. Improved and harmonized methods to monitor and quantify vector activity are required to support the development of criteria that more accurately define periods of low vector abundance, especially those relevant to BTV transmission. It is recommended that harmonized vector monitoring and comprehensive BTV surveillance systems are developed, integrating local vector abundance and BT incidence data, to establish more accurately the level of vector activity required for virus transmission in their area.

The vector activity should be recorded by using an annual monitoring programme based on *Culicoides* adult capture with UV light traps placed outside stables or animal housing. Pending additional studies, it is recommended that all suspect and confirmed vector species be considered equally capable of transmitting all BTV serotypes. The analysis of age grading of captured *Culicoides* females is recommended to be carried out.

In regard to the over-wintering mechanisms and the duration of BTV viraemia, recent data from BTV infections of ruminants regardless of serotype (including serotype 8) do not show that duration of viraemia as indicated by virus isolation or RT-PCR, is different from that already described in the previous EFSA Report\(^4\). Importantly, the central finding of the previous EFSA Report\(^4\) is confirmed that animals remain RT-PCR positive after they no longer circulate infectious virus so PCR-positive status and viraemia are not synonymous. Transmission of field strains of BTV serotype 8 from dam to progeny amongst ruminants in northern Europe has been demonstrated in both field and experimental studies. However, the role of vertical infection of foetal or neonatal ruminants in the epidemiology of BTV-8 infection, including any role in virus overwintering, is currently uncertain. Field investigations of natural BTV-8 infections of pregnant animals are required to reliably establish the role of vertical infection, if any, in the natural transmission cycle.

Regarding the measures that can be used to protect animals against attacks by vectors, insecticides may be used to limit the population of *Culicoides* and their biting rates, thereby reducing the risk of sequential BTV transmission. Historical testing of toxicity worldwide has demonstrated that pyrethroid-based products are more effective in the laboratory against *Culicoides* than organophosphate-based (OP) products. Hence, the use of the former is preferred except where other issues (environmental impact and legislation) preclude it.

However, pyrethroid-based insecticides should not be used as a stand-alone measure to protect animals against *Culicoides* attacks. A common result after treatment with pour-on formulations or ear tags was the decreasing insecticidal efficacy from the back line to the belly and legs of treated animals. This is related to the limited spread of the insecticide and is common to topically applied products of non-systemic activity. Dipping products have not been assessed to date for this role in Europe. Systemic products have also not been assessed, but based on past experience, are unlikely to be useful for lowering *Culicoides* population levels thereby preventing BTV transmission on a significant scale. No new data have been provided regarding the treatment of housing or transport for animals. Treatment of breeding sites remains difficult as habitats are poorly defined for most species.

Studies should be carried out in a proper manner to actually correlate the use of insecticides with the magnitude of the decrease of the risk of BTV transmission in treated animals. No insecticidal products are currently authorised specifically against *Culicoides* in the EU although a wide range of untested products are available. In the absence of any valid data on the efficacy and safety of veterinary medicinal products or biocidal product for the control of *Culicoides*, no treatment protocols have been formally approved in the EU for specifically protecting animals against *Culicoides* attacks. When using substances authorised for other indications or claims (e.g. against nuisance flies), label instructions use should be followed. When using veterinary medicinal products under the “cascade”, a standard withdrawal period of at least 28 days for meat and 7 days for milk must be applied, when the product is used in another animal species, or, when the dosing instructions in the product literature are not followed in the target species. All use of chemical treatments should be carefully assessed with regard to environmental impact, user risk and the potential for development of resistance in *Culicoides* populations.

**Key words:** Bluetongue, Overwintering, Viremia, *Culicoides* Ecology, *Culicoides* Control, Insecticides.
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ABBREVIATIONS
BT Bluetongue
BTV Bluetongue Virus
CT cycle threshold value
CVMP Committee for Medicinal Products for Veterinary Use
EIP Extrinsic Incubation Period
MRL Maximum Residue Limits
OP Organophosphate
OVI Onderstepoort Veterinary Institute
RT-PCR Real-Time Polymerase Chain Reaction
SVFP Seasonally Vector-Free Period
UV Ultra Violet Rays
VICH International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products
BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION (DG SANCO)


This Regulation has been drawn up on the basis of experience gained and the scientific advice that has already been provided by recent EFSA opinions to the Commission supporting the legislative decision making process and risk management. The Regulation amended certain existing EU measures for bluetongue, to make them more sustainable, proportionate and science-based. It brings EU rules more into line with international standards and reduces as far as possible obstacles to trade that bluetongue may cause while maintaining the adequate level of guarantees.

However, during the discussions with the Member States and consultations with stakeholders some gaps in the scientific knowledge and scientific advice on bluetongue have been identified. Furthermore, the experience gained during the two last epidemic waves with incursions of different serotypes and the dynamics of the disease under different conditions needs to be considered in order to have updated scientific information.

In view of the evolution of the disease and the previous scientific opinions provided by EFSA, the current situation as regards bluetongue in Europe requires special consideration as regards i) revision and update of previous opinions on vectors and viraemia and over-wintering mechanisms, ii) insecticides and repellents, iii) protection against vectors attack, and iv) the risk posed by the transit of animals from restricted zones.

In order to cover the above mentioned issues the Commission is in need of:

1. A review and an update of previous opinions as regards vectors ecology. In particular a complete overview of the vectors distribution in the EU and the density of vector population over the year would be needed. It is necessary to clarify the likelihood of finding vectors throughout the year by using certain key variables (temperature, altitude, latitude, winds...) and other indicators (surveillance data) so accurate and applicable criteria for the determination of the seasonally free period are identified.

2. A review and update of previous opinions as regards the risk of re-occurrence of bluetongue by assessing any new scientific information as regards the over-wintering mechanisms of the BT virus and the length of viraemia of all BTV serotypes relevant to the EU situation.

3. Scientific advice on the effectiveness and suitability of insecticides and repellents for Culicoides species, including adequate protocols for its use, including different combinations, in order to reduce the number of vectors and the probability of transmission of the virus; so that they are used as effective risk mitigation measures.

4. Scientific advice on the different measures that can be used to protect animals against attacks by vectors including chemical and physical means taking into account the combined effect of different elements and measures that could reduce the risk.
An assessment of the risk of transit as defined in Regulation 1266/2007. Scientific advice should be provided in particular on the appropriateness of the treatments of the animals in addition to the treatments of the means of transport with insecticide/repellents.

**TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION (DG SANCO)**

In view of the above, and in accordance with Article 29 of Regulation (EC) N° 178/2002, the Commission asks EFSA:

- To review and to update previous opinions as regards vectors ecology (models for distribution/density), in order to have more accurate and applicable criteria for the determination of the seasonally free period.

- To review and to update previous opinions as regards over-wintering mechanisms and the length of viraemia of BT virus.

- To review and to update previous opinions and to provide a scientific assessment of appropriateness of insecticides and repellents for *Culicoides*, including adequate protocols for its use.

- To provide a scientific assessment on the appropriateness of a set of measures, different elements and the combinations of which that can be used to protect animals against attacks by vectors.

- To assess the risk of transit as defined in Regulation (EC) N°1266/2007, in particular taking into account the treatment with insecticides/repellents of animals and/or the means of transport.

This opinion (EFSA-Q-2008-201) responds to the first 4 questions (terms of reference) whereas the last question will be dealt with and responded to in a separate opinion (EFSA-Q-2008-436).
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In accordance with the provisions of Article 30 of the Regulation 178/2002 (EC, 2002) and Article 59 of Regulation 726/2004 (EC, 2004), the European Food Safety Authority (EFSA) involved the European Medicines Agency (EMEA) in the preparation of this report in order to avoid divergence between the opinions of their scientific committees. The EFSA AHAW Panel is very grateful for the constructive input of the EMEA in this report. DG Environment was also consulted as far as biocides were concerned. The AHAW Panel gratefully acknowledge their input.
ASSESSMENT

1. Duration of Viraemia and Potential Role of Animals in Overwintering of BTV

1.1. Duration of Viremia

The prolonged but not persistent viraemia that occurs in BTV-infected ruminants is described in the 2007 EFSA Report (EFSA, 2007a; pages 15-16). The strain of BTV-8 currently circulating in Europe shows biological properties somewhat different from those of field strains of other BTV serotypes, including a heightened ability to induce clinical signs in cattle (Elbers et al., 2008; Appendix A), to cross the placenta and infect the foetus (Wouda et al., 2008), and in the putative vectors responsible for virus transmission (Dijkstra et al., 2008; Meiswinkel et al., 2007). However, the dynamics of BTV-8 infections of livestock (duration of viraemia by virus isolation and by RT-PCR) are largely uncharacterized and there are few reports of experimental infections with the virus (Darpe et al., 2007, Backx et al., 2007).

Darpe et al. (2007) experimentally infected 4 polled Dorset sheep and 4 calves with a strain of BTV-8 isolated in the Netherlands in 2006 (passaged once each in KC and BHK cells, inoculum 1.5 ml x 10^6.5 TCID50/ml). Viraemia in sheep as shown by quantitative (real-time) RT-PCR began at D3-D4 in all 4 animals, and continued until D28-33 (end of experiment) whereas a conventional RT-PCR assay was less sensitive (negative results in one sheep by D10 and another by D25). The blood of infected calves was positive by real time RT-PCR from D1-D6 until at least D30 (end of the experiment), also with greater sensitivity than conventional RT-PCR. No comparison was made between viraemia measured by RT-PCR and by virus isolation.

Backx et al. (2007) used blood from an infected Texel sheep to inoculate additional Texel sheep (n=3) and Dutch dairy goats (n=2). As much as 24 ml of washed erythrocytes was intravenously administered to each animal. All sheep were RT-PCR positive by D4. No virus isolation was attempted in this study.

Experimental infection of 4 Ripollesa sheep with a low titre (2ml of 10^4.3 TCID50/ml) of low passage BTV-8 (1 x embryonated egg, 2 x Vero cells) caused fever (>40ºC) from D8 to D11, dyspnoea and conjunctivitis but no other clinical signs (Domingo, 2008). Viraemia (as determined by virus isolation) was first detected in 2 sheep at D6 and in all 4 animals from D10 until D14. Virus was irregularly isolated from one sheep after D14, specifically at day 28, but viraemia as assessed by conventional RT-PCR was detected in all 4 sheep until D51 (end of the experiment), which is consistent with the results obtained with other serotypes of BTV (EFSA, 2007a; pages 15-16). Sheep blood collected at D36 and 51 after infection that was RT-PCR positive/VI negative was inoculated into susceptible sheep (n=2 for each sampling day). The PCR positive/virus isolation negative bloods did not cause either viraemia or seroconversion by 25 days after inoculation of susceptible sheep, whereas blood that was both RT-PCR and virus isolation positive (collected from an inoculated sheep at D20) caused both viraemia and seroconversion in 2 inoculated sheep.

As part of a vaccine challenge study, 14 control sheep were inoculated with a high challenge dose (10 ml of 10^7.3 TCID50) of the same BTV-8 inoculum. BTV nucleic acid was consistently detected by conventional RT-PCR in blood on the majority of sampling days from D2 until the end of the experiment (D24) (164 out of 168 samples RT-PCR positive, 97.6%). In comparison, virus was isolated from 131 of 168 (78%) samples from D2 to D24 post challenge. Thus, as with other BTV serotypes (EFSA, 2007a; pages 15-16) BTV nucleic acid more
consistently can be detected by RT-PCR, and for a longer interval after infection than virus can be isolated. Specifically, at the end of the experiment (D24 after challenge) most sheep were still positive by RT-PCR but only approximately half of them still were viraemic as determined by virus isolation.

Reduced duration and level of viraemia is a criterion of vaccine efficiency, and available data on vaccine efficacy were published by EFSA (2007a). During this year new data related to BTV-1 and BTV-8 vaccine testing have been published (see Appendix B).

Conclusions:

Recent data from BTV infections of ruminants regardless of serotype (including serotype 8) do not show that duration of viraemia as indicated by virus isolation or RT-PCR, is different from that already described in the 2007 EFSA Report (EFSA, 2007a; pages 15-16).

Recommendations:

There is a clear need for ongoing evaluation of duration of viraemia following natural BTV infection of all ruminant species. Importantly, the central finding of the 2007 EFSA Report is confirmed that animals remain RT-PCR positive after they no longer circulate infectious virus so PCR-positive status and viraemia are not synonymous.

1.2. Role of animals in overwintering of BTV

Whereas BTV is transmitted between ruminants throughout the year in tropical regions, infection is distinctly seasonal in temperate zones where the vast majority of infections occur during the late summer and autumn months. The term “over-wintering” is somewhat of a misnomer as infectious virus largely disappears from early winter until mid-summer (mid-December until July in the Northern Hemisphere), whereas PCR positivity is extended from late summer until early spring (March/April) of the following year (Appendix A). The precise mechanism of this highly seasonal nature of BTV infection remains poorly defined (EFSA, 2007a; pages 36-37, 48-50), including the relative contributions of animals and insects therein. Although duration of viraemia in BTV-infected ruminants can be prolonged, it is not persistent (EFSA, 2007a; pages 15-16) and of insufficient duration to overwinter the virus in a single infected animal. Nevill (1971) investigated the overwintering of BTV on the high veldt of South Africa and proposed several theories that might explain the highly seasonal nature of infection: a) transovarial transmission of the virus in vector insects, b) a complicated overwintering cycle that involves some unidentified intermediate host such as reptiles or birds, c) prolonged survival of infected adult Culicoides insects, d) prolonged infection of cattle, and e) an ongoing low level cycle of infection between cattle and Culicoides insects throughout the overwintering period, a theory favoured by Nevill based on his extensive field studies.

Findings from studies conducted in the 1970s lead to the hypothesis that cattle infected with BTV in utero (following experimental infection of pregnant heifers) sustained an inapparent, persistent (perhaps lifelong) postnatal infection with specific immune tolerance to the infecting virus (Luedke et al., 1977; 1982). Persistently infected cattle clearly would provide an ideal mechanism of over-wintering of BTV, and a mechanism for widespread dissemination of the virus through movement of cattle. However, Luedke’s suggestions of persistent BTV infection have not been sustained by intensive experimental and field studies in several countries. Barzilai et al. (1975) reported that the progeny of 25 heifers that were naturally infected with BTV serotype 4 or 16 were normal and both virus isolation negative and seronegative at birth.
Similarly, in an extensive study in the western United States, BTV was isolated from 206 of 8,751 healthy cattle and the virus was not isolated from samples collected between mid-December and June (when any putatively persistently infected calves would be born; Maclachlan et al., 1989). Furthermore, more than 8,000 aborted bovine foetuses have been screened for BTV infection at the California Diagnostic Laboratory in the last 20 years, only 10 of which were determined to have been infected with BTV and these foetuses all had severe brain lesions (Maclachlan and Osburn, 2008). Congenital BTV infection has not been described in aborted foetuses in other regions of the United States where BTV infection is endemic and cattle regularly are infected with BTV during pregnancy (Kirkbride, 1992; T. McElwain and E. Howarth, personal communication), whereas calves in California very sporadically are detected with brain lesions consistent with in utero BTV infection (Brown and Maclachlan, 1983; McKercher et al., 1970; Richards et al., 1971). These rare cases of congenital BTV of calves have been attributed to natural infections with live attenuated vaccine strains of the virus that circulate in nature or field viruses that have acquired individual genes of vaccine viruses through reassortment (Maclachlan and Osburn, 2008). Extensive surveillance in Italy since 2003 (after vaccination began) has failed to demonstrate vertical transmission of BTV serotypes 1, 2, 4, 9 or 16, other than rare isolation of vaccine viruses from aborted foetuses (G. Savini, personal communication). Intensive surveillance for some 20 years has never identified vertical transmission (congenital infection) of the progeny of sentinel cattle in the Northern Territory of Australia where BTV infection is endemic (L. Melville, personal communication), and congenital BTV infection has also only rarely been described amongst cattle in South Africa where live attenuated vaccines are used (Zumpt et al., 1978). Experimental studies by Roeder et al. (1991) showed that the vertical transmission did not occur following infection of 20 pregnant cows at either 40 or 60 days of gestation with BTV serotype 11. Similarly, Parsonson et al. (1994a; 1994b) showed that vertical transmission did not occur amongst the progeny of 11 cows or 47 ewes infected with various BTV serotypes at the time of breeding or specified stages of gestation. Importantly, the studies of Roeder and Parsonson utilized the same virus as that used by Luedke, and a bull that Luedke claimed to be persistently infected did not transmit virus to susceptible heifers in Parsonson’s studies (1994b). Other investigators were unable to isolate BTV or demonstrate virus by PCR from putatively persistently infected cattle described by Luedke (R. Bowen, T. Howard, I. Parsonson, personal communications).

