Chapter 5
Bluetongue Disease: An Analysis of the Epidemic in Germany 2006–2009

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Abstract In August 2006, bluetongue virus of serotype 8 (BTV-8), which had occurred before in the sub-Saharan region, Asia and South America, was introduced into Central Europe. The virus hit an area with a high population density of BTV-naive ruminants, suitable vectors (*Culicoides* spp.) and climatic conditions favourable for virogenesis and transmission. In 2006 and 2007, the disease spread over wide parts of western Germany and had a high economic impact on sheep and cattle farms. To reduce animal losses, mitigate the clinical symptoms and stop the further spread of the disease, Germany decided to implement a compulsory vaccination programme with a monovalent, inactivated vaccine against BTV-8 in May 2008 which has apparently led to the eradication of the disease. This chapter reviews the pathogenesis of bluetongue disease, the clinical signs, diagnosis, the course of the epidemic, control measures and the economic impact of the BTV-8 epidemic in Germany.

Keywords Bluetongue disease • Culicoides • Epidemiology • Germany • Vector

5.1 Introduction

Bluetongue disease (BT) is a non-contagious vector-borne disease that mainly affects ruminants but also camelids. It is caused by the BT virus (BTV), which belongs to the genus *Orbivirus* within the family *Reoviridae* and of which 24

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serotypes are known; further serotypes awaiting confirmation were described in Switzerland (Toggenburg virus) (Hofmann et al. 2008) and Kuwait (Maan et al. 2011a, b). BTV is transmitted between hosts almost exclusively through the bites of female *Culicoides* biting midges. BT is a notifiable disease under the German Animal Disease Act, reportable in the European Union via the Animal Disease Notification System (ADNS) and at the global level notifiable to the World Organisation for Animal Health (OIE).

BT had never been reported in Germany before it occurred in the region of Aachen in North Rhine-Westphalia in August 2006, almost simultaneously with outbreaks in Belgium and the Netherlands (EU Rapid Press release Nr. IP/06/1112, ProMedMail, 20060828.2448, Mehlhorn et al. 2007; Toussaint et al. 2007).

5.2 Pathogenesis

BTV is spread by infected haematophagous insects, mainly biting midges (*Culicoides* spp.) that excrete the virus in their saliva. In addition to the transmission itself, it has been suggested that immunomodulatory proteins in the midge saliva aid in the initial infection of the host (Darpel et al. 2009). During a blood meal, BTV is inoculated into the skin, which may be both an important site for replication and a source of virus for blood-feeding vectors (Darpel et al. 2009). After inoculation, migrating dendritic cells transport the virus to the draining lymph node (Barratt-Boyes et al. 1995; Hemati et al. 2009), from where it disseminates to other lymphoid tissues (Pini 1976; Barratt-Boyes and Maclachlan 1994). Replication occurs principally in mononuclear phagocytic cells, proliferating lymphocytes and endothelial cells (Mahrt and Osburn 1986; Maclachlan et al. 1990; Barratt-Boyes et al. 1992; DeMaula et al. 2001; Drew et al. 2010b). Accordingly, bluetongue pathogenesis is characterized by virus-mediated immune suppression, endothelial injury and dysfunction (DeMaula et al. 2002a; Maclachlan 2004; Maclachlan et al. 2009; Umeshappa et al. 2010).

Virus replication in endothelial cells causes direct cell injury and necrosis. Vascular blockage leads to haemorrhage and tissue infarction (Mahrt and Osburn 1986) that can manifest as myonecrosis and mucosal ulceration (Drew et al. 2010b). The activation of pulmonary endothelial cells and macrophages (DeMaula et al. 2002a, b; Drew et al. 2010b), on the other hand, and the subsequent release of host-derived inflammatory and vasoactive mediators (Hemati et al. 2009) increase vascular permeability, potentially leading to the widespread oedema often seen in fatal BT, African horse sickness and other virus-induced haemorrhagic fevers (Maclachlan et al. 2009; Drew et al. 2010a; Maclachlan 2011). A confirmation of this hypothesis, however, will require a deeper understanding of the highly complex interplay of cytokines in infected ruminants beyond the isolated findings of groundbreaking in vitro studies.