The absence or very rare occurrence of natural vertical transmission of most BTV serotypes and strains investigated to date in North America, Australia and South Africa, stands in marked contrast to findings during the current BTV-8 outbreaks in northern Europe where vertical transmission of virus following infection of pregnant ruminants occurs with some considerable frequency. Specifically, laboratory studies have confirmed that aborted bovine foetuses in northern Europe frequently are PCR positive and/or seropositive to BTV-8 and sometimes have teratogenic brain lesions (Wouda et al., 2008; De Clercq et al., 2008, Vercauteren et al., 2008). Newborn calves also have been identified that are both virus isolation and PCR positive (Menzies et al., 2008). BTV is highly pathogenic to ruminant foetuses if it gains access to foetal tissues during early gestation, following either transplacental infection or direct inoculation (see the following section). Bovine foetuses that were directly inoculated with one of several different BTV serotypes prior to midgestation (80 – 125 days of gestation) either died or were born seropositive, virus negative and with severe cerebral malformations (Barnard and Pienaar, 1976; Maclachlan and Osburn, 1983; Maclachlan et al., 1985a; 1985b, Thomas et al., 1986; Waldvogel et al., 1992a). It was concluded that such animals could have no realistic role as virus reservoirs (Maclachlan et al., 1985b; 1989). Inoculation of foetal calves after midgestation did not cause severe teratological lesions although these animals sometimes were born prematurely as weak calves, and calves infected after mid-gestation can be virus isolation positive at birth (Jochim et al., 1974; Waldvogel et al., 1992b). Similarly, BTV infection of
pregnant ewes at mid-gestation lead to the birth of lambs that were viraemic for as long as 2 months after birth (Gibbs et al., 1979). However, viraemia in these animals ended at various periods up to 2 months after birth and they were not persistently infected or immunotolerant to BTV.

Based on evaluation of data available at the time it was concluded at the second symposium on bluetongue held in Paris in 1991 that natural BTV infection of cattle is transient and neither persistent nor immunotolerant (Maclachlan and Gard, 1992). The current OIE Code is based on the conclusion that although venereal and congenital transmission of BTV can occur in ruminants, these mechanisms are unimportant to the long term perpetuation of the virus (reviewed: Barratt-Boyes and Maclachlan, 1995; Gibbs and Greiner, 1994; Maclachlan and Osburn, 2006). However, further studies are needed to fully assess the epidemiological importance of transplacental BTV-8 infection of ruminants in Europe given the uniqueness of this occurrence as compared to other field strains of BTV (Maclachlan and Osburn, 2008). Of particular importance is definition of the role, if any, of congenitally infected calves in maintaining virus between seasons. The Working Group does note, however, that cursory evaluation of available data (Appendix A) suggests that the highly seasonal occurrence of BTV serotype 8 infection in northern Europe is not markedly different from that of other BTV serotypes (-1, -2, -4, -9 and -16) that occur within Europe in which vertical transmission has not been identified or occurs at a very low frequency (see figures in Appendix A).

1.2.1. Congenital BTV infection and virus-induced teratology

Transplacental transmission of BTV was first definitively described in California in the early 1950s following the immunization of pregnant ewes with a live attenuated BTV vaccine (Schultz and DeLay, 1955). Large numbers of “dummy lambs” were delivered from ewes immunized during pregnancy with a live attenuated BTV serotype 10 vaccine, and vaccination during the fifth and sixth weeks of gestation had particularly adverse consequences. The brains of affected lambs had distinctive cavitating lesions in the subcortical white matter, and the cerebellum was involved in severe cases (Cordy and Schultz, 1964). In subsequent studies, lesions of acute necrotizing meningoencephalitis progressing to hydranencephaly and subcortical cysts were described in approximately 20% of the foetuses born to ewes that were vaccinated on the 40th day of gestation (Young and Cordy, 1964). Similarly, Flanagan and Johnson (1995) showed that whereas unadapted BTV serotype 23 did not cross the placenta of pregnant sheep, vaccination of ewes in early gestation with a BTV serotype 23 vaccine that had been attenuated by cell culture passage resulted in a high incidence of reproductive failure as well as hydranencephaly in lambs that survived to birth. It is very clear that modification of field strains of BTV by growth in either embryonated eggs or in cell culture can markedly increase their ability to cross the placenta, to cause foetal infections and/or excretion through semen (reviewed Murray and Eaton, 1996; Kirkland and Hawkes, 2004; Maclachlan et al., 2000).

The consequences of transplacental infection of foetal ruminants are dependent on foetal age at infection. Infection of foetal lambs with a vaccine strain of BTV serotype 10 at 50 – 59 days of gestation caused a severe necrotizing encephalopathy and retinopathy that manifest at birth as hydranencephaly and retinal dysplasia (Osburn et al., 1971a; 1971b; Osburn and Silverstein, 1972; Silverstein et al., 1971). In contrast, infection of foetuses at approximately 75 days of gestation produced multifocal encephalitis and selective white matter vacuolation that at birth manifest as cerebral cysts. Foetuses inoculated after 100 days of gestation developed only nonsuppurative meningoencephalitis. A similar age-dependence of lesions has been described after BTV infection of foetal cattle, with hydranencephaly resulting from infections between
approximately 80 and 125 days of gestation and milder lesions thereafter (Barnard and Pienaar, 1976; Maclachlan and Osburn, 1983; Maclachlan et al., 1985a; 1985b; Thomas et al., 1986; Waldvogel et al., 1992a; 1992b).

Data on BTV infections of foetal ruminants (prior to advent of real time PCR, and with regard to foetal infections with BTV serotypes other than serotype 8) have been reviewed previously during international symposia at which it was concluded that although venereal and congenital transmission of BTV can occur in ruminants, these mechanisms are unimportant to the long term perpetuation of the virus. Furthermore, it was concluded that transplacental transmission is a property of certain BTV strains, especially virus strains that have been propagated in embryonated eggs and/or cell culture, and that transplacental infection rarely or never occurs with the majority of field strains of BTV. Studies based on several different, laboratory propagated BTV serotypes have shown that BTV infection of foetal ruminants in early gestation leads to either foetal death or to teratogenic abnormalities of the brain identical to those recently described following natural BTV8 infection. Foetal ruminants infected with such viruses in early gestation are typically born serologically positive to BTV and virus isolation negative (either PCR positive or negative), as apparently are foetuses that are naturally infected during early gestation. In contrast, infection of foetuses after midgestation does not result in severe teratogenic abnormalities, but infected animals can be viraemic at birth although these animals do clear all infectious virus after a viraemia of similar duration to that which occurs following some infections of postnatal animals.

Transplacental BTV serotype 8 infection has been unequivocally documented following field (natural) infection of pregnant livestock in northern Europe (Wouda et al., 2008; Menzies et al., 2008; De Clercq et al., 2008), or experimental infection (Backx et al., 2008), and the brain lesions described in congenitally infected calves are identical to those previously described in calves naturally or experimentally infected with other serotypes of BTV (Barnard and Pienaar, 1976; Brown and Maclachlan, 1983; Maclachlan and Osburn, 1983; Maclachlan et al., 1985a; 1985b; Richards et al., 1971; Zumpt et al., 1978). Foetuses with teratogenic brain lesions caused by congenital BTV infection are born seropositive and can be either PCR negative or positive (Wouda et al., 2008). Unexplained infection of neonates prior to ingestion of colostrum or milk also has been described (P. Mellor, personal communication).

Conclusions:

Transmission of field strains of BTV-8 from dam to progeny amongst ruminants in northern Europe has been demonstrated in both field and experimental studies. However, the role of vertical infection of foetal or neonatal ruminants in the epidemiology of BTV-8 infection, including any role in virus overwintering, is currently uncertain. Transmission from dam to progeny could occur by transplacental transfer of virus, and by oral infection of neonates by infected milk or colostrum.

Transplacental transmission is a property of certain BTV strains, especially virus strains that have been propagated in embryonated eggs or cell culture, however, transplacental infection rarely or never occurs with the majority of field strains of BTV.

Currently, analysis of existing data do not support the concept of immunotolerant BTV-8 infection which, however cannot be discounted

Recommendations:
Although vertical transmission of BTV-8 from mother to foetus has been unequivocally confirmed, field investigations of natural BTV-8 infections of pregnant animals are required to accurately establish the role of vertical infection, if any, in the natural transmission cycle.

**Recommendations for future research:**

Although transmission of BTV in semen is highly unusual (EFSA, 2007a; page 16), given other distinctive and unusual properties of BTV serotype 8 the possibility of transmission of virus in semen also should be investigated.

1.3. **Role of wildlife including wild ruminants and carnivores**

BTV infection of wild life has been extensively reviewed in the previous EFSA Report on bluetongue (EFSA, 2007a; page 17). Initial observations showed that BTV-8 can infect wild ruminants in affected MS.

In an extensive study carried out in the Southern half of peninsular Spain in the epidemic seasons of 2005-2006 and 2006-2007, five species of wild ruminants were confirmed to have been infected by BTV-4 (Ruiz-Fons *et al.*, 2008). Blood samples were collected from Red deer (*Cervus elaphus*) and roe deer (*Capreolus capreolus*), which are the widest distributed wild ruminants in Spain, as well as from mouflon (*Ovis aries*), fallow deer (*Dama dama*) and aoudad (*Ammotragus lervia*). Sera were examined for BTV antibodies using a commercial serum antibody test kit. Seropositivity was found in red deer (309/1409; 21.9%), fallow deer (34/96; 35.4%), roe deer (2/39; 5.1%), mouflon (9/68; 13.2 %), and aoudad (1/4; 25%). An increased seroprevalence was found from south to north. In another survey made in Andalusia 9 out of 93 (11%) Spanish ibex (*Capra pyrenaica*) were seropositive (Garcia *et al.*, 2008).

Recent publications document the occurrence of BTV-8 infection in yaks (Mauroy *et al.*, 2008), camelids (Henrich *et al.*, 2007) and wild red deer (Linden *et al.*, 2008). Also affected in Belgium or Germany were European bison, roe and fallow deer, and mouflon (C. Staubach, personal communication), and possibly even carnivores in zoos (P. Mellor, personal communication). In south-west Spain, an outbreak of BTV-1 has been described in a wild population of mouflons (*Ovis aries musimon*), with clinical disease and mortality (Fernández-Pacheco *et al.*, 2008).

**Conclusions:**

BTV-1, -4 and -8 infection and clinical disease of various wildlife and non domestic species has recently been described, indicating that wildlife may play a role in the spread of BTV.

**Recommendations:**

Further investigation of the role of wild ruminants, other herbivores and carnivores are needed, to determine their impact on the spreading of the disease and intervention strategies.
2. Vector ecology

2.1. Updating of the taxonomy, distribution and vector status of the Culicoides species in Europe

In Europe, Culicoides species that have been implicated as potential vectors of BTV generally belong to the subgenera Avaritia and Culicoides, although in most cases the evidence implicating them in this role is little more than circumstantial. Studies that implicate species of Culicoides in BTV transmission in Europe are presented in Table 1.

2.1.1. Subgenus Avaritia

To date, the majority of studies of vector ecology in Europe have concerned the Avaritia subgenus, due to their often high abundance at farm locations. In Europe, this subgenus includes Culicoides (Avaritia) obsoletus (Meigen) 1818, Culicoides (Avaritia) imicola Kieffer 1913, Culicoides (Avaritia) chiopterus (Meigen) 1830, Culicoides (Avaritia) scoticus Downes and Kettle 1952, Culicoides (Avaritia) dewulfi Goetghebuer 1936, and Culicoides (Avaritia) montanus Shakirzjanova, 1962.

Historically, the Obsoletus complex (named also Obsoletus group by some authors), included the following species C. obsoletus, C. scoticus, C. montanus, C. chiopterus and C. dewulfi and was always used instead of the subgenus Avaritia in Europe (excluding C. imicola, which at that time was not known in Europe). However, the taxonomic situation has now been refined further using molecular tools. Today we understand that the first three species given above comprise the Obsoletus complex whereas the remaining two taxa are separate. These five species, together with C. imicola, form the subgenus Avaritia in Europe.

Of these species, Culicoides imicola is the most easily distinguished by wing pattern from the rest of the European Avaritia species. Where doubt in taxonomic identity occurs, a recently published RT-PCR assay with a specificity of 92% and sensitivity of 95% has been developed for quantifying this species from light traps captures without need of classical identification (Cêtre-Sossah et al., 2008).

The difficulty of morphologically separating females of the other Avaritia species in Europe, prior to the advent of molecular marker differentiation, led to the use, and sometimes creating some confusion, of the term ‘Obsoletus complex’ as a grouping of convenience. As has been mentioned, only when examining historical literature, therefore, this should be interpreted as being a synonym of the Avaritia subgenus (excluding C. imicola). This approach was adopted by many BTV vector monitoring programmes in different countries where the Culicoides were mostly separated uniquely by the wing pattern. Recent molecular marker studies based around ITS1, ITS2 and COI sequences indicate that the species C. obsoletus and C. montanus appear to be very closely related. Similarly, while C. scoticus does form a separate phylogenetic clade, it is morphologically very similar to C. obsoletus and at present cannot easily be separated from this species (Meiswinkel et al., 2004; Nolan et al., 2007; Mathieu et al., 2007). Based on molecular and limited morphological classification, however, C. chiopterus and, particularly, C. dewulfi are only distantly related to these species (Meiswinkel et al., 2004; Nolan et al., 2007; Mathieu et al., 2007) and are themselves not closely related to each other so should be considered as two separate taxa. For the present report, therefore the following notation will be used:
Obsoletus complex = *C. obsoletus*, *C. scoticus* and *C. montanus* used as a grouping of those species that can only be differentiated in the female form by molecular markers.

2.1.1.1. *Culicoides imicola*: distribution, vector status and larval habitat

*Culicoides imicola* has an Afro-Asiatic distribution that was until recently restricted in Europe to the south of the Iberian Peninsula and a few Greek islands located close to Anatolian Turkey. Recently many authors have described an apparent and on-going northward range expansion in this species (Mellor and Wittmann, 2002). Much of the early work regarding the ability of this species to transmit BTV was carried out in Africa. Transmission of BTV to sheep via the injection of homogenised, field collected *C. imicola* (=*C. pallidipennis*) and by the bites of *C. imicola* was initially carried out in South Africa (Du Toit, 1944) with later studies using membrane-based virus feeding techniques, also in South Africa to confirm susceptibility to infection (e.g. Venter et al., 1998). In Europe, BTV PCR-based positives have been recorded from pools of *C. imicola* (e.g. Ferrari et al., 2005). In Italy, BTV-4 and BTV-1 have been isolated from parous females of *C. imicola*, respectively in 2003 and 2006 (M. Goffredo, personal communication). Other data confirming the isolation of infectious BTV from *C. imicola* in Europe have not been published. The implication of *C. imicola* as a vector of BTV in Europe is thus based on its distribution and abundance on farms, in outbreak areas, isolation of BTV from parous individuals and historical evidence of its role in transmission elsewhere. Few direct vector competence experiments with European *C. imicola* have been undertaken and results remained limited (Biteau-Coroller, 2006, PhD thesis), due to the difficulties in feeding and maintenance in the laboratory. The breeding sites of *C. imicola* have been clearly identified at the farm level. Moist soil enriched with organic matter appears to be the most suitable habitat for larval development (Braverman et al., 1974).

2.1.1.2. The Obsoletus complex: vector status

The first indication of the potential for northern Palaearctic species of *Culicoides* to act as BTV vectors was the isolation of BTV-4 from pools of the Obsoletus complex in Cyprus in 1977 (Mellor and Pitzolis, 1979) and later in Italy (BTV-9 and BTV-2; Savini et al., 2005). Additionally, a laboratory study had shown that pools of this group could be artificially infected and would replicate BTV (Jennings and Mellor, 1988) and a later study using BTV-9, demonstrated that this ability varied according to population, and that, in some cases, the proportion of individuals of these groups capable of supporting replication was higher than originally thought (Carpenter et al., 2006). Hence, prior to the BTV-8 incursion, it was known that populations of the complex would be likely to be able to transmit the virus. Following the BTV-8 outbreak, pools of the Obsoletus complex and molecularly identified adults of *C. obsoletus* have been recorded as BTV positive via real-time RT-PCR in Germany in 2006 (BTV-8; Mehlhorn, 2007) and in Spain in 2008 respectively (BTV-1; J.M. Sánchez- Vizcaíno and M. A. Miranda, personal communication).

2.1.1.3. The Obsoletus complex: distribution, vector status and larval habitat

Within the Obsoletus complex, *C. obsoletus* and *C. scoticus* are distributed widely throughout the Palaearctic region but *C. montanus* appears to be less common (not being recorded in the UK, for example: Boorman, 1986). It is unclear at present whether the difficulties in separating this species by morphology from the other members of the complex have led to this species being under-reported. In Italy, *C. montanus*, identified using multiplex PCR, have been found mainly in Sardinia, Sicily and southern mainland in relatively low abundances and at low altitude (M. Goffredo, personal communication). The distribution of *C. obsoletus* and *C. scoticus* across Europe is not well documented, although populations of these species appear
extremely widespread, as based upon the collection and identification of males (e.g. the former species has also been recorded from the US and widely across Russia). Multiplex PCR molecular techniques (as described by Gomulski et al., 2006; Nolan et al., 2007; Mathieu et al., 2007), combined with an increase in the use of standardised surveillance across affected countries, should allow more detailed assessments of the distribution of the species to be considered in the future.

During the 2006 BTV-8 outbreak in the Netherlands, 2,500 parous Obsoletus complex midges were screened for virus in groups of 100 using an in-house RT-PCR assay (Meiswinkel et al., 2007). These groups did not yield any positive results. In 2007, Carpenter et al. demonstrated that C. scoticus was capable of supporting replication of both BTV-9 and BTV-8 to high levels (>3 log TCID₅₀) under laboratory conditions using a pledget feeding method paired with a species specific PCR. In this study C. obsoletus did not yield high titre infections (300+ individuals tested to May 2008). To date, C. montanus has not yet been examined in a BTV transmission role.

The breeding sites for C. obsoletus include a wide variety of habitats, including rotten banana stumps (Mellor and Pitizolis, 1979), forest leaf litter, stagnant water and marshy areas (Dzhafarov, 1976), horse dung (but not cow dung), and heaps of garden compost (Campbell & Pelham-Clinton, 1960). In the case of C. scoticus there is no clear information on the major breeding sites despite reports of developmental stages in the fungi Lactarius turpis and Armillaria mellea (Downes and Kettle, 1952; Buxton, 1960) and in marshy habitats in southern England (Boorman and Goddard, 1970). Recently, C. obsoletus/scoticus larvae have also been found in maize silage residues (Zimmer et al., 2008). The major breeding sites of this species, and of C. montanus across Europe, therefore remain largely unknown or unconfirmed.

2.1.1.4. Culicoides dewulfi Goetghebuer (1936)

This species has a Palearctic distribution, and has been recorded from across northern Europe (EFSA, 2007a; 2007b; 2007c; 2007d). This species appears to be less common and abundant in southern Europe than the Obsoletus complex although it is found across a similar geographical range. The morphological distinction of this species from species of the Obsoletus complex based only on wing pattern remains problematic for inexperienced operators. Nevertheless, separation in most cases can be achieved morphologically using certain characteristics (e.g. asymmetry of spermathecae, patterns on the abdomens) or via molecular markers.

BTV-8 has been recently detected using real-time RT-PCR in a single C. dewulfi pool of 40/50 individuals in the Netherlands (Meiswinkel et al., 2007).

Breeding sites for C. dewulfi have been reported as horse and cattle dung in the UK (Kettle and Lawson, 1952; Campbell and Pelham-Clinton, 1960) and in dung/soil ploughed mixtures in Belgium (Zimmer et al., 2008).

2.1.1.5. Culicoides chiopterus Meigen (1830)

This species has a Palearctic distribution being recorded from across northern Europe. Similarly to C. dewulfi, it’s abundance in southern Europe has not been well defined in comparison to the north. Identification of this species is usually through its size (commonly being much smaller than the other Obsoletus complex species) and lack of clear markings on wings.
This species has been recently implicated in the transmission of BTV-8 in northern Europe by the detection of RT-PCR-positive non-engorged blood females in The Netherlands and in France (Dijkstra et al., 2008; ProMED-mail 20080403.1222).

The breeding sites for C. chiopterus are similar to those described for C. dewulfi. Cow dung is the main larval habitat for this species, as cited by Kettle and Lawson (1952), Campbell and Pelham-Clinton (1960), and more recently by Zimmer et al. (2008).

2.1.2. Subgenus Culicoides

The subgenus Culicoides comprises about 50 species, and recently Gomulski et al. (2006) have proposed this subgenus should be split into four taxa: the subgenus Culicoides sensu stricto, the subgenus Silvicola Mirzaeva and Isaev 1990, the subgenus Hoffmania Fox 1948 and, the Fagineus complex. These authors recognized at least 14 European species from the subgenus Culicoides sensu stricto, among these the most common in Europe are: Culicoides pulicaris Linné, 1758; Culicoides punctatus Meigen 1818; Culicoides impunctatus Goetghebuer 1920; Culicoides newstead Austen 1921; Culicoides delta (Edwards) 1939; Culicoides lupicaris Downes and Kettle 1952.

Based on morphological and molecular (ITS2) considerations, the subgenus Culicoides can be further subdivided into 3 complexes: the Pulicaris complex, including C. pulicaris and C. lupicaris, the Newsteadi complex including C. newsteadi and C. punctatus, and the Impunctatus complex including C. impunctatus and C. delta (Gomulski et al., 2006; J.-C. Delécolle, personal communication). However, the taxonomic status remains unclear; for instance, morphological and molecular (ITS2) considerations differ about the inclusion of C. flavipulicaris into the Pulicaris complex (J.-C. Delécolle, personal communication) or into the Fagineus complex (Gomulski et al., 2006). Moreover, the taxa C. pulicaris and C. newsteadi may include further as yet undescribed species (Gomulski et al., 2006).

For convenience, it seems useful to group the species of the subgenus Culicoides sensu stricto as defined by Gomulski et al. (2006) under the term of Pulicaris complex. The Pulicaris complex has a Palearctic distribution being recorded from across northern and southern Europe and North Africa.

BTV has been isolated from pools of Pulicaris complex midges in Italy (Caracappa et al., 2003). The importance of this complex in BTV transmission may be reduced, however, relative to other potential vectors e.g. the Obsoletus complex because in general it seems to be less abundant and widespread. In southern Europe (specifically Italy and Spain), C. newsteadi is by far the most abundant species of this group but has to date not been demonstrated to be a vector. However, Carpenter et al., (2006) showed that populations of C. pulicaris/C. punctatus in the UK were capable of supporting the replication of BTV-9 to high levels when orally infected by this virus. Meiswinkel et al., (2007) tested 96 C. pulicaris/C. lupicaris and 20 C. punctatus, caught in the field in The Netherlands but failed to find RT-PCR positives for BTV-8 in these species.
Table 1. Summary of the main conclusions on potential Culicoides vectors of BTV in Europe. Much of this information is based on collections of Culicoides using light traps only.

<table>
<thead>
<tr>
<th>Species</th>
<th>Easy to identify morphologically on wing pattern*</th>
<th>Virus studies</th>
<th>Virus isolation/PCR detection</th>
<th>Abundance in Mediterranean Europe</th>
<th>Abundance in temperate Europe</th>
<th>Breeding sites</th>
<th>Diurnal activity recorded</th>
<th>Indoor/Outdoor presence recorded</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Culicoides imicola</em></td>
<td>Yes</td>
<td>Field</td>
<td>+/+</td>
<td>+++</td>
<td>-</td>
<td>Sun-exposed organically enriched mud in the farmyard</td>
<td>-</td>
<td>+/+</td>
</tr>
<tr>
<td>Obsoletus complex</td>
<td>Yes</td>
<td>Field/Lab</td>
<td>+/+</td>
<td>+++</td>
<td>+++</td>
<td>Forest, organically enriched soil.</td>
<td>+</td>
<td>+/+</td>
</tr>
<tr>
<td>C. dewulfi</td>
<td>No</td>
<td>Field</td>
<td>-/+</td>
<td>+</td>
<td>+++</td>
<td>Dung pats</td>
<td>+</td>
<td>+/+</td>
</tr>
<tr>
<td>C. chiopterus</td>
<td>Yes</td>
<td>Field</td>
<td>-/+</td>
<td>+</td>
<td>+++</td>
<td>Dung pats</td>
<td>+</td>
<td>+/+</td>
</tr>
<tr>
<td>Pulicaris complex</td>
<td>Yes</td>
<td>Field/Lab</td>
<td>+/+</td>
<td>+++</td>
<td>+++</td>
<td>Sun-exposed, vegetated, organically enriched mud in the farmyard</td>
<td>-</td>
<td>+/+</td>
</tr>
<tr>
<td>C. pulicaris</td>
<td>No</td>
<td>Field</td>
<td>+/+</td>
<td>+</td>
<td>+(+</td>
<td>Sun-exposed, organically enriched mud in the farmyard</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. newsteadi</td>
<td>Yes</td>
<td>Field</td>
<td>-/-</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. lupicaris</td>
<td>No</td>
<td>Field</td>
<td>-/-</td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C. punctatus</td>
<td>Yes</td>
<td>Field</td>
<td>-/-</td>
<td>(+)</td>
<td>+++</td>
<td>Sun-exposed organically enriched mud in the farmyard</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Individual species of *Culicoides* are relatively easy to assign to the correct species complex, but more difficult to identify down to species level (e.g. *Culicoides obsoletus/scoticus*)
* + low abundant species; +++ high abundant species; (+) and (-): suspected according to expert’s opinion but not confirmed by references
2.1.3. Assessing vector status: Methodology and interpretation

Four criteria have been described to identify an insect species as vector (WHO, 1967):

1) association in the field between the species and the disease;
2) virus recoverable from wild-caught non-engorged insects;
3) species able to become infected after natural or artificial infection;
4) species able to biologically transmit the infection.

The species fulfilling all these criteria should be considered “proven vectors”, while the species that fulfil some of the criteria could be considered “suspected vectors”. The other species, associated with the vertebrate hosts, remain “potential vectors”.

The BTV-8 incursion in northern Europe has highlighted the difficulties in integrating standard laboratory diagnostics into studies of vector competence. To date the only field examinations carried out have relied upon the use of real-time RT-PCR based techniques which identify the presence of viral RNA rather than infectious virus, to implicate virus presence in pools of potential vector species. The validity of these techniques is at best debatable for the following reasons:

- It is unknown what proportion of the parous Culicoides caught at light has actually fed upon a viraemic animal.
- It is not known how many midges within a pool that tests positive for BT viral RNA are infected.
- A large proportion of individuals of even competent Culicoides vector species are known to possess barriers that prevent virus dissemination and can result in persistent, non-transmissible infections (see Mellor, 2000 for review). Pool-based identification of viral RNA cannot differentiate between these and fully disseminated infections (i.e. those potentially capable of subsequently infecting a susceptible host).

Where isolation rates are very low, as has been the case in the studies published to date, these issues can entirely invalidate studies aiming to implicate new vector species. Additionally, when using real-time RT-PCR it is not known:

- If the ‘positive’ result represents the presence of infectious virus or merely the presence of non-infectious RNA. To date it has not been demonstrated that Culicoides that are non-susceptible to infection are capable of entirely clearing a virus-infected blood meal and subsequently producing a negative result via real-time RT-PCR, or if they can, how long this process takes.
- If a low cycle threshold (CT) value, indicative of high levels of viral RNA, represents a transmissible virus infection

The recent publication of a technique for rapid throughput of individual Culicoides should allow most of the above difficulties to be addressed (see Veronesi et al., 2008). This technique could be paired with cell-based isolation of live virus from individuals (including the use of insect cell lines), and a multiplex PCR to differentiate vectors to species level. Full dissemination of virus is not easy to demonstrate in Culicoides due to the difficulties in removing salivary glands from such a small insect and the fact that field virus load is unlikely to be able to be determined accurately due to the requirement of adaptation to cell culture (which frequently requires blind passages). Once the isolation of live virus has been
convincingly demonstrated from particular species, however, these species can be targeted for further investigation to determine the proportion of transmissible infections.

**Conclusions:**

In the last two years outbreaks of BTV-8 and BTV-1 have occurred in Europe outside the range of *C. imicola*, confirming earlier findings from southern Europe and laboratory-based studies from the UK, and demonstrating that northern Palaearctic species of *Culicoides* are able to transmit BTV.

To date, the specific vector(s) of BTV in these areas have not been identified, although strong circumstantial evidence implicates members of the Obsoletus complex, *C. dewulfi*, *C. chiopterus* and the Pulicaris complex as the likeliest candidates. This list is probably not exhaustive and the identification of additional vector species is likely.

To date, standardised and appropriate testing protocols to determine the vector competence levels of *Culicoides* species for BTV in Europe have not been applied. In northern Europe this has led to the use of pool-based real-time RT-PCR investigations on field caught parous female midges to imply vector competence levels. These methods have several technical drawbacks and do not provide a measure of vector transmission in the field.

Similarly, studies from southern Europe, based around cell-based isolation of virus, while superior to those using real-time RT-PCR, are still difficult to interpret due to the use of pool-based isolation methods and an inability to accurately assess viral dissemination levels. Recent publications that allow high-throughput processing of *Culicoides* for virus isolation may allow some of these issues to be addressed and also enable standardisation between laboratories.

Laboratory-based studies on vector competence remain time consuming and difficult to perform outside the areas of BTV transmission as they require specialist laboratory accommodation.

Vector competence is just one element of the vector capacity of a species for BTV transmission. Other elements some of which have been assessed in southern Europe, include host preferences, biting rates, vector survival, location of breeding sites, temporal and spatial distribution, and abundance. An integrated assessment of all of these elements is required to gain a realistic idea of the importance of each potential vector species.

**Recommendations:**

Molecular techniques should be used for the differentiation of species from the Obsoletus and Pulicaris complexes, especially in epidemiologically relevant areas. Where possible these techniques should be integrated into surveillance schemes.

Training on dipteran taxonomy is additionally also recommended to be conducted at a European level.

Varying levels of circumstantial evidence has linked *C. imicola*, the Obsoletus complex, *C. dewulfi*, *C. chiopterus* and the Pulicaris complex with BTV transmission in Europe. Consequently, targeted surveys should be undertaken for defining their temporal and spatial distributions across Europe.

A standardisation of techniques to implicate field-caught *Culicoides* vectors in BTV transmission is required. It is recommended that this be used to harmonise studies across Europe as, to date, testing methods, in northern Europe particularly, have been inadequate.

Laboratory testing for vector competence should be carried out, where possible, in parallel with field-based testing.
Analyses of the vector competence of particular species should be made with reference not only to the ability of the vector to become infected by, replicate and transmit the virus but also to its wider ecological requirements (i.e. its vector capacity), which may vary with region and season.

2.2. Adult feeding habits/host preferences - an update

After reviewing the information presented in the previous EFSA Report (EFSA, 2007a) and Opinion (EFSA, 2007b), no significant updates have been identified on this topic. Most knowledge concerning the adult feeding habits was obtained some 20 years ago (e.g. McCall and Trees, 1993; Mellor and McCaig, 1974; Nielsen, 1971). However, it is known that current research on this topic is being conducted by several scientific institutions and universities in France, Germany, Spain and United Kingdom.

2.2.1. Host Preferences

No standardised tests of host preference exhibited by Culicoides species in northern Europe have been carried out to date. In C. imicola examined in Israel, amongst the species considered horses are the most preferred host, while sheep, dogs, birds respectively are the least preferred hosts (Braverman et al., 1971; Braverman et al., 1977; Braverman and Chizov-Ginzburg, 1996). Some of these findings were based on indirect calculations by comparing the rate of blood engorged females captured inside horse stables with the rate of blood engorged females trapped near other hosts (Braverman and Frish, 1984).

Conclusions:

From a practical point of view and considering the risk of BTV transmission, the Culicoides species that transmit BTV are considered to be opportunistic blood feeders upon birds and mammals, but they are strongly attracted to large mammals.

2.2.2. Biting areas on hosts

To summarize information already included (EFSA, 2007a), it is well documented that there is a difference in adult Culicoides habits in relation to the zones of the host they bite. This seems to be related to the type of host: sheep are attacked mainly on the bare areas (head, ears, belly and legs; S. Carpenter, personal communication). Biting sites on cattle appear to be less restricted. The dorsum seemed to be the preference zone for C. imicola (Braverman et al., 1983; Braverman et al., 2003), whereas biting areas for species from the Obsoletus complex on cattle are more general (e.g. McCall and Trees, 1993; Mellor and McCaig, 1974; Nielsen, 1971). C. obsoletus prefers to bite the belly. This information should be taken into account when developing protocols for insecticidal treatment of cattle and sheep.