5.3 Clinical Signs

Before the first outbreaks of BT in Central Europe in 2006, clinical signs of the disease were mainly described for sheep (Erasmus 1990). However, it is known since many years that BTV can infect several domestic and wild ruminant species (Tabachnick 1996; Darpel et al. 2007). The severity of the disease in sheep may depend to a marked extent on environmental conditions, most notably exposure to sunlight, a frequently ignored fact (Erasmus 1990). Other authors doubt whether there is an ill-defined interaction with the environment (Mellor and Wittmann 2002). The clinical picture also varies depending on the strain of the virus as well as the breed and age of the infected animals with older age groups being more susceptible (Tabachnick 1996; Mellor and Wittmann 2002). In general, the clinical picture of BT can be extremely variable (EFSA 2007).

5.3.1 Sheep

Irrespective of the region of the world from where BTV serotypes originate, clinical disease of sheep follows a similar pattern (Parsonson 1992). The severity of clinical signs depends on both the breed of sheep and the strain of virus (Darpel et al. 2007). All breeds of sheep are susceptible to BTV infection, although the clinical outcome may vary remarkably. Especially indigenous African breeds have been reported as resistant (Erasmus 1990). However, not only African but also other indigenous breeds seem to be less susceptible than introduced European breeds and Merino sheep (Parsonson 1992) or may only be affected subclinically (Darpel et al. 2007). Febrile reactions with fever exceeding 41°C are common (Erasmus 1990). This could also be reproduced with a BTV-8 strain in experimental infections (Darpel et al. 2007). Within 1–2 days after the onset of the disease, the skin of the muzzle and lips as well as the oral mucosa became hyperaemic and oedematous (Erasmus 1990). This was also frequently observed during the BTV-8 epidemic in Central Europe between 2006 and 2009. Figure 5.1 shows a ewe from a backyard farm in North Rhine-Westphalia in September 2006. During the BTV-8 outbreak in Germany 2006–2009, clinical disease was often very severe in sheep.

Nasal discharge, which became later sometimes mucopurulent, and resulting dyspnoea could often be observed.

Foot lesions developing with the subsidence of fever represented a frequently detected disease manifestation. The coronary band is hyperaemic often combined with petechial haemorrhages.

An investigation in sheep farms in 2007 revealed that stillborn lambs showed clinical signs such as crusts and lesions of the oral mucosa (Fig. 5.2a). One of the farmers reported that the newborn lambs were physically and motorically retarded for several weeks. Feet lesions were apparently so painful that some of the sheep were reluctant to walk for weeks even after the acute symptoms had healed. Instead, they crawled on their carpal joints (Fig. 5.2b). When the animals were supported or

Fig. 5.1 Ewe with hyperaemic muzzle and shallow erosions and crusts on the nostril

when they got shooed, they walked on their feet for a little while; however, they immediately fell back to their previous behaviour when they were left alone.

5.3.2 Goats

Reports on clinical signs with BTV-8 virus strains in goats are rare. During experimental studies with BTV-8 in Dutch dairy goats, fever, signs of general illness, apathy, dysphagia, diarrhoea and lameness were observed (Backx et al. 2007). The discrepancy between the observations in the field and in laboratory experiments might be explained by different routes of infection, e.g. intravenous injection vs. the natural route of infection via biting midges or by vector preferences or the types of husbandry systems (Backx et al. 2007). During the BTV-8 epidemic in Germany in 2006–2009, a total of 26,954 BTV-8-infected premises were recorded (TSN database; 15.11.2011, 1135 hours), among which there were 132 holdings where goats were reported as clinically affected (TSN database; 15.11.2011, 1135 hours). This shows that goats were affected by this epidemic in Germany, but only to a limited extent.

5.3.3 Cattle

It is thought that cattle have now largely replaced antelopes as a maintenance host of the virus in Africa (Gerdes 2004). However, before the BTV-8 epidemic in northern

Fig. 5.2 Clinical signs of BT in sheep; (**a**) lips and tongue of a stillborn lamb with crusts and lesions in the oral mucosa; (**b**) sheep walking on carpal joints

Europe, natural and experimental BTV infection of cattle was considered asymptomatic in the vast majority of cases (Maclachlan et al. 1992; Maclachlan 2011). A transient febrile response, lacrimation and salivation were occasionally observed in infected animals (Erasmus 1990).

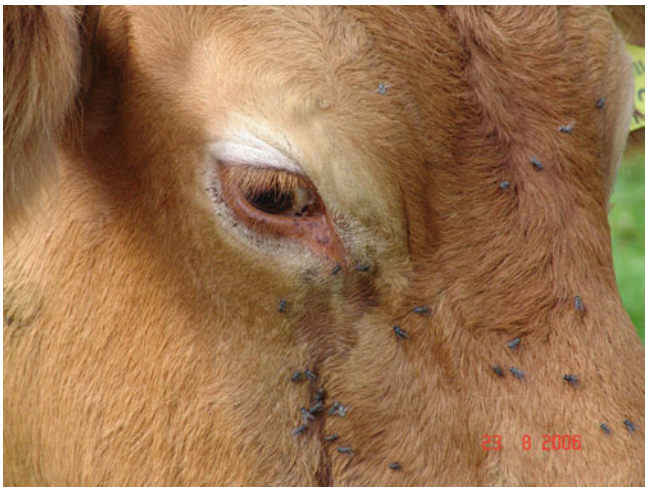

Fig. 5.3 Limousin cow with swollen eyelids, lacrimation and petechial haemorrhages

At the beginning of the BTV-8 epidemic in Central Europe, clinical symptoms such as fever (40–41°C) for 2–14 days, severe nasal discharge, lacrimation, facial oedema and nasal excoriations were described in adult cattle (Mehlhorn et al. 2007). However, using a field virus strain that originated from a BT outbreak in the Netherlands in an experimental infection, no pyrexia was recorded in any of the cattle (four 6-month-old male Holstein-Friesian calves) at any stage of the experiment (Darpel et al. 2007). One of the first German BTV-8 outbreaks was detected in a beef suckler herd (57 cattle, Limousin breed) in North Rhine-Westphalia. One cow showed a bilateral conjunctivitis, oedema of the eyelids combined with petechial haemorrhages on the swollen mucous membranes of the eyelids. Additionally, strong lacrimation was obvious as a predominant clinical sign (Fig. 5.3). The local tissue damage was complicated by bacterial infection.

Ulcers and erosions may occur in the oral mucosa (Fig. 5.4). The skin of the muzzle was inflamed at the beginning of the disease; later, cracks and peels could be observed (Fig. 5.5).

Large-scale erosions or haemorrhagic lesions in the skin of the teats were quite often reported in dairy cattle (Fig. 5.6). These lesions sometimes resulted in detachments of bigger parts of the skin of the affected teats. This caused pain during milking or nursing. Later on, these lesions were also affected by bacterial infection or characterized by the formation of crusts.

Lesions involving coronitis and inflammation of the whole foot region were also frequently reported. The coronary bands were hyperaemic, swollen and inflamed. Therefore, stiffness or lameness is common in BTV-infected cattle (Erasmus 1990).

Fig. 5.4 Erosion on the oral mucosa in healing after BTV-8 infection

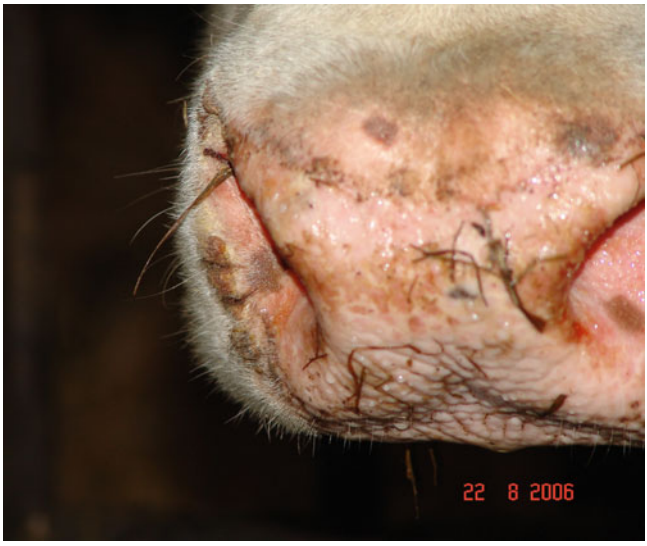

Fig. 5.5 Erosions, hemorrhagic lesions accompanied by bacterial superinfections and inflammatory foci of on the muzzle of a BTV-infected cattle