2.3. Indoor / outdoor activity

In northern Europe, it has been reported by several authors (see EFSA, 2008c) that the species from the Obsoletus complex and C. dewulfi may feed on animals kept indoors. There are previous studies suggesting that the degree of openness of a building is directly associated with the numbers of individual insects entering (Barnard, 1997; Meiswinkel et al., 2000; Calvete et al., unpublished data). Thus, an open/closed surface ratio could be calculated in order to compare the indoor and outdoor characteristics of premises. Unfortunately, most recent papers, pertaining to this issue are brief notes or letters and do not include such a ratio. Thus, meaningful comparisons across different farming systems in different countries are extremely difficult to conduct in the present update. Indoor activity by Obsoletus complex has recently
been reported in France (T. Baldet in EFSA, 2007a), Belgium (Losson et al., 2007) and the UK (M. Baylis, personal communication). In the UK, Baylis et al. (unpublished data) reported a 6-fold reduction of Obsoletus complex midges collected inside cow sheds compared to outside early in the season. However, later in the season, as ambient temperatures decreased, this advantage was lost though the overall numbers of Culicoides collected was also significantly reduced at this time. These data have been acquired by comparing captures obtained by outdoor and indoor light traps. During surveys of Culicoides in parts of Brandenburg state (Germany), blood engorged females were regularly caught by means of traps located inside pens during summer (up to 60%) and winter periods (Bauer et al., unpublished data). It is still not clearly demonstrated whether inside-outside behaviour is modulated by the presence of the animals only inside stables or by the difference of temperature between outside and inside stables. In addition, it is important to point out that all the studies about indoor/outdoor activity are indirect measures based on light trapping. In this sense, it should be taken into account that the response of Culicoides species to UV stimuli is still not fully understood. Further, the trapping efficiency of light traps indoors, as opposed to outdoors, is likely to be far higher. When indoor activity has been described in some areas (i.e. France), it seemed to extend the period of activity of the Culicoides for several weeks during which time activity outdoors was absent (T. Balenghien, personal communication). Until recently, there has been no evidence concerning the relevance of indoor activity to the transmission of BTV, despite blood-fed females frequently being collected inside stables. However, recent work conducted in Germany using emergence traps (B. Bauer, personal communication), has raised the possibility that some Culicoides species may breed inside premises. The effect that this may have on BTV transmission is at present uncertain and it is unclear how widespread this phenomenon is.

To date, the endophilic behaviour of the Obsoletus complex populations in southern Europe has not been assessed. However, there is some evidence based on light trap collections indicating that C. imicola also can be captured inside stables (C. Calvete, personal communication). Similarly, the use of suction light traps indoors in Israel has shown that small numbers of C. imicola can enter “closed” cowsheds (Y. Braverman, personal communication).

Conclusions:
The major potential vector species/groups are all known to feed upon cattle and sheep but vary in the body region upon which they feed from species to species and from host to host.

There is clear evidence of indoor activity (including blood feeding) of the Culicoides vector species, especially during winter time, which may extend the seasonal activity of such species when outdoor activity is absent by an, as yet, undefined period. This has been mostly suggested by comparing indoor and outdoor light trap captures.

To date, no clear scientific evidence has been reported concerning the role of adult Culicoides surviving inside stables in prolonging the transmission period of BTV. Further, the period of survival of such adults inside premises is also unknown.

Recommendations:
The information about preferred biting areas requires confirmation to vector species level including the effect of host breed and fleece length (in the case of sheep).

When indoor activity of Culicoides vector species is investigated, unimpeded and impeded (i.e. open-closed) access ratios are recommended to be calculated to facilitate comparisons among premises of different countries.

Further data are required to determine the significance of indoor biting and species of Culicoides involved, housing conditions and survival of adult Culicoides inside premises.
These data should be collected using less biased trapping methods than light traps (e.g. direct collections from hosts or suction traps).

2.4. Adult biting rates

The absolute number of adults of the different species of Culicoides which may occur in the geographical region of a particular host species is extremely difficult to estimate. As presented in the previous EFSA Report on bluetongue (EFSA, 2007a), biting rates on each host species can vary widely from place to place and from vector species to vector species. In general, it can be assumed that when animals are close to the breeding sites of an abundant species/complex (i.e.: C. imicola; Obsoletus complex), the host would be attacked by a large number of Culicoides females of those species, depending on the time of the season. It is also important to point out that light trap captures provide inadequate information regarding animal biting rates. Blood-engorged females are rarely captured when traps are placed adjacent to breeding sites, although they may be more readily taken in traps directly adjacent to animals.

Recent studies conducted by Carpenter et al. (2008) in UK investigated Culicoides biting Poll Dorset ewes at a site using a drop trap system and a modified multiplex PCR (Mathieu et al., 2007) to identify all individuals caught to species level. In 192 standardised catches 2,184 Culicoides were caught and all four Obsoletus complex species present in the UK were found to feed on sheep, together with C. dewulfi, C. chiopterus and C. nubeculosus. Culicoides pulicaris and C. punctatus were not found on the sheep. Culicoides obsoletus was the most common species trapped (51.8%), followed by C. chiopterus (31.5%), C. scoticus (10.2%) and C. dewulfi (6.5%). Light trap catches (carried out according to standard protocol using the Ondestepoort Veterinary Institute[OVI] manufactured trap) at the same location did not accurately reflect these results, with C. chiopterus constituting only approximately 1% of the total catch, and with individuals of the Pulicaris complex being recorded at light even though they were absent in the biting rate trials. Culicoides chiopterus has also been found in large numbers on cattle by previous authors (e.g. Nielsen, 1971). Attempts to catch Culicoides spp. in a commercial mosquito trap, emitting a warmed stream of carbon dioxide (0.5 L/min) and 1-octen-3-ol (6-8 mg/h), and a delta trap design emitting 0.2 L/min, failed almost entirely, catching less than 5 C. obsoletus s.l. individuals/night at the site. Recent studies in Spain (Sarti Monteys and B. Mullens, personal communication) also confirm that species relative abundance and composition widely differ when different sampling methods, such as CO₂ traps, UV traps and animal traps are compared at the field level.

Conclusions:

From the information presented above, it is clear that light trap results can be biased towards some Culicoides species, and away from others, thereby missing species that may play an important role in the BTV transmission.

Furthermore, light trap captures do not provide reliable information on host biting rates or feeding preferences, since non-engorged nulliparous and parous females, are predominantly captured and at unknown rates compared with blood engorged females.

Therefore, inferences regarding relative biting levels from light trap collections are likely to be highly approximate at best. If they are undertaken, such inferences must be made very carefully and with an appreciation of the above facts.
The adult biting rate of the different species of *Culicoides* is difficult to measure; however, it is important to do so to be able to make a realistic assessment of the relative importance of the different vector species.

**Recommendations:**

Results from animal traps and light traps in field studies should be compared directly across Europe, in order to estimate on-animal vector diversity, to assess the biting rates of the different *Culicoides* spp., and hopefully to relate animal biting rates to light trap collection data.

### 2.5. Hours of attack and dispersal

In general, the biting activity of the different *Culicoides* spp. in Europe and most of the rest of the world is highest during crepuscular and nocturnal hours. For example, crepuscular and nocturnal activity of *C. imicola* and of species of the Obsoletus complex seems to be widely assumed in southern Europe. In a recent study conducted in the Balearic Islands using a light trap equipped with a catch jar rotator (D. Borràs *et al.*, personal communication), there was continuous activity of *C. imicola* and Obsoletus complex, with nulliparous and parous females detected from sunset to sunrise. In Israel, 43 hourly suction night trappings with black lights showed that *C. imicola* was on the wing throughout the night and the largest numbers were captured during the first 3 hours of night (Braverman *et al.*, 2008). However, light traps, while convenient, work only in relative darkness. Recent findings suggest a limited level of diurnal feeding activity of the species from the Obsoletus complex and other species such as *C. dewulfi*, especially in northern Europe and particularly in late autumn. A large number of other studies, all carried out in mid-summer, have claimed that biting activity of northern European species is primarily crepuscular (e.g. Zhdanova, 1975; Olbrich and Liebisch, 1988 Wulfsberg, 1989). Obviously light traps are not adequate for detecting such activity, hence other methods, such as bait animals (host seeking) or vehicle-mounted or suction traps (general activity), could be used. However such studies are difficult and therefore are relatively uncommon. There is also no information about what proportion of each *Culicoides* population may exhibit such diurnal behaviour and the relevance for BTV transmission, see below.

In recent research conducted in the UK by Carpenter *et al.* (2008), animal baited traps showed some evidence of diurnal activity. However, catches were only made between 6:00 pm and 10:00 pm (i.e. in the period leading up to dusk) in July. Definite conclusions regarding the importance of dial periodicity cannot at present be made, although the highest numbers of all four species recorded were at low light intensities (<200 W m$^{-2}$). It was noticeable, however, that low levels of midge activity were recorded with all Obsoletus complex species even at $>$26°C and 360 W m$^{-2}$ solar intensity, indicating that there could well be biting throughout the day, particularly in late season (as suggested by Service, 1969). There is also the possibility that periodicity patterns change over the seasons (as described for *C. sonorensis* in the US: Barnard and Jones, 1980).

Regarding dispersal, little new information has been published from the last EFSA Report on bluetongue (EFSA, 2007a). It is still assumed that adult *Culicoides* usually disperse only short distances from the breeding sites (several hundred meters to a few kilometres). It is also fairly well accepted that adults can passively fly on air streams and thereby dispersed from one area to another, even overseas, by wind streams (e.g. Sellers, 1980; Hardy and Cheng, 1986 Gloster *et al.*, 2007, Hayashi *et al.*, 1979). Specifically, it has been shown that *C. imicola* is vulnerable to air stream carriage, as a proportion of the adult population has a tendency to fly upwards (Braverman and Linley, 1993). Similarly, Sanders *et al.*, (unpublished), captured *C. obsoletus*...
and *C. pulicaris* at 200m altitude using a meteorological balloon at Cardington in the UK, demonstrating that these potential vector species can be carried on airstreams.

Carpenter *et al.* (2007) recently reported that males and nulliparous females of the potential vector *C. impunctatus*, following emergence, rest upon downy birch (*Betula pubescens*), which are used for facultative mating and the development of the initial autogenous egg batch. Such information could be of value for the targeted use of approved insecticides. However, to date there is no clear information about resting sites for the main vector species in Europe. This lack of information on where *Culicoides* adults rest is an important gap in our knowledge of the biology of this genus worldwide.

**Conclusions:**

Present data indicate that the biting activity of the majority of the vector species in Europe primarily occurs during the crepuscular and nocturnal hours.

There are indications, however, that under suitable meteorological conditions and particularly during the later part of the season, diurnal feeding activity of potential vector species of *Culicoides* may occur.

The implications for BTV transmission of vector daylight activity are at present unclear as trapping programmes based only on light traps are not adequate to provide information regarding the daylight biting activity.

Dispersion of vector species of *Culicoides* at the farm level is still very poorly understood, but assumed to be short distances from the breeding sites.

Long distance dispersion of vector *Culicoides* on winds over scores or even hundreds of km has been reported by several workers but the proportion of a population that are involved is thought to be very small.

**Recommendations:**

Daylight activity of certain species of *Culicoides* is recommended to be further investigated in order to assess its relevance for the transmission of BTV.

Alternative sampling methods to light traps such as animal bait, suction and truck traps are recommended to be used when daylight activity of *Culicoides* is being investigated.

Dispersal of *Culicoides* could be examined both by viral movement in space and time and in terms of the genetics of populations on farms of known distance from each other.

2.6. **Overwintering – an update**

In temperate areas, most *Culicoides* species overwinter as fourth-instar larvae, leading to the virtual absence of adults during winter months (Mellor *et al.*, 2000). On the contrary, in the Mediterranean countries of southern Europe, *Culicoides* adults (e.g. *C. imicola*; species of the Obsoletus complex) can occur throughout the winter, as for example in the south of Spain, Portugal and Italy. However, in some areas of these countries, a complete absence of adult captures in light traps can be observed during the winter time especially in the case of *C. imicola* (Miranda *et al.*, 2004). In northern Europe during the winter 2006-2007, entomological surveillance systems collected very few active adult *Culicoides*, and those that were captured were almost exclusively from the Obsoletus complex and the clear majority (> 95%) was nulliparous (EFSA, 2007a; Losson *et al.* 2007). Moreover, during the 2007-2008 winter at lower latitudes, few adult *Culicoides* were collected in France from the middle of December to February, of these 97% were Obsoletus complex, and 82% of these were females with a parity rate of 48% (T. Balenghien, personal communication). The same parity rate also occurs during
winter in Italy (M. Goffredo, personal communication). These findings suggest that very small numbers of Obsoletus complex adults may be active at any time during the winter in both northern and southern Europe. The collection of nulliparous females during the winter suggests either the continuous emergence of small numbers of individuals or long-term persistence of nulliparous females without blood feeding. The occasional collection of parous females suggests that some emerged nulliparous females do engorge and lay eggs and/or that old ‘previous season’ adults can survive through most or all of the winter. Indeed, it has been observed that midges from the Obsoletus complex can survive up to 92 days at 17-25 °C, provided only with sucrose solution without blood feeding, and they can survive and quickly recover after 10 days at 4°C (Goffredo et al., 2004b). In another study, they were shown to have survived up to 3 months at cool temperatures (Boorman, 1991).

With regard to the overwintering of BTV in vectors, this could occur either by the survival of the virus in overwintering virus-infected adults or in vertically infected larvae. To date the evidence of transovarial transmission of BTV is extremely weak and no isolation of live BTV has been made from overwintering populations of Culicoides. However, Beer and Hoffmann, FLI, (personal communication) observed an infection of a bovine between January 20th – February 10th 2008. Initially the animal tested negative but on February 10th it was diagnosed positive by PCR. Hence, the hypothesis of virus survival in long lived Culicoides females (i.e. >120 days in northern Europe) cannot be entirely excluded, until studies of Culicoides lifespan at low temperatures have been completed. Indeed, if a lifespan of >120 days is envisaged small numbers of infected ‘previous season’ female Culicoides could survive during the winter and/or newly emerged females could engorge on viraemic hosts in the first winter months and harbour the virus until the seasonal increase in temperature allows virogenesis to restart and be completed. This would be likely to result in sporadic overwintering in locations where the virus was last active in the previous transmission season. However, the observed pattern of BTV-8 in northern Europe does not fit this picture as overwintering occurred widely and was concentrated around areas of intense transmission rather than most recent transmission. Subclinically infected viraemic vertebrate hosts are unlikely to play a role in the overwintering of the virus, as the maximum period of infectivity of such hosts has been set at only 60 days, which is insufficient, in northern Europe, to bridge the winter period. The occurrence of persistently infected sheep has been reported in laboratory experiments (Takamatsu et al., 2003) but this phenomenon, though looked for has not been confirmed in laboratory experiments elsewhere or in the field (White and Mecham, 2004; Melville et al., 2004).

Conclusions:
In southern Europe, adult C. imicola, Obsoletus complex and Pulicaris complex may be absent for variable periods of the winter or present continuously in low numbers depending on location, and weather conditions.

In northern Europe, adult C. dewulfi, C. chiopterus, Obsoletus complex and Pulicaris complex virtually disappear for a period of the winter (> three months), although very low numbers may be recorded at any time during the winter dependent upon weather conditions.

No single mechanism has been demonstrated to be responsible for the overwintering of BTV in northern Europe. Year-round presence of infected Culicoides remains the most likely although transplacental transmission cannot be excluded.

Recommendations:
Due to geographical and inter-annual variations in Culicoides activity, systematic and extensive entomological monitoring is advisable to be implemented to detect overwintering adults.
Besides light traps, alternative sampling methods such as animal bait, suction and truck traps are recommended to be used for detecting overwintering adults as these are likely to be diurnally active.

Analysis of the parous females captured during winter time is advisable in order to detect BTV infected individuals. This analysis is recommended to be coordinated across Europe as numbers are likely to be extremely small.

The survival rates of adults Culicoides at lower temperatures is recommended to be further investigated under laboratory conditions.

2.7. Distribution and abundance of Culicoides vectors in the EU

In general, all the European countries affected by BT during the last 10 years already have distribution maps of the main vector species. However, this information is still not available on any of the official websites dedicated to BTV at the European level, much information being available only in scientific publications.