5.4 Diagnosis

5.4.1 BTV Isolation

For the direct detection of bluetongue virus, antigen or genome, whole blood is preferred over plasma because BTV is closely associated with red blood cells

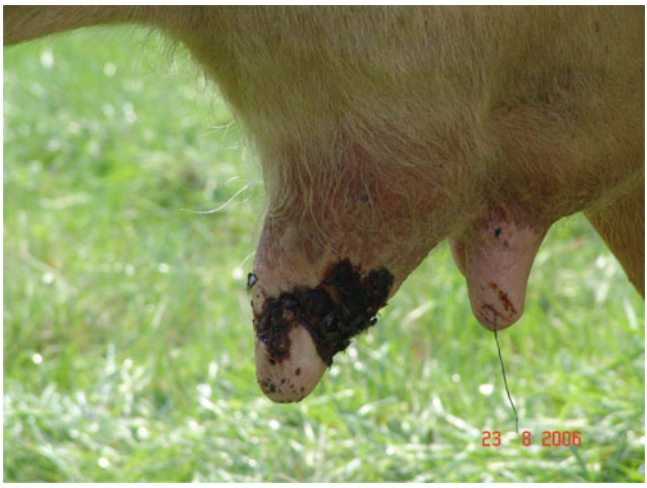

Fig. 5.6 Large-scale hemorrhagic lesions on the teats of BTV-8 infected cattle

(Brewer and Maclachlan 1992; Nunamaker et al. 1992). Recommended specimens for the isolation of bluetongue virus are blood of live animals or spleen samples collected at necropsy. Heparinized or EDTA-treated blood that has been washed several times with phosphate-buffered saline to remove BTV antibodies is best suited for virus isolation. The release of virus particles from the erythrocyte membrane by sonication after washing can increase the success of the isolation. BTV-positive spleen samples are homogenized in cell culture medium and cleared by centrifugation. The purified supernatants can then be used for the inoculation of cultured cells or embryonated chicken eggs. For the latter, most laboratories use the intravenous inoculation method published by Goldsmit and Barzilai (1985). BTV-infected chicken embryos usually die within 2 and 7 days, and appear cherry red as a result of massive haemorrhages.

Besides mammalian cell lines (e.g. baby hamster kidney cells [BHK-21], African green monkey kidney cells [Vero] or bovine aorta endothelial cells), several insect-derived cell lines (e.g. *Aedes albopictus* clone C6/36 or *Culicoides variipennis* larval [KC] cells) can be used for isolation and propagation of BTV (Clavijo et al. 2000). The passage of virus in cell culture typically results in an adaptation of the virus to the in vitro conditions (Gould et al. 1989).

A highly sensitive and reliable method for the isolation of BTV is the inoculation of susceptible animals, mainly sheep or cattle. Although animal experiments are very expensive and ethical considerations have to be taken into account, an important advantage can justify this approach. Based on the large volume of inocula (up to 500 ml sample material are tolerated during intravenous injection), the sensitivity is very high. For samples with borderline infectivity (blood from animals in a late stage of infection or semen samples with a low viral load), the inoculation

of susceptible animals can be the only way to propagate the virus (Hourrigan and Klingsporn 1975; Eschbaumer et al. 2010a, b).

Laboratory animals such as mice are not regularly used for BTV diagnosis and research. Nevertheless, the intracerebral inoculation of BTV isolates in 2–3 days old suckling mice causes clinical signs and death within 2–5 days after inoculation. Recently, a novel mouse model for BTV using type I interferon-receptor-deficient (IFNAR−/−) mice was developed (Calvo-Pinilla et al. 2009b; Eschbaumer et al. 2010b). Owing to the high susceptibility of these mice to fatal infection with BTV, they can also be used for virus isolation from samples harbouring a small viral load.

5.4.2 Molecular Diagnosis

Historically, confirmation and classification of BTV isolates have been based on immunological assays such as the indirect immune fluorescence test (Ruckerbauer et al. 1967; Jochim and Jones 1983), the complement fixation test (Shone et al. 1956), the haemagglutination assay (van der Walt 1980; Hübschle 1980), electron microscopy (Gould et al. 1989; Nunamaker et al. 1992), virus neutralization assays (Howell 1960) and competitive antigen-capture ELISAs (Mecham 1993; Mecham and Wilson 2004). With the development of nucleic acid detection methods in the late 1980s, cloned cDNA segments of several BTV serotypes were used to define the genetic relationships between and within the BTV serotypes (Unger et al. 1988; Ritter and Roy 1988). The introduction of the polymerase chain reaction (PCR) in the beginning of the 1990s revolutionized the molecular diagnosis of BTV (Wade-Evans et al. 1990; McColl and Gould 1991). In the following years, several improvements of BTV genome detection by PCR were published. Besides one-step RT-PCR assays, nested PCR systems and multiplex PCR systems for the identification of different BTV serotypes circulating in one region were developed (Wilson and Chase 1993; Katz et al. 1994; Aradaib et al. 1998; Zientara et al. 2004). Primers of different segments were used for the amplification and characterization of BTV strains (see Hoffmann et al. 2009a, for a review). For group-specific assays, conserved regions of the segments 5, 6, 7 and 10 were identified (Aradaib et al. 1998; Pierce et al. 1998; Bandyopadhyay et al. 1998; Anthony et al. 2007). Specific primers for the VP2 gene were developed and used in singleplex or multiplex assays to determine the BTV serotype (Wilson and Chase 1993; Eschbaumer et al. 2011b).

A new era for the molecular diagnosis of BTV began with the development of the real-time RT-PCR technology in the 1990s (Higuchi et al. 1993; Wittwer et al. 1997). Prior to the BTV-8 outbreak in Europe in the summer of 2006, only very few real-time quantitative RT-PCR (RT-qPCR) assays for the detection of BTV had been published. The first used primers were designed for the detection of the NS1 gene (Seg-5) (Wilson et al. 2004). However, this assay detected only 11 out of the 19 serotypes tested. The same year, another RT-qPCR was published using Förster resonance energy transfer (FRET) probe technology targeting genome segment

2 (VP2) (Orru et al. 2004). In 2006, an RT-qPCR assay was developed using a conserved region in RNA segment 5 of BTV-2 and BTV-4 (Jimenez-Clavero et al. 2006). This assay detected all of the recent Mediterranean isolates that were tested, BTV vaccine strains for serotypes 2 and 4 as well as 15 out of the 24 BTV reference strains. In the European outbreak of BTV-8, however, this assay showed a reduced sensitivity for the field strain of BTV-8 compared to other assays (Batten et al. 2008a). In the same year, an RT-qPCR was developed using a molecular beacon (MB) fluorescent probe designed within the NS3 conserved region of segment 10 (Orru et al. 2006).

Since the start of the northern European outbreak in August 2006, many RT-qPCR assays have been developed. Most of these were assays for the detection of all 24 serotypes identified at this time (Toussaint et al. 2007; Shaw et al. 2007), using conserved regions of the VP1 (seg-1) and NS1 (seg-5) genes. Nevertheless, the reduced sensitivity for the novel BTV serotypes 25 and 26 (Hofmann et al. 2008; Maan et al. 2011a, b) confirms the necessity for regular verification of the functionality of such pan-BTV assays. The parallel use of independent assays with equivalent diagnostic sensitivity and specificity can overcome the limitations of one assay. Another option is the application of pan-orbivirus assays, which use primer binding sites in the polymerase gene (seg-1) that are conserved among all currently known orbiviruses (Palacios et al. 2011). In addition to the group-specific (pan-BTV) real-time RT-PCR assays, serotype-specific BTV assays were developed and validated by diagnostic laboratories (Mertens et al. 2007; Hoffmann et al. 2009a, b). Furthermore, the advantages of the real-time PCR technology can be used for high-throughput analyses (Vandemeulebroucke et al. 2010). The use of robotics for automated extraction of BTV RNA combined with the co-amplification of the BTV target and an internal control RNA ensures a high diagnostic reliability during the investigation of large sample batches (Toussaint et al. 2007; Vandenbussche et al. 2010). These can be blood and tissue specimens in outbreak situations or insect samples from entomological monitoring programmes. High-throughput RNA extraction and real-time RT-PCR are particularly indispensable for the BTV analysis of extensive numbers of midges (Hoffmann et al. 2009c; Vanbinst et al. 2009).