The distribution of C. imicola in Italy (Goffredo et al., 2001; 2003; 2004a; 2004b; Scaramozzino et al., 2002; Calistri et al., 2003, De Liberato et al., 2003; Torina et al., 2004), France (Corsica, south-eastern Var department in Delécolle and De la Rocque, 2002), Spain (Ortega et al., 1997; Ortega et al., 1998; Miranda et. al., 2003, Sarto i Monteys and Saiz-Ardanaz, 2003; Sarto i Monteys et al., 2004; Sarto i Monteys et al. 2005; Calvete et al., 2006, Calvete et al., 2008), Greece (Patakakis, 2004), Portugal (Capela et al., 2003) and Malta (Goffredo et al., 2004c) is well known. The northern limits of its range are likely to change from year to year due to local climatic variations, climate-change and/or increased surveillance effort. There is clear evidence that C. imicola is expanding its distribution to the north (Purse et al., 2005). In some southern European countries, as for example Italy, France and Spain, the possible spread of C. imicola to higher latitudes is evaluated by means of the national entomosurveillance programmes. In Italy, on the basis of permanent collection sites (1/1,600 km²) in the last 6 years, C. imicola did not show any northward spread. In Spain, there is evidence suggesting a northward expansion of this species (Calvete et al., 2006) based upon new records of C. imicola that have recently been made in the north of the country. In France, despite established populations of this species have been detected, only recently (2004), on the mainland C. imicola remained localised in some parts of the Var department and there is apparently no further evidence of its northwards spread through the mainland (T. Balenghien, personal communication).

The Obsoletus complex, has been shown to be ubiquitous across the whole of Europe, and data are also available from most of the affected countries. The group has been recorded across all of the BTV-affected countries independent of BTV serotype and is very common in Italy (Goffredo et al., 2004b; 2008; De Liberato et al., 2005; Torina et al., 2004), France (T. Balenghien, personal communication), Portugal (Capela et al., 2003) and Spain (Miranda et al., 2004; Sarto i Monteys et al., 2004, Calvete et al., 2008), and virtually all of northern Europe (EFSA 2007c). Although widespread across most of southern Europe, the Obsoletus complex seems to be more abundant in the northern areas of those countries (e.g. Italy and Spain) (Calvete et al., 2008). The distribution of the Obsoletus complex has been reported to be closely associated with deciduous forests in Italy (Conte et al., 2007). It is also worth mentioning that in Corsica, Greece, Italy, Spain, Portugal and some areas of mainland France (Var department) there are areas where the distribution of the Obsoletus complex overlaps with that of C. imicola (Goffredo, 2004a; Calvete et al., 2008). Despite this overlap, the transmission of BTV-2, -4, and -16 has been restricted within the distribution of C. imicola. BTV-9, however, is transmitted both within the C. imicola distribution and also further north in
the Balkans in areas dominated by Palaearctic species and where *C. imicola* has not been recorded. BTV-1 is transmitted mainly within the *C. imicola* distribution but recently has expanded into northern Spain, where *C. imicola* is rare and SW France where *C. imicola* is apparently absent. Furthermore, BTV-1 PCR-positive, *C. obsoletus* have recently been identified in the south of Spain (J.M. Sánchez-Vizcaíno and M.A. Miranda, personal communication), in an area where both *C. imicola* and Obsoletus complex overlap in time and space.

The most common species in UV traps in northern Europe are species from the Obsoletus complex, being about 80% of the collection in France, (T. Balenghien, personal communication), 50% in the UK and 90% in Northern Italy (Goffredo et al., 2008). This is also true in The Netherlands (EFSA, 2007c), Belgium (Losson et al., 2007) and Germany (Mehlhorn, 2008). In The Netherlands in 2006 the Obsoletus complex represented the 37% of total *Culicoides* (Meiswinkel et al., 2008 in press). The presence of males in collections is considered to indicate the presence of breeding sites nearby.

*Culicoides dewulfi* is also a species with a Palearctic distribution, cited in Belgium, Italy, France, Germany, Netherlands, Portugal, Spain and UK. In Europe, this species seems to be more abundant in northern than in southern countries. For example, during surveillance in 2006 in the Netherlands it was found to comprise around the 13% of the total capture of *Culicoides* (Meiswinkel et al., 2008 in press). It has been also found to be widely distributed in Belgium (De Deken and others, unpublished data, in Losson et al., 2007). In France, this species represents 6% of the total captures of *Culicoides* in north-eastern France, but only 1% in south-eastern France (T. Balenghien, personal communication). In Northern Italy *C. dewulfi* is less than 2% of total *Culicoides* (Goffredo et al., 2008). Finally, in Spain it has not been detected in the national entomological monitoring programme over the last two years.

*Culicoides chiopterus*, recently identified as a potential BTV vector, is another well distributed species across the whole of Europe, being cited from Belgium, Italy, France, Germany, Netherlands, Portugal, Spain and the UK. In general, this species seems to be more abundant in northern than in southern countries but is usually captured in lesser numbers than the Obsoletus complex. Indeed, *C. chiopterus* represents about 6% of the total light trap captures in northern France and it is rare in the south (T. Balenghien, personal communication). In 2006 in The Netherlands this species represented the 7% (Meiswinkel et al., 2008 in press). However, as stated above, this and other species may be underestimated by light trap samples (Carpenter et al., 2008)

In the case of the Pulicaris complex, *C. pulicaris* seems to be widely distributed in Europe, being cited from both northern and southern European countries, but being less abundant than the Obsoletus complex. For example, only 29 females have been captured in the Balearic Islands from 2004 to 2007 (D. Borràs and M.A. Miranda, personal communication). It has been cited as comprising less than the 5% of total *Culicoides* in the Netherlands and in northern France, whereas in Belgium it seems to be a relatively more abundant species (EFSA, 2007c). However, other species included in the Pulicaris complex, such as *C. newsteadi* and *C. punctatus* are more abundant than the Obsoletus complex in Spain (D. Borràs and M.A. Miranda, personal communication). In Italy the Pulicaris complex is common and abundant in Southern Italy (Goffredo et al., 2004a) and, within the complex, *C. newsteadi* and *C. punctatus* are the most abundant. Meanwhile in northern Europe, as for example in The Netherlands in 2006, *C. punctatus* was the most abundant species (30%) after the Obsoletus complex (36%) (Meiswinkel et al., 2008 in press; EFSA 2007c). Other species from the Pulicaris group, such as *C. lupicaris*, are also well distributed in Europe and cited in many countries, but are less abundant than other subgenus *Culicoides* species.
As has been already mentioned in chapter 3.4 (Adult biting rates), there is evidence demonstrating that all the species of *Culicoides* in Europe are not equally attracted to UV light traps, particularly when their relative abundance is compared with alternative sampling methods such as animal bait traps. This may result in a biased estimation of the abundance and distribution of important species for the transmission of BTV in Europe.

**Conclusions:**

The distribution of the main vector species is well known in each of the BTV affected countries, however the information has not been compiled and made available collectively at a European level.

The distribution of *C. imicola* is well documented in the southern European countries. The northward expansion of this species is detected each year by the entomological monitoring programmes operating in some countries. It is one of the most abundant species in the southern Europe.

The distribution of the Obsoletus complex includes all the countries in Europe, although it is relatively more abundant in the northern regions. In the southern regions of Europe, the distributions of *C. imicola* and the Obsoletus complex frequently overlap.

*C. dewulfi, C. chiopterus* and Pulicaris complex are also widespread in Europe, especially in the northern countries. Nevertheless, their abundance as estimated on the basis of light traps has been always reported as being lower than the Obsoletus complex.

Distribution and abundance data solely obtained by only using UV light traps may underestimate some species important for the transmission of BTV.

**Recommendations:**

In order to better understand the current distribution of the species included in the Obsoletus complex, it is recommended to perform co-ordinated European surveys using the molecular identification of Obsoletus complex females to species level. In addition, the routine identification of males from these complexes is also advisable to have a clearer picture of each species distribution. These data should be made available in the EU centralised database (BT-Net).

An increased number of sampling sites around the known northern limits of the range of *C. imicola* is recommended to improve understand of the role of this species in the northward spread of BTV.

**Recommendations for research:**

Alternative sampling methods to UV light traps, such us animal bait, suction and truck traps are recommended to be used where possible to improve our understanding of the relative abundance and distribution of the different *Culicoides* species in Europe.

2.8. **Vector seasonality and age grading of the vector populations – an update**

In general, and depending on the local climate *Culicoides* species can be uni-, bi-, tri- or multi-voltine (Birley and Boorman, 1982; Braverman et al., 1985; Holmes and Boorman, 1987). To date, entomological surveillance programmes in the different BT affected European countries have provided a reasonably complete picture of the *Culicoides* spp. seasonality. As was reviewed in the previous EFSA Report on bluetongue (EFSA, 2007a), the seasonality pattern of *C. imicola* and Obsoletus complex has been widely studied in southern Europe, showing differences between both species. *Culicoides imicola* and Pulicaris complex midge populations
peak usually in September/October, meanwhile Obsoletus complex peaks usually in June/July (Ortega et al., 1997; Miranda et al. 2004). In contrast in northern European countries, the species from the Obsoletus and Pulicaris complex as well as C. dewulfi, C. chiopterus and C. pulicaris are usually more abundant during May and June. The emergence of populations is driven by meteorological factors and the species-specific biology. For example, in Spain it has been estimated that the number of days from the last detection of Culicoides spp. activity from the previous year to the first detection of adults the next year may range from 87.2 ± 66.2 days (South Spain; Málaga region) to 142.0 ± 73.5 days (North Spain; Lugo region) (M.A. Miranda and C. Calvete, personal communication). The first detection of Culicoides adult activity, particularly of parous females, is therefore of great importance for the estimation of risk of transmission of BTV, however it has been never numerically estimated and no references are available about the annual first detection of vectors across Europe.

The seasonal pattern of the different Culicoides species may vary from year to year due to changes in the meteorological conditions, breeding sites or host availability. This has been demonstrated in Corsica by Gerbier et al., (2008) where during year 2003 the peak of abundance of C. imicola was different from that of 2002. It is widely accepted that the seasonal peak of BTV transmission is linked with the period of high abundance of the competent vector species (Mellor, 2000) and thus could be considered and important factor when risk of transmission is needed to be estimated.

There is little published information in Europe about the annual variation of the gonotrophic development (age grading) of the Culicoides females, especially regarding parous females, which are accepted to be the only transmitter of the virus. In the Netherlands, it was found that parous females were more abundant in relation to nulliparous females in late autumn- early winter (EFSA, 2007a). In the case of C. imicola in Spain (D. Borrás and M.A. Miranda personal communication), it has been estimated that no parous females were detected from January to March from 2004 to 2007. The highest proportion of parous females was found in August, when they represented 40 % of the total capture. The technique described by Dyce (1969) is the simplest system for a quick age grading of the samples obtained by the UV light traps.

In Europe, vector seasonality has been exclusively assessed by using light traps placed mainly outdoors, thus there are no long term data sets available today on the implications of indoor activity and daylight activity in the reduction or extension of the seasonal annual pattern of Culicoides vector species.

**Conclusions:**

In Europe, there is a clear understanding of the seasonality of C. imicola and Obsoletus complex species. Similar information related to other species such as C. dewulfi, C. chiopterus and Pulicaris complex is much more incomplete. The annual seasonal pattern of emergences in the spring and die off in the winter is modulated by the climate, species specific biology, breeding sites and host availability.

There is little information available in Europe regarding the annual seasonal variations in abundance of nulliparous and parous females, despite the importance of age grading of Culicoides females as a factor related to the risk of transmission of BTV.

To date long term data sets about the implications of indoor activity and day light activity on the reduction or extension of the seasonal annual pattern of Culicoides vector species are not available.
Recommendations:

Due to the importance of defining the seasonal pattern of the Culicoides vector species, their monitoring on a year-round basis using permanent sampling sites is recommended. It is also important to link data obtained from entomological sampling with data obtained from the serological data of BTV surveillance programmes.

A harmonisation of the above entomological monitoring programmes across Europe is recommended for a better assessment of the annual seasonal pattern, including age grading of females, the likely total absence of adults during winter and the first detection of Culicoides activity the following season.

Further research is recommended in order to estimate the relevance of the indoor and day-light activity in either the extension or reduction of the seasonal pattern of the different vector Culicoides species. Indoor and day-light activities should be defined and standardized for research purposes.

3. Approach to a vector low abundance season definition – an update

According to Annex V of Commission Regulation EC 1266/2007 (EC, 2007), the definition of the Seasonal Vector Free Period (SVFP) is mainly based on the complete absence of adult Culicoides (specifically C. imicola) captured in light traps and the determination of a certain threshold of Culicoides abundance. When the cited threshold was not possible to be determined, then a general criterion of less than 5 captured parous females of those suspected vector species has been recommended to be adopted (EFSA, 2007b). Cessation of vector activity has been determined until now by means of light trap collection of Culicoides spp. When no catches of adult Culicoides were detected it was assumed that adult activity and/or population density had significantly decreased. This relative cessation of activity is mainly due to abiotic parameters, such as temperature, as well as the intrinsic biology of the different species, as for example the different seasonal pattern showed by C. imicola and the Obsoletus complex (see also previous chapters 3.6 and 3.8)

The current definition of the SVFP in Regulation (EC) No 1266/2007 (EC, 2007) was then developed in line with the OIE Code according to the epidemiological situation regarding certain serotypes and vectors, as well as specific geographical areas in the EU. However, in the light of the actual situation in Europe, it is important to review the concept of SVFP as well as the general and specific criteria involved. In particular, the complete cessation of vector activity measured by means of light traps seems to be restricted during winter to Afro-tropical species such as C. imicola and only in specific areas of southern Europe, meanwhile in other areas such cessation is too short (less than 15 days) or never reached (Ortega et al., 1997; Miranda et al., 2004; Calvete et al., 2006). In addition, it has been demonstrated that other species such as those species included in the Obsoletus and Pulicaris complexes can be captured throughout the year both in southern and northern Europe, indicating that in certain areas, their activity does not stop during the winter time (Rawlings and Mellor, 1994; Miranda et al., 2004; Losson et al., 2007). Temperature modulates the immature stages development, as well as the abundance, survivorship and activity of the adults. Temperature is also a very useful predictive tool for insect development especially when the concept of degree-days (DD) is applied to the target species. In the case of the European species, the lack of information about the basic life history of the different species limits the use of this concept. For some nearctic species, e.g. C. sonorensis, the theoretical threshold for adult activity in Canada has been reported to be 2.5 ºC and the ovarian development to be 9.3 ºC (Lysyk and Danik, 2007; Lysyk, 2007). This means that 2.5 ºC is the minimum threshold that adults of C. sonorensis may need to start activity. In Europe, the temperature thresholds for activity of C. imicola and the
Obsoletus complex are not well known. Furthermore, temperature has a direct relationship with the longevity of the adult *Culicoides*. Recent work in Canada conducted by Lysyk and Danik (2007) has shown that the theoretical longevity for *C. sonorensis*, the major BTV vector in North America, ranges from a maximum of 28 days at 30 °C to 84 days at 10 °C. Similar work has been not conducted in Europe and only some general estimates are available (see Chapter 3.6).

To date, there is insufficient scientific information to determine a general threshold for the definition of the SVFP across Europe. The specific value of 5 parous females of the suspected or identified vector species is partially supported by some work conducted during the BTV-8 outbreaks in northern Europe (EFSA, 2007a). However, when considering the last winter 2007-2008, there is evidence that at least one transmission occurred even below the threshold of 5 parous females in Germany (B. Bauer, personal communication). Based on these facts, it seems that the concept of an absolute SVFP is unrealistic for defining the role of the different vector species during winter when transmission is supposed to be absent or very low. Nevertheless the seasonal occurrence of BT in Europe is clearly related to the seasonal pattern of the vectors throughout the year. Indeed, it has been described that the BTV outbreaks in Europe are linked to periods when the suspected vector species have a peak of abundance (Mellor et al., 2000; Capela et al., 2003; De Liberato et al., 2003; Miranda et al., 2004). Based on this, the periods of low abundance of the different vector species, mainly during winter time, seem suitable for animal movements since the risk of transmission is apparently very low during that season. To date, the periods of low abundance of *Culicoides* spp. are assessed by the entomological monitoring programmes developed in all the affected MS. In some cases (Spain, Italy, France, etc.) there are more than five years of monitoring of the *Culicoides* populations. However, there is a lack of harmonization of the different monitoring programs across Europe. As has been already mentioned above, the periods of low abundance of the vectors has been fully characterized in many affected countries during the last years (see chapter 3.8). These historical data about the annual seasonal pattern of the *Culicoides* species are also linked with the data obtained from the serological data that are systematically conducted in many countries around Europe.