5.4.3 Serology

Historically, BTV antibody detection in serum relied on complement fixation and agar gel immunodiffusion. Both, however, proved inferior to enzyme-linked immunosorbent assays (ELISAs) and were eventually replaced (Afshar 1994; Hamblin 2004). Highly sensitive ELISAs can pick up the humoral immune response to BTV as early as 1 week after infection (Batten et al. 2008a; Oura et al. 2009). Several systems are commercially available, mostly detecting antibodies against VP7, a BTV structural protein that is largely conserved across all serotypes. Three competitive ELISA kits (by ID VET, IDEXX and VMRD) are currently licensed for use in Germany. A latex agglutination test (Yang et al. 2010a) and immunochromatographic strips

(Yang et al. 2010b) have recently been proposed, promising faster sample turnover and the possibility of pen-side testing.

Apart from the competitive ELISAs, an indirect assay is available for individual and bulk milk samples (Kramps et al. 2008; Chaignat et al. 2010; Mars et al. 2010). Double-antigen sandwich ELISAs, which use peroxidase-labelled antigen to detect captured antibody (Laman et al. 1991), display superior sensitivity early in infection and more reliably detect vaccine-induced antibody (Eschbaumer et al. 2009; Oura et al. 2009). Two kits (by ID VET and Prionics) are commercially available in Germany.[1] However, their bias for multimeric antibody molecules such as immunoglobulin (Ig) M may have a negative impact on sensitivity during the transition from IgM to IgG in the development of the immune response (Eschbaumer et al. 2011a).

Beyond the performance of a group-specific assay, serotyping usually requires labour-intensive neutralization tests against a panel of reference viruses (Hamblin 2004). A serotype-specific antibody ELISA for BTV-8 has recently been implemented, but data on its performance are not yet available.

Regardless of the test format, the widespread use of inactivated whole virus vaccines in Europe (Zientara et al. 2010) interferes with serological surveillance. The commercially available VP7-based tests are unable to differentiate between infected and vaccinated animals (Mertens et al. 2009). Theoretically, inactivated vaccines should only elicit antibody responses to structural proteins. The discrimination potential of ELISAs based on non-structural proteins NS1 or NS3 has been demonstrated (Anderson et al. 1993; Barros et al. 2009) but is highly dependent on the purity of the vaccine. The carryover of non-structural proteins from the culture system used to produce the vaccine may result in antibodies to those proteins in vaccinated animals (Alpar et al. 2009), particularly after repeated vaccinations. Recent attempts at establishing a commercial NS1 ELISA were not successful. Vaccination with inactivated vaccines from different companies led to an increased number of unspecific results in the test, which, consequently, was not released by the manufacturer.

5.5 Epidemiology

5.5.1 Disease Transmission

When BTV serotype 8 (BTV-8) first appeared in Central Europe, no data on the putative vectors were available. Transmission of BTV had generally been supposed to be associated with *Culicoides imicola*, the most efficient and widespread vector in the Old World (Meiswinkel et al. 2007). Since this species is restricted to Africa and southern Europe, the occurrence of BT in more northern countries and its

[1] According to the list of products certified by the Friedrich-Loeffler-Institut pursuant to §17c of the Animal Diseases Act; see http://www.fli.bund.de/en/startseite/services/licensing-authority.html for the most recent version.

effective transmission by indigenous vectors was surprising, despite evidence from Italy suggesting that also *C. pulicaris* and midges of the *C. obsoletus* group can harbour BTV (Caracappa et al. 2003; Savini et al. 2004).