In the light of the information on the seasonal pattern of adult *Culicoides* vectors in Europe, it is evident that a period of the year with a total cessation of activity of the adult vectors may not occur. However, it is possible to identify periods of the year when the risk of transmission of BTV is considered to be very low. Such a period coincides with very low captures of the different vector species and with low temperatures during winter that theoretically precludes virus replication in the vector. This assumption is mainly based on laboratory observations regarding virus replication demonstrating that virogenesis is faster at higher temperatures (Mullens et al., 1995, Pauweska et al., 2002; Wittmann et al., 2002; Carpenter et al., unpublished). This low transmission period will vary across Europe depending on the timing and duration of the local climate given the fact that these vectors are dependent primarily on favourable temperature condition. Therefore, the timing and duration of the low transmission period should be established for all MS using data for the BTV serotypes circulating in the region provided by the serological surveillance, the systematic surveillance of the vector abundance and local temperature data provided by national or international meteorological services.

There are few field based data on the comparative efficiency of different vector species or populations of species at transmitting BTV, and none in Europe. However, laboratory-based experimental studies (O’Connell, 2002; Baylis et al., 2008) demonstrate that a single dispersing infectious female of a *Culicoides* vector species could initiate a BT epidemic from a single bite. A recent laboratory study by Baylis et al. (2008) using the American vector *C. sonorensis*, has confirmed this assumption.
As it has been mentioned already (see chapter 3.8), light traps placed outside stables are the main trapping method used to date for the monitoring of the seasonal pattern of the *Culicoides* species and consequently the periods of low abundance of the *Culicoides* spp. Recent findings obtained by using animal traps indicated that light traps could bias the abundance and presence of important vector species. In addition, the indoor activity suggested in some parts of Europe may extend the presence of the *Culicoides* during winter and thus being undetected by outside placed traps. In the same sense, a day-light pattern of activity has been also suggested in some parts of Europe that could clearly be underestimated by using light traps. However, it should be borne in mind that the current data are not sufficient for determining the relevance of all the aforementioned phenomena in the assessment of the abundance and seasonal pattern of the *Culicoides* species across Europe, and particularly during the period of low abundance in winter time. Finally, the relevance of such data for BTV transmission is also unclear, even assuming that these two phenomena may extend the period of vector activity.

**Conclusions:**

The current definition of the SVFP was based on the epidemiological situation of certain areas in Europe for certain serotypes and certain species of vectors. The importance of some species (i.e.: *C. dewulfi*, *C. chiopterus*, *C. pulicaris* and the Obsoletus complex) implicated in the transmission of the BTV was not specifically included in the current definition.

The complete cessation of vector activity depends on the different vector species and the climatic conditions of each country. In certain areas of Europe, the complete cessation of *C. imicola* and Obsoletus complex species for more than 2 months has been documented, whereas very low level, activity of adults from the Obsoletus and Pulicaris complexes species and *C. dewulfi* has been detected in many parts of Europe during winter time.

Recent data demonstrate that some *Culicoides* species, in some geographical areas in Europe are active to a greater or lesser extent throughout the year and that an absolute vector free period may not exist.

However, there are periods of the year when the abundance of the *Culicoides* vector species is very low, mainly coinciding with winter time, and long-standing practical experience demonstrates that transmission of BTV is substantially reduced or halted during these periods.

The main tool for determining the relative abundances of the different *Culicoides* vector species is the UV light trap placed outside a stable. However, there are some findings based on biting collections from animals which suggest that the density and occurrence of some *Culicoides* species may be either under or overestimated by light traps.

There is insufficient scientific evidence to confirm that the daylight pattern of activity and the indoor activity of the *Culicoides* may have relevance for the assessment of the abundance and seasonal pattern of the *Culicoides* across Europe. Its relevance for BTV transmission is also unclear, even assuming that these two phenomena may extend the period of vector activity.

**Recommendations:**

Improved and harmonized methods to monitor and quantify vector activity are required to support the development of criteria that more accurately define periods of low vector abundance, especially those relevant to BTV transmission.

It is recommended that harmonized vector monitoring programmes be developed and that MS implement comprehensive BTV surveillance systems, integrating local vector abundance and BT incidence data, to establish more accurately the level of vector activity required for virus transmission in their area.
The vector activity should be recorded by using an annual monitoring programme based on *Culicoides* adult capture with UV light traps placed outside stables or animal housing. At least 3 years of data, preferably including climatic data, are needed to provide sufficiently meaningful information for determining the period of low abundance.

Pending additional studies, it is recommended that all suspect and confirmed vector species be considered equally capable of transmitting all BTV serotypes. The analysis of age grading of captured *Culicoides* females is also recommended.

Training courses to harmonize the taxonomy, the age grading and monitoring procedures for vectors across Europe are recommended.

It is recommended to compare the current light traps with other sampling methodologies such as animal baited traps to provide reliable estimates of *Culicoides* abundances in the monitoring programmes. This comparison should be conducted during the periods of low and high *Culicoides* abundances throughout the year.

Indoor and outdoor *Culicoides* activity, both by day and by night, should be investigated further to assess their relevance. If their relevance is confirmed, then the current vector monitoring systems may need to be adapted. The term indoor activities should be standardized.
4. Vector control

4.1. Introduction
The methods commonly used to control *Culicoides* worldwide were reviewed as part of the previous EFSA report on bluetongue (EFSA, 2007a). These methods can be broadly divided into those that involve a chemical application and those that utilise physical methods.

Chemical methods include:
- treating livestock with insecticides, repellents or systemic antiparasitic drug (e.g. avermectins)
- treating larval breeding sites or adult resting areas with insecticides
- treating animal housing and/or transport with insecticides

Physical methods include:
- removal or reduction of larval breeding sites on farm holdings
- housing livestock in screened buildings at times of high levels of *Culicoides* activity.

There is a need to differentiate between repellent effects and irritation. Repellent effects will prevent an insect to land (and feed) on a treated host. It has no killing effect and the insect will be able to land and feed on an untreated host.

Irritation does not prevent the insect from landing and, sometimes, feeding. However, due to irritation (“hot feet effect”) the insect may eventually be so irritated that it will take off prior to feeding. But it has picked up insecticide and may consequently become paralyzed before it will die. Most of the pyrethroids do not possess repellent effects.

4.2. Chemical Methods
There are currently no veterinary medicinal products or biocidal products authorised in the EU specifically for use in any control role against *Culicoides*. Hence, interest has centred upon the large number of products that are available, licensed and in widespread use against other Dipteran pest species. It is important to note that the use of certain type of product formulation has been restricted recently, particularly in northern Europe, due to both environmental and operator safety concerns.

4.2.1. Treatment of animals
A wide range of compounds are used to control both ecto- and endo-parasites on ruminants and some of these have the potential to impact upon *Culicoides* that attempt to feed upon them. These can be divided into products for topical use, such as pour-on formulations (usually applied along the back-line of the animal), ear tags attached to animals, dipping formulations (where the animal is immersed in the product) or injectable systemic formulations. Pour-on, ear tags and dipping products are usually based around synthetic pyrethroids (e.g. cypermethrin, deltamethrin, permethrin, flumethrin, cyfluthrin, cyhalothrin) or organophosphates, although other active ingredients are also sometimes used (e.g. macrocyclic lactones). These compounds exert their effect in two ways: primarily they are highly toxic to insects landing on the treated animal, often killing within minutes of their landing; secondarily they exert a contact irritation that may reduce the probability of the arthropod successfully initiating or completing a blood meal from the host. While toxicity of different active ingredients is usually relatively easy to
define, this latter effect is not usually so well defined. However, it is usually far less effective than that exerted by dedicated repellent compounds (e.g. DEET). Injectable systemic compounds are most commonly based around macrocyclic lactones (e.g. avermectins) and toxicity occurs following ingestion of a blood meal containing the active ingredient.

4.2.1.1. Pour-on products

Since the publication of the previous EFSA opinion on bluetongue (EFSA, 2007b), several studies have examined the efficacy of pour-on products on ruminants. Two in vitro studies of lethality effects of deltamethrin and cyfluthrin on Culicoides spp. were published by Mehlhorn et al. (2008). In this study, deltamethrin (7.5 g/100 ml) or cyfluthrin (1 g/100ml) containing pour-on solutions were administered to cattle and sheep. Hairclippings from legs of treated animals were removed at regular intervals up to 35 days following application and field-caught Culicoides exposed to these clippings for 15 seconds-2 minutes. For both, cyfluthrin and deltamethrin, lethal effects were recorded at up to 21 and 28 days post-application, respectively.

Additionally, a 1% deltamethrin-based pour-on formulation and a 1.25 % w/v high-cis cypermethrin-based pour-on formulation were tested by Carpenter et al. (2007) on UK sheep and cattle at maximum recommended dosage. Insecticide persistence and effect was examined by taking fleece samples at known times, post application, and exposing them to a colony of C. nubeculosus, a non-vector species, in an assay similar to that of Mullens et al. (2000). Additionally, samples were analysed for active compound using GC-MS and the decay of active ingredient rates calculated over time, demonstrating actual presence of insecticide in the areas of sampling. In contrast to the Mehlhorn et al. (2008) study, field caught C. obsoletus collected from treated and control animals did not live long enough to allow direct comparisons of mortality rates (which would have been desirable given variation in susceptibility from species to species). Additionally, while both formulations were highly toxic to Culicoides in the laboratory, neither were found to spread sufficiently on sheep to protect the primary regions of biting (belly, face, legs). These results were not significantly improved by applying the products to the flanks of the animals rather than along the back line (i.e. the recommended route of administration). While applications to cattle were found to spread much further and hence provide higher knockdown and kill rates, results were still vastly inferior to those presented by Mehlhorn et al. (2008). This contrast may be associated with the lack of negative controls for mortality in the former study, which would be likely to be high when using field caught specimens.

In a third study, Culicoides obsoletus and, probably, C. punctatus were caught with BioGents UV light sentinel traps inside the buildings of a stud bull farm, located in Schmergow, Brandenburg, Germany. Bulls were regularly treated at intervals of 4–6 weeks with deltamethrin (0.75%) pour on (30ml of the commercial product/bull). Following a topical treatment on August 2nd, 2007 a total of 13 catches were performed during the subsequent fortnight. Between 5 – 36% of all midges had succeeded to feed (average 13.2%). The maximum of 36% was recorded on August 8th, 6 days after the treatment. Similar observations were made during the subsequent months of September and October, respectively. It is noteworthy that despite an internal fumigation with a mixture of cypermethrin and chlorpyrifos and a simultaneous topical treatment on October 4th between 17 – 37% of the midges succeeded to feed during the subsequent fortnight. The maximum of 37% was observed on October 18th, two weeks after the treatment (Bauer et al., unpublished data). The effects of two deltamethrin-based pour-on products were also tested on Holstein Friesian cattle, at maximum recommended dosages. Here, again, relatively little amounts of the insecticide spread from the back line to the belly where a significant number of midges feed. Of the two products tested, only one produced significant mortalities across different body regions, including the belly of
treated cattle for up to seven days after treatment (corresponding largely to the results of Carpenter et al., 2007).

In another 5-month clinical field study on cattle performed in North-West Germany in 2007, the efficacy of a cyfluthrin containing pour-on formulation was investigated in the control of Culicoides (Liebisch et al., 2008a). On treated animals, a repellent effect lasting for 14 days was observed. The toxic effect on biting midges turned out to be 23 days. The persistence of insecticidal effects of cyfluthrin was confirmed by the results of bioassays using hair clippings from treated animals. The results confirm the decreasing insecticidal efficacy from dorsal to ventral and distal body regions of treated animals which is related to the reduced distribution of the active compound from the back line to the belly and legs. No serologically positive and no animals with BT were recorded after termination of the study. The findings of this study are difficult to interpret. Firstly, the lack of untreated control groups of cattle that meant that natural mortality in captured Culicoides for insecticidal effects was not accounted for. This is important as Culicoides caught via pooter are often damaged and generally do not survive for long periods. Additionally, the absence of a control group of untreated cattle for testing the repellent effects makes it impossible to determine whether the results can be generalised to other populations.

4.2.1.2. Ear Tags

Liebisch et al. (2008b) carried out a field trial on a total of 165 heifers and 20 dairy cows tagged with either one or two Permethrin-impregnated ear-tags (1.2 g permethrin per tag) in North-West Germany in 2007. Repellence and toxic effects against midges in cattle were observed for up to 19 days after tagging. Cattle with one ear tag were less protected. However, due to the same shortcomings as described for the study of Liebisch et al. (2008a) it is impossible to determine whether the results can be used for generalised conclusions.

Another 5-month clinical field study on the use of cypermethrin containing ear tags (Cypermethrin 1.067 g/tag) in cattle was carried out by Liebisch et al. in Germany in the same region in 2007 (Liebisch and Liebisch, 2008). Two herds with a total of 40 young cattle and 140 dairy cows were included. The animals were treated with one or two ear tags/animal. Study design and methodologies were similar to those of the previous studies (Liebisch et al. 2008a, 2008b). Insecticidal efficacy on the animals was observed for up to 14 days and 21 days in animals treated with one and two ear tags, respectively. Persistency was confirmed in bioassays using hairclippings taken from different body regions. There were no meaningful differences in the results between animals treated with one or two ear tags, respectively. Again, it was evident that insecticidal efficacy reduces from the back line to the belly and legs of treated animals. After study termination, one serologically positive animal was identified. However, due to shortcomings in the study design and methodologies as described above, it is not possible to draw conclusions from this study which can be generalised to other populations.

4.2.1.3. Dipping products

Since the publication of the previous EFSA opinion on bluetongue (EFSA, 2007b), no further assessment has been made of dipping products. Given the lack of spread experienced for three studies of pour-on products discussed above these products may provide an alternative. However, pyrethroid dips have been withdrawn from use in a number of northern European countries due to environmental concerns, and few organophosphate products are now available due to operator safety concerns.

4.2.1.4. Repellents

True repellents have rarely been used in livestock as they require re-application on a daily basis (unfeasible except for the most valuable of livestock), usually can only be used in relatively
low concentrations in comparison to humans due to more rapid absorption of products. Since the publication of the previous opinion (EFSA, 2007b), no further work on these compounds has been carried out.

Some historical evidence of a true repellent effect has been provided from work carried out in Israel. The following substances were tested against C. imicola over one night using an in vitro field model of impregnated polyester bed-nets covering DuToit suction light traps (Braverman and Chicov-Ginzburg, 1987): Alphacypermethrin, Permethrin, cypermethrin, pyrethrin and lambdacyhalothrin in various preparations and concentrations. Only alpha-cypermethrin 1.5% pour-on showed any repellence for the entire night. The other substances did not show any effect or only weak repellency. However, it will be difficult to conclude from the results that treating livestock with any one of the compounds will lead to comparable results.

4.2.1.5. Systemic compounds

Systemic compounds such as macrocyclic lactones have been suggested as a means of control primarily due to data that examined mortality of Culicoides feeding on treated cattle in Australia, although, as discussed in the previous opinion (EFSA, 2007b), these results were equivocal. They have several drawbacks by not being useable in dairy herds and possessing long withdrawal times for meat. Additionally there are concerns regarding the environmental impact of these products upon non-target fauna and also the development of anthelmintic resistance. Since the previous opinion (EFSA, 2007b), no further work regarding their use has been published.

4.2.2. Treatment of breeding sites

As outlined by a previous EFSA opinion on bluetongue (EFSA, 2007b), in the absence of a clear identification of breeding sites of species (as for example from the Obsoletus complex) in Europe, larval control remains not feasible. Although a recent publication gave some information about the breeding sites of Culicoides on European farms (Zimmer et al., 2008), these observations require both extension and repetition before they can be generalised across the region of interest. Broad-scale Ultra Low Volume (ULV) insecticidal treatment of potential larval development sites with synthetic pyrethroids or organophosphates is not acceptable under current legislation in the UK, particularly given the poor level of knowledge regarding adult resting and larval development habitats of most of the potential vector species. Culicoides obsoletus presents a particularly difficult problem in that it inhabits a wide range of habitats as larvae at low densities, making wide-scale removal unrealistic. The only potential role for insecticides in the UK is in extremely contained habitats (e.g. dung or compost heaps) where run-off to water sources are less likely to occur. Trials of the effect of both, chemical and husbandry changes, have not been carried out to date.

Further information has been obtained regarding the larval habitats of C. imicola in Israel. This species breeds almost entirely within the perimeter of the animal compounds (Braverman et al., 2001). Besides weekly manure removal, oral treatment of cattle with tetrachlorvinphos reduced the numbers of adult C. imicola (as monitored by suction light trap) by killing the larvae in the animals’ faeces (Braverman, unpublished data). However, since no MRL is established, the use of tetrachlorvinphos, as a veterinary medicinal product, is not currently permitted in food producing animals in the European Union.