Meanwhile, entomological monitoring has shown that *C. imicola* is still not present in Germany and that members of the *C. obsoletus* and *C. pulicaris* complexes are relevant vectors for BT in Germany and Central Europe (Hoffmann et al. 2009c; Mehlhorn et al. 2009a). They are small midges of approximately 1–2 mm length which tend to be active in the evening, night and dawn. The length of time between ingestion of virus by a midge and its appearance in the saliva, the so-called extrinsic incubation period, is influenced by both temperature and salivary proteases of the vectors. At temperatures of 10–30°C, the extrinsic incubation period becomes progressively shorter with increasing temperatures since:

- Midges feed more frequently.
- Both the reproductive cycle of midges and virus replication in midges are faster.
- A greater proportion of the midge population becomes vector competent.
- Possibly more midge species become vector competent (Hateley 2009).

In Germany, transmission of BT is apparently interrupted or at least significantly reduced during the cold season (late autumn, winter and early spring) as a consequence of the low temperatures, which reduce the activity of *Culicoides* biting midges and BTV replication in the midgut of the biting midges. However, BTV might persist during the winter either in the vector population or in the host population (Wilson et al. 2008). Another possible way of overwintering is persistence in an as yet unknown wild ruminant population, e.g. red deer. In Belgium, relatively high levels of seroprevalence have been observed in red deer during 2007 and 2008. By contrast, the seroprevalence in roe deer was low, which was explained by the fact that red deer live in large groups, move more and therefore might be more exposed to insects (Linden et al. 2010). The results of the German wildlife monitoring on BT provide no evidence for the conclusion that a reservoir for BTV might have formed in wild ruminants in Central Europe. However, the relative importance of the remaining potential overwintering mechanisms remains unclear, too (Napp et al. 2011). As active midges were also found in the cold season, although in reduced numbers and mostly close to or within stables, a true midge-free period does not exist in Germany (Mehlhorn et al. 2009b).

Several investigation programmes were set up to share European BT outbreak data, e.g. in the BT51 group and as part of EPIZONE, an EU-funded Network of Excellence. Special attention has been paid to both mechanistic and stochastic predictive modelling which included a wide range of predictor variables to assess the spread of BTV (Faes et al. 2011; de Koeijer et al. 2011; Ducheyne et al. 2011; Willgert et al. 2011).

5.5.2 *Introduction of Bluetongue Disease into Germany*

In 2006, the first infections with bluetongue virus of serotype 8 (BTV-8), which was initially restricted to the sub-Saharan region, Asia and South America, invaded

Central Europe. On August 18th, BTV-8 was confirmed in the Netherlands (EU Rapid Press release Nr. IP/06/1112, ProMedMail, 20060828.2448) and Belgium (Toussaint et al. 2007). On 21 August 2006, the first outbreak was reported in the neighbouring district of Aachen on the German side of the border (OIE, immediate notification, Conraths et al. 2007). The virus hit an area with a high density of BT-naive animals, presence of suitable vectors (*Culicoides* spp.), climatic conditions favourable for virogenesis in local biting midges and for transmission. So far, the epidemiological situation in Germany has been dominated by BTV-8 (Conraths et al. 2009; Gethmann et al. 2010), but a few cases of infections with BTV-6 and a single case of BTV-1 in an imported animal were reported (Eschbaumer et al. 2010a).

In addition to entomological patterns, much research has been devoted to the determination of the most likely time and place of introduction of BTV-8 (Saegerman et al. 2010). The following hypotheses to explain the introduction of BTV-8 to Western Europe were taken into consideration:

- Legal or illegal import of viraemic susceptible animal species.
- Legal or illegal import of infected non-susceptible animal species, especially against the background that the World Equestrian Games took place in Aachen at the time when the first BT cases were noticed.
- Introduction via infected vectors, especially because midges are so light that they can drift by wind over hundreds of kilometres (Ducheyne et al. 2007). Alba et al. (2004) confirmed the possibility of introduction of infected midges to the Balearic Islands from Sardinia during the BT outbreak in the year 2000. It has also been proposed that BTV-infected biting midges might have been moved from northern Africa to Spain, Portugal or Italy by wind across the Strait of Gibraltar and the Mediterranean Sea in 2006 (Gloster et al. 2007; Hendrickx 2008). Another alternative is that infected vectors