4.2.3. Treatment of Animal Housing/Transport

Insecticides recommended for use in vehicles in the UK during animal movement all contain synthetic pyrethroids as active ingredient. None have been tested against the northern European Culicoides, but they outperform organophosphates in wind tunnel trials carried out in other
regions with other vector species (Floore, 1985; Kline et al., 1981). Spraying is limited to animal housing/abattoirs (or similar) and the vehicle itself.

In Sardinia (2000), pyrethroids were applied on the ground and stables of several farms, in order to control the C. imicola population. In two farms, with low and high abundance of the vector, blacklight traps were placed and insect collections were made before and after the spraying. A drop of the C. imicola was detected after the treatment, but the population recovered after a week in both places (M. Goffredo, personal communication).

In Spain, trials using insecticide impregnated nets on stable openings conferred a relatively protection to animals by reducing the abundance of C. imicola into stables when compared with a control stable (Calvete et al., 2007).

Insecticide-treated nets have been found to reduce Culicoides numbers by 70 – 80% within suction traps placed inside pens in which animals were stabled in Kumasi, Ghana (Maia et al., 2005).

4.2.4. Considerations to be made when applying chemical treatments

4.2.4.1. Licensing in the EC

Across the community, differences exist in the licensing classification of the substances and as a result, the same compound/product might be available in some Member States as a veterinary medicinal product and in others as a biocidal product.

For veterinary medicinal products used in a food-producing animal, only substances can be used for which a maximum residue limit (MRL) has been established, even if this MRL is for another species. Under the so-called “cascade”, it is possible to use a veterinary medicinal product, which is authorised for another animal species, if there is no other authorised product for this indication/species available.

A list of pyrethroids authorised as veterinary medicinal product for use in cattle, sheep or goats in the European Union is appended to this document (Appendix C)

4.2.4.2. Environmental Impact

Due to their broad spectrum insecticidal activity, many substances such as synthetic pyrethroids or avermectines are highly toxic to non-target species such as bees, fish and aquatic life. Hence, the impact of insecticidal compounds on the environment has to be carefully considered when using these products. For example, while synthetic pyrethroids have relatively low mammalian toxicity and hence present little risk to the user, they are extremely toxic to aquatic life which can have a major environmental impact when contamination of water bodies occurs from run-off after use. This has led to their withdrawal as dipping products from several European countries. The weighing up of the impact of each product is generally done by the country concerned rather than on a Europe-wide basis.

4.2.4.3. Insecticide resistance in Culicoides and other insects of veterinary or medical importance

While resistance has not been documented in European Culicoides to date, there exists a clear risk of development due to the limited classes of insecticides that are registered and available for use in the Member States. In the UK, for example, only one dipping product, diazinon (an OP) is currently licensed and, if used on a wide scale, resistance in treated populations could easily present problems. These problems are currently an issue in other European nuisance fly populations, e.g. for Musca domestica it takes about 5 – 6 generations to develop tolerance and, ultimately, resistance against insecticides. In moderate climates there may be as many as 12 generations in one year.
Conclusions:
No insecticidal products are currently authorised specifically against *Culicoides* in the EU although a wide range of untested products are available.

Historical testing of toxicity worldwide has demonstrated that pyrethroids-based products are more effective in the laboratory against *Culicoides* than organophosphate-based products. Hence, the use of the former is preferred except where other issues (environmental impact and legislation) preclude it.

Novel data concerning the efficacy of pyrethroid-based pour-ons and ear-tag products have been obtained but are equivocal. While all trials showed some mortality in *Culicoides* feeding on treated animals, the extent of this mortality was poorly measured and was not related to reduction in BTV transmission.

Thus the development of efficacious pyrethroid-based insecticides for reducing *Culicoides* attack rates (and thus the sequential transmission of BTV) has yet to be realised. Nevertheless, a number of recent studies, despite shortcomings in experimental design and the lack of controls, have shown promise and leads us to conclude that they may prove useful following additional future development.

However, a common result after treatment with pour on formulations or ear tags was the decreasing insecticidal efficacy from the back line to the belly and legs of treated animals. This is related to the limited spread of the insecticide and is common to topically applied products of non-systemic activity.

Dipping products have not been assessed to date in this role in Europe. Systemic products have also not been assessed, but based on past experience, are unlikely to be used to lower *Culicoides* population levels thereby preventing BTV transmission on a wide scale.

No new data have been provided regarding the treatment of housing or transport for animals. Treatment of breeding sites remains difficult as habitats are poorly defined for most species.

Recommendations:
Further research is required to investigate products with the potential for use against *Culicoides*. Preference should be given to products that already fulfil legislative criteria for use in EU member states.

For testing of insecticidal/repellent activity against *Culicoides*, it is recommended to use the standardised *in vitro* method of the WHO for lethality and repellency testing where this is compatible with field populations.

Field studies should always include a negative, untreated control group and, if available, a positive control. Abbot’s (Abbot, 1925) correction for mortality should be implemented and the efficacy trials should be performed in accordance with relevant VICH (International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products) and European CVMP (Committee for Medicinal Products for Veterinary Use) guidelines for veterinary medicinal products.

Although clear, confirmatory data are not at present available, pyrethroid-based pour-ons or ear tags may limit the population of *Culicoides*, thereby reduce the risk of sequential BTV transmission. Pyrethroid-based insecticides should not be used as a stand-alone measure to protect animals against *Culicoides* attacks.

The use of sprays or dipping may be more suitable for immediate effects and short term treatment. Also, these methods should be preferred in order to ensure equal distribution over...
the entire body surface. The use of these products, however, should be considered with regard to adverse environmental and health effects.

All use of chemical treatments should be carefully assessed with regard to environmental impact, user risk and the potential for development of resistance in *Culicoides* populations.

4.3. Physical (mechanical) control

4.3.1. Breeding site removal/reduction

In Mediterranean countries, *C. imicola* breeds in close proximity to animals, particularly in intensive cattle farms. Removal of the manure, which is an important component of the breeding site, should therefore reduce drastically the population of *C. imicola*. Manure removal can be done by converting the manure to compost, biogas and/or by ploughing the manure in the animal yard, as it is already practiced in Israel in some dairy farms. Wherever there are manure heaps that can’t be entirely removed, the top 6 cm should be removed every 10 days (*Culicoides* being likely to breed on the surface of heaps).

Keep animal constructions and premises as dry as possible by preventing leakage of water installation and overflow of water troughs.

In northern Europe the situation is more complicated as potential vector species breed in a wide variety of habitats and due to higher levels of precipitation it is more difficult to identify the wetter areas that support *Culicoides* larvae. In addition at least one species, *C. obsoletus*, is known to breed in a large diversity of habitats. Therefore, measures to reduce population density will be difficult to define. Given the knowledge regarding *C. scoticus* is even poorer than that for *C. obsoletus* it will be similarly difficult to target this species. In the case of *C. dewulfi* and *C. chiopterus*, the literature, to date, indicates that these species breed in dung, as indicated in the previous EFSA report on bluetongue (EFSA, 2007a), and hence population numbers might be reduced by dung removal, however, to date no quantitative evidence of this has been provided. Inferences could be drawn from the fact that manure removal from animal yards reduces dramatically the numbers of houseflies and it is reasonable to assume that it will reduce also the dung breeding *Culicoides* and species that develop in dung contaminated development sites. Other potential vector species including the subgenus *C. pulicaris* have a wide range of habitats that are not presently well enough defined to allow treatment or removal of breeding areas that are difficult to target e.g. marsh land.

Conclusions:

Dung removal/treatment especially in countries with Mediterranean climate when feasible and practical has the potential to reduce populations of *C. imicola*. Additionally, preventing overflow of water from water troughs and leaking water installations and keeping the animal premises as dry as possible would prevent/reduce creation of developmental sites of *C. imicola*.

In northern Europe, targeting of larval habitats is more difficult due to the wide range of habitats used by potential vector species. While some species are restricted to dung and hence could be targeted by removal of these habitats, this only reduces probability of transmission by these species and not others utilising less specific larval development sites.

Recommendations:

In order to minimise *Culicoides* breeding where possible, animal dung should be removed from sheepfolds, cowshed and their premises and suitably managed.

Research should be done to evaluate the effectiveness of different manure management options to minimise *Culicoides* breeding under different husbandry conditions existing in the EU.
4.3.2. Preventative stabling – an update

When the stable is totally closed (from the sunset to the morning after), it is still possible to collect small numbers of Culicoides inside, possibly because they may be active during the day. In stables in which 85% of surface (walls, doors) is closed, the abundance of the Obsoletus complex inside can be 20% lower than outside (M. Goffredo, Personal Communication).

Baylis and colleagues have undertaken a study in North Wales into the possible benefits of stabling animals at night to protect them from attack by Culicoides. A 3x replicated 4x4 Latin Square design was used, on 4 farms, with 4 treatments, over 12 nights. It was undertaken in June 2007 and again in October 2007. The four treatments were the combinations of inside versus outside stables, and animals present/absent. In June, over 70,000 Culicoides were captured; 92% were C. obsoletus, and 99.5% were female. A model of farm, night (nested within 4-day block), treatment and the F x T interaction explained 78% of variance in the (log-transformed) catch of C. obsoletus. Regardless of inside/outside, the presence of cattle increased catches by about 2x. Regardless of the presence/absence of cattle, catches inside were about one-sixth those of outside. The strength of the protective effect (inside versus outside) varied across farms, perhaps in line with how enclosed the stables were. In October, ~4700 Culicoides were captured; 86% were C. obsoletus, and 98% were female. A model of farm, night (nested within 4-day block) and treatment explained 44% of variance in (log-transformed) catch of C. obsoletus. The presence of cattle did not affect catches outside; but increased them by 3.7x inside. Catches inside were one-quarter those of outside in the absence of cattle, but not different in the presence of cattle. These results point to an important conclusion. Stabling may partially protect animals from attack by Culicoides early in the season but not later in the season. Further analysis showed that this effect is probably driven by the weather. When conditions are warm and calm (common in June; occasional in October) outside catches can be high – and exceed those inside. When conditions are cold, or windy (occasional in June, common in October), outside catches are suppressed and match those inside.

In closed stables in Israel only small numbers of C. imicola were caught, but in animal sheds they fly uninterrupted what shows that stabling animals could prevent/reduce their infection with BTV. (Braverman, personal communication)

Keeping animals day and night in stables protected by nets will probably be the best way to protect them from the vector attacks and therefore to prevent BTV transmission to animals due for export. Nevertheless, the application of this method is unrealistic on a large scale under many situations, for example the extensive sheep breeding areas and should be weighed against animal welfare and potential negative consequences.

Recommendations:
The endophily of all the suspected European vector species should be better assessed, and considered when decisions have to be taken in areas at risk of BTV circulation.

4.3.3. Decoy hosts – an update

Horses are very good decoy animals they are much more attractive to C. imicola than sheep and goats and probably more than cattle. At present it is difficult to recommend the use of decoy hosts due to a lack of knowledge regarding the host-prefereence of the potential Culicoides vectors of BTV in northern Europe. It is also unclear whether these techniques will exacerbate transmission by increasing available larval habitat. Animal welfare issues are also important to consider in the use of this technique.
4.4. Protocols for the use of insecticides/repellents as risk mitigation measures (type of product / frequency)

Insecticides may be used to limit the population of *Culicoides* and their biting rates, thereby reducing the risk of sequential BTV transmission. However, studies should be carried out in a proper manner to actually correlate the use of insecticides with the magnitude of the decrease of the risk of BTV transmission in treated animals.

In the absence of any valid data on the efficacy and safety of veterinary medicinal products or biocidal product for the control of *Culicoides*, no treatment protocols have been formally approved in the EU for specifically protecting animals against *Culicoides* attacks.

When using substances authorised for other indications or claims (e.g. against nuisance flies), label instructions use should be followed.

When using veterinary medicinal products under the “cascade”, a standard withdrawal period of at least 28 days for meat and 7 days for milk must be applied, when the product is used in another animal species, or, when the dosing instructions in the product literature are not followed in the target species.
REFERENCES


Downes J.A., Kettle D.S. (1952). Descriptions of three species of Culicoides Latreille (Diptera: Ceratopogonidae) new to science, together with notes on, and a revised key to the British species of the Pulicaris and Obsoletus groups. pp. 61-78.


EFSA, European Food Safety Authority (2007b). Opinion of the Scientific Panel on Animal Health and Welfare (AHAW) on request from the Commission on bluetongue vectors and


APPENDICES

APPENDIX A

Seasonality of Bluetongue in Europe.

Data: The information were obtained from the ADNS (Animal Disease Notification System) and includes all cases declared in Europe in the last two years. Other information available were: Country, administrative unit, date of outbreak confirmation, affected species and number of animals affected, dead, slaughtered and destroyed.

The cases declared in Spain in 2005 and in Corsica in 2003-05 came from the Spanish Government (http://rasve.mapa.es/Publica/Focos/Focos_Consultar.asp)

Between January 2006 and March 2008 the total number of affected farms was 50,797 (2,708 in 2006, 44,293 in 2007 and 3,796 during the first three months of 2008)

Table 1 summarizes the number of cases per country and species.

<table>
<thead>
<tr>
<th>Country Name</th>
<th>CATTLE</th>
<th>GOATS</th>
<th>SHEEP</th>
<th>WILD</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>BELGIUM</td>
<td>980</td>
<td>2</td>
<td>1,575</td>
<td>0</td>
<td>2,557</td>
</tr>
<tr>
<td>BULGARIA</td>
<td>11</td>
<td>13</td>
<td>11</td>
<td>0</td>
<td>35</td>
</tr>
<tr>
<td>CZECH REPUBLIC</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>DENMARK</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>FRANCE</td>
<td>17,489</td>
<td>278</td>
<td>1,472</td>
<td>0</td>
<td>19,239</td>
</tr>
<tr>
<td>GERMANY</td>
<td>7,084</td>
<td>152</td>
<td>5,261</td>
<td>44</td>
<td>12,541</td>
</tr>
<tr>
<td>ITALY</td>
<td>5</td>
<td>33</td>
<td>233</td>
<td>0</td>
<td>271</td>
</tr>
<tr>
<td>LUXEMBURG</td>
<td>1,062</td>
<td>17</td>
<td>315</td>
<td>1</td>
<td>1,395</td>
</tr>
<tr>
<td>NETHERLANDS</td>
<td>2,951</td>
<td>54</td>
<td>3,318</td>
<td>1</td>
<td>6,324</td>
</tr>
<tr>
<td>PORTUGAL</td>
<td>1</td>
<td>3</td>
<td>157</td>
<td>0</td>
<td>161</td>
</tr>
<tr>
<td>SPAIN</td>
<td>476</td>
<td>862</td>
<td>6,804</td>
<td>1</td>
<td>8,143</td>
</tr>
<tr>
<td>SWITZERLAND</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>UNITED KINGDOM</td>
<td>101</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>120</td>
</tr>
<tr>
<td>TOTAL</td>
<td>30,169</td>
<td>1,415</td>
<td>19,166</td>
<td>47</td>
<td>50,797</td>
</tr>
</tbody>
</table>

Table 1. Number of BTV cases between January 2006 and March 2008 per country and species in Europe.

Temporal distribution of BT in Europe (2006-2008)

Figure 1 represents the distribution of these cases by week. Most of the cases were declared in the second semester, between January 1st and June 30th 2007 only 132 cases were declared (90 in January, 17 in February, 5 in March 9 in April, 9 in May and 2 in June) (figure 2).
Figure 1. Weekly distribution of BTV cases between January 2006 and March 2008 (source ADNS).

Figure 2. Weekly distribution of BTV cases in the first semester of 2006 (source ADNS).
Figures 3 and 4 show the evolution of the infection in different countries. The peak of cases appeared between September and October, except in the case of France in 2007 where it appeared in November. This difference can be explained by the criteria used in this country to declare the disease: PCR positive animals were considered cases independently of the presence of clinical signs (taken into account the duration of the positivity to PCR, probably most of these cases would have been infected some weeks or even months before their declaration).

Figure 3. Monthly distribution of BTV cases in some Northern and Central European countries between January 2006 and March 2008 (source ADNS).

Figure 4. Monthly distribution of BTV cases in Italy, Portugal and Spain between January 2006 and March 2008 (source ADNS).
Figure 5 represents the temporal pattern of declaration of cases in different regions of France.

The evolution of the outbreaks in Sardinia in 2000/2001 followed a similar pattern with only few cases in the first semester of 2001 (Figure 6)

Figure 6. Weekly distribution of BTV cases in Sardinia (2000/2001)
Differences in the mortality between zones.

In the countries affected by BTV-8, a 2.8% of the cattle farms affected declared that at least one animal died as a consequence of the disease. In the Southern countries (affected by other serotypes) no deaths were declared (see Table 2).

Table 2. Number of cases with one or more dead animals by species and serotype.

<table>
<thead>
<tr>
<th></th>
<th>BTV-8*</th>
<th>Other serotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No deaths</td>
<td>1 or more deaths</td>
</tr>
<tr>
<td>CATTLE</td>
<td>28,846</td>
<td>830</td>
</tr>
<tr>
<td>GOATS</td>
<td>487</td>
<td>17</td>
</tr>
<tr>
<td>SHEEP</td>
<td>9,376</td>
<td>2,585</td>
</tr>
<tr>
<td>WILD RUMINANTS</td>
<td>19</td>
<td>27</td>
</tr>
<tr>
<td>TOTAL</td>
<td>38,728</td>
<td>3,459</td>
</tr>
</tbody>
</table>

* includes all countries except Bulgaria, Italy, Portugal and Spain
Duration of the seasonally free periods in different regions of Europe:

The periods without any declared case of Bluetongue in different countries or regions of Europe are summarized in the next table:

Table 3. Seasonal periods without declared cases.

<table>
<thead>
<tr>
<th>Country/Region</th>
<th>Year</th>
<th>Last case</th>
<th>First case</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardinia</td>
<td>2001</td>
<td>21/03/2001</td>
<td>24/05/2001</td>
<td>64</td>
</tr>
<tr>
<td>*CA (Sardinia)</td>
<td>2001</td>
<td>19/01/2001</td>
<td>24/05/2001</td>
<td>125</td>
</tr>
<tr>
<td>*NU (Sardinia)</td>
<td>2001</td>
<td>09/02/2001</td>
<td>24/05/2001</td>
<td>104</td>
</tr>
<tr>
<td>*OR (Sardinia)</td>
<td>2001</td>
<td>16/03/2001</td>
<td>10/09/2001</td>
<td>178</td>
</tr>
<tr>
<td>*SS (Sardinia)</td>
<td>2001</td>
<td>21/03/2001</td>
<td>07/08/2001</td>
<td>139</td>
</tr>
<tr>
<td>Corsica</td>
<td>2004</td>
<td>16/12/2003</td>
<td>14/09/2004</td>
<td>273</td>
</tr>
<tr>
<td>Corsica</td>
<td>2005</td>
<td>16/03/2004</td>
<td>31/08/2005</td>
<td>&gt;365</td>
</tr>
<tr>
<td>Spain (mainland)</td>
<td>2005</td>
<td>10/01/2005</td>
<td>29/07/2005</td>
<td>200</td>
</tr>
<tr>
<td>*Extremadura</td>
<td>2005</td>
<td>14/12/2004</td>
<td>04/08/2005</td>
<td>233</td>
</tr>
<tr>
<td>Belgium</td>
<td>2007</td>
<td>14/12/2006</td>
<td>09/07/2007</td>
<td>207</td>
</tr>
<tr>
<td>Netherlands</td>
<td>2007</td>
<td>20/03/2007</td>
<td>25/07/2007</td>
<td>127</td>
</tr>
<tr>
<td>Germany</td>
<td>2007</td>
<td>12/02/2007</td>
<td>06/06/2007</td>
<td>114</td>
</tr>
<tr>
<td>Germany</td>
<td>2008</td>
<td>13/12/2007</td>
<td>17/03/2008</td>
<td>95</td>
</tr>
<tr>
<td>Luxemburg</td>
<td>2007</td>
<td>16/01/2007</td>
<td>17/08/2007</td>
<td>213</td>
</tr>
<tr>
<td>France(^1)</td>
<td>2007</td>
<td>14/11/2006</td>
<td>27/07/2007</td>
<td>255</td>
</tr>
</tbody>
</table>

\(^1\) 24 cases between those dates, but all of them were farms with seropositive animals by ELISA (but not viraemic).

Until now, no cases have been detected in the period between March 21st and May 24th (64 days) in those countries.

Periods with less than 0.1% of the total cases considering the previous and the next year (i.e. In Sardinia during the years 2000 and 2001 there were 12,235 cases and only 8 of them were between February and May) in different countries or regions of Europe are shown in the next table (Table 4). Only few cases have been declared between February and June.

Table 4. Periods with less that 0.1% of the declared cases in two years.
<table>
<thead>
<tr>
<th>Country/region</th>
<th>Dates cases of</th>
<th># Cases*</th>
<th>Begin period</th>
<th>End</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sardinia</td>
<td>(see below)</td>
<td>8 / 12235</td>
<td>26/01/2001</td>
<td>07/06/2001</td>
<td>132</td>
</tr>
<tr>
<td>*CA (Sardinia)</td>
<td>19-Jan, 24-may</td>
<td>2 / 3083</td>
<td>27/12/2000</td>
<td>07/06/2001</td>
<td>162</td>
</tr>
<tr>
<td>*NU (Sardinia)</td>
<td>5-feb, 9-feb, 24-may</td>
<td>3 / 3012</td>
<td>26/01/2001</td>
<td>01/08/2001</td>
<td>187</td>
</tr>
<tr>
<td>*OR (Sardinia)</td>
<td>16-mar</td>
<td>1 / 2491</td>
<td>10/01/2001</td>
<td>10/09/2001</td>
<td>243</td>
</tr>
<tr>
<td>*SS (Sardinia)</td>
<td>1-feb, 2-feb, 21-mar</td>
<td>3 / 3649</td>
<td>10/01/2001</td>
<td>07/08/2001</td>
<td>209</td>
</tr>
<tr>
<td>Corsica</td>
<td>-</td>
<td>0 / 36</td>
<td>16/12/2003</td>
<td>14/09/2004</td>
<td>273</td>
</tr>
<tr>
<td>Corsica</td>
<td>-</td>
<td>0 / 36</td>
<td>16/03/2005</td>
<td>31/08/2006</td>
<td>&gt;365</td>
</tr>
<tr>
<td>Spain (mainland)</td>
<td>-</td>
<td>0 / 410</td>
<td>10/01/2005</td>
<td>29/07/2005</td>
<td>200</td>
</tr>
<tr>
<td>*Andalusia</td>
<td>-</td>
<td>0 / 316</td>
<td>10/01/2005</td>
<td>29/07/2005</td>
<td>200</td>
</tr>
<tr>
<td>*Extremadura</td>
<td>-</td>
<td>0 / 78</td>
<td>14/12/2004</td>
<td>04/08/2005</td>
<td>233</td>
</tr>
<tr>
<td>Belgium</td>
<td>-</td>
<td>0 / 2557</td>
<td>14/12/2006</td>
<td>09/07/2007</td>
<td>207</td>
</tr>
<tr>
<td>Netherlands</td>
<td>20-mar</td>
<td>1 / 6324</td>
<td>16/02/2007</td>
<td>25/07/2007</td>
<td>159</td>
</tr>
<tr>
<td>Germany</td>
<td>12-feb, 6-jun, 18-jun</td>
<td>3 / 12541</td>
<td>02/02/2007</td>
<td>06/07/2007</td>
<td>154</td>
</tr>
<tr>
<td>Luxemburg</td>
<td>-</td>
<td>0 / 1395</td>
<td>16/01/2007</td>
<td>17/08/2007</td>
<td>213</td>
</tr>
</tbody>
</table>

* number of cases during the “free” period divided by the total cases (considering both, the previous and current years).
APPENDIX B

New BTV vaccines against recent introduced serotypes in EU are finalizing their development. Latest information comes from the recent Bluetongue Satellite Symposium “Bluetongue in Europe, back to the future!!” held in Brescia, Italy, on 7th June 2008, and this has been incorporated in the present report because no detailed information on vaccine protective immunity was available at the moment. These products are intended to safely clinical protection and could control the spread of BTV-1 and -8 if significantly reduce or abrogate completely the viraemia after infection. Also the minimum period after vaccination to achieve the protective immunity of cattle and sheep animals is of capital importance for animal trade.

A brief resume on vaccine characteristics is reviewed in the Table 1 This new developed vaccines are addressed to vaccinate cattle and sheep in front serotype 1 or 8. This inactivated vaccines contains full virus with adjuvant. The virus is produced on cellular cultures with further purification and inactivation steps. Few or not details are given on adjuvants and antigenic concentration used to formulate the vaccines. Most relevant results are presented on efficacy tests but no mentions are given about quality or security assays.

Efficacy studies were done in official laboratories (NRL-Algete, Spain; IZS A&M, Teramo, Italy), research institutes (CReSA, Spain) or by own companies. Vaccines were applied on cattle, two vaccine applications, and in sheep, single or double vaccination, at intervals of 3 to 4 weeks in double vaccinated animals. Animals were challenged 15 to 60 days after the last vaccination.

Efficacy was evaluated, before challenge by serum neutralising (SNT) antibodies, and clinical score and virology analysis after challenge. In challenged animals strong clinical protection were reported with reduction or absence of clinical signs and fever on vaccinated groups with BTV-1 and BTV-8 vaccines. Viraemia results between 3 to 28 days after challenge was estimated in many cases by quantitative RT-PCR, but one study used classical RT-PCR compared to virus isolation.

All BTV-1 and BTV-8 vaccines may reduce or abrogate the viraemia after single or double vaccination by subcutaneous or intramuscular route in cattle and sheep, at appropriate antigen content. In sheep viraemia exclusion for BTV-8 could be obtained 21 days after single or double vaccination and for BTV-1 after two vaccinations. Whereas, in cattle, negative viraemia results were reported after double BTV-8 or BTV-1 vaccination. Field experiences of use of these vaccines in the field are not yet available (Domingo, 2008; Hammers et al., 2008a, 2008b; Plana Duran et al., 2008; Paradell et al., 2008a, 2008b; Puentes et al., 2008).
Table 1. New information received from Bluetongue Symposium, Brescia 7 June 2008, on inactivated vaccines against BTV-1 and BTV-8 for cattle and sheep.

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>SEROTYPE</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>1</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titre</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adjuvant</td>
<td>n.e.</td>
<td>n.e.</td>
<td>Saponine &amp; AL(OH)3</td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experimental conditions</th>
<th>Species</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Sheep</th>
<th>Cattle</th>
<th>Sheep</th>
<th>Cattle</th>
<th>Sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nº of vaccinated animals</td>
<td>4</td>
<td>5-8</td>
<td>10</td>
<td>21</td>
<td>3x7</td>
<td>3x7</td>
<td>n.e.</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Nº of control animals</td>
<td>2</td>
<td>7</td>
<td>11</td>
<td>7</td>
<td>n.e.</td>
<td>5</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of animals</td>
<td>10w</td>
<td>4w</td>
<td>6 to 7w</td>
<td>10w</td>
<td>4w</td>
<td>n.e.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nº of vaccinations</td>
<td>Twice</td>
<td>Twice</td>
<td>Twice</td>
<td>Single</td>
<td>Twice</td>
<td>Twice</td>
<td>Single</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Route of administration</td>
<td>Subcutaneous</td>
<td>Intramuscular</td>
<td>Subcutaneous</td>
<td>Subcutaneous</td>
<td>Intramuscular</td>
<td>Subcutaneous</td>
<td>Subcutaneous</td>
<td></td>
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</tr>
<tr>
<td>Vaccine interval</td>
<td>D0 and D21</td>
<td>D0 and D21</td>
<td>D0 and D21</td>
<td>D21</td>
<td>D0 and D21</td>
<td>D0 and D28</td>
<td>D0</td>
<td></td>
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<tr>
<td>Challenge</td>
<td>Virus strain</td>
<td>BTV1/Algeria/2006</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
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<tr>
<td>Dose</td>
<td>n.e.</td>
<td>n.e.</td>
<td>10ml x 10^7TCID50/ml</td>
<td>n.e.</td>
<td>n.e.</td>
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<tr>
<td>Route</td>
<td>n.e.</td>
<td>n.e.</td>
<td>s.c</td>
<td>n.e.</td>
<td>n.e.</td>
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<tr>
<td>Challenge days after last vaccination</td>
<td>60d</td>
<td>15d</td>
<td>25d</td>
<td>21d</td>
<td>25d</td>
<td>23d</td>
<td>31d</td>
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<tr>
<td>Efficacy results on vaccinated</td>
<td>Antibodies</td>
<td>Yes</td>
<td>Yes 14d or 15d after 2nd vaccine</td>
<td>Yes after second vaccine</td>
<td>Yes 15d after 2nd vaccine</td>
<td>n.e.</td>
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<tr>
<td>Fever</td>
<td>No</td>
<td>Lower than controls</td>
<td>No</td>
<td>Lower than controls</td>
<td>No</td>
<td>Lower than controls</td>
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<td>Signs</td>
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<td>Lower than controls</td>
<td>No</td>
<td>Lower than controls</td>
<td>Lower than controls</td>
<td>No</td>
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<tr>
<td>Viremia</td>
<td>Negative</td>
<td>Negative</td>
<td>Group 1: neg; Group 2: neg; Group 3: one of 7 was viremic by 1day</td>
<td>Group 1: neg; Group 2: neg; Group 3: one animal of 7 was viremic more than 1day</td>
<td>Group &quot;high antigen&quot;: negative; Group &quot;low antigen&quot;: viremia reduction</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
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<td>-------------------------</td>
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<td>----------</td>
<td>---------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
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<tr>
<td>Results on controls</td>
<td>Viremia and fever</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes, 7 of 7</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
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<tr>
<td></td>
<td>Viremia peak</td>
<td>10dpi</td>
<td>7-8dpi</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
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<tr>
<td></td>
<td>Viremia length</td>
<td>&gt;28dpi</td>
<td>n.e.</td>
<td>&gt;24d</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
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<td>Diagnostic techniques</td>
<td>Viremia detection</td>
<td>Real time PCR²</td>
<td>Real time PCR²</td>
<td>RT-PCR, Virus isolation</td>
<td>Real time PCR²</td>
<td>Real time PCR</td>
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<td></td>
<td>Antibodies</td>
<td>ELISA, SNT</td>
<td>n.e.</td>
<td>cELISA, SNT</td>
<td>n.e.</td>
<td>n.e.</td>
<td>n.e.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>References</td>
<td>Authors and communication title</td>
<td>Puentes et al.: Inactivated vaccines against bluetongue; protection of cattle and sheep from exp challenge with BTV1 and 4</td>
<td>Paradell et al.: Efficacy of Zulvac® 1-Inactivated and adjuvanted vaccine- against bluetongue virus serotype 1</td>
<td>Domingo M.: Laboratory efficacy of an inactivated BTV-8 vaccine adjuvanted with saponine and Al(OH)3 in sheep</td>
<td>Plana Duran et al.: Efficacy of Zulvac® 8-Inactivated and adjuvanted vaccine- against bluetongue virus serotype 8</td>
<td>Hamers et al.: Efficacy of an inactivated BTV-8 vaccine against a virulent BTV-8 challenge in cattle and in sheep</td>
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</table>

(¹) n.e.: not specified; (²) Toussaint et al. (2007).
APPENDIX C
List of authorised\(^5\) veterinary medicinal products in Europe

<table>
<thead>
<tr>
<th>Active ingredient</th>
<th>Species</th>
<th>Route of administration</th>
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</thead>
<tbody>
<tr>
<td>Alphacypermethrin,</td>
<td>Cattle, sheep</td>
<td>Pour-on</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>Cattle</td>
<td>Pour-on</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pour-on</td>
</tr>
<tr>
<td>Cyhalothrin</td>
<td>Cattle</td>
<td>Pour-on</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>Cattle</td>
<td>Ear tags</td>
</tr>
<tr>
<td></td>
<td>Cattle, sheep</td>
<td>Pour-on</td>
</tr>
<tr>
<td></td>
<td>Cattle, sheep, horse</td>
<td>Concentrate for cutaneous solution /</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Spray, dip</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>Cattle, sheep</td>
<td>Pour-on</td>
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<tr>
<td></td>
<td></td>
<td>External solution: dip, shower or spray</td>
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<tr>
<td>Fenvalerate</td>
<td>Cattle</td>
<td>Spray; Solution for local application</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(spraying or dipping)</td>
</tr>
<tr>
<td>Flumethrin</td>
<td>Cattle, sheep</td>
<td>Pour-on</td>
</tr>
<tr>
<td>Permethrin</td>
<td>Cattle</td>
<td>Ear tag</td>
</tr>
<tr>
<td></td>
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<td>Pour-on</td>
</tr>
<tr>
<td></td>
<td>Cattle</td>
<td>Concentrate for pulverisation / spray</td>
</tr>
</tbody>
</table>

\(^5\) Authorised in at least one Member States in the EU for the control of *Diptera*. The product might not be on the market in all Member States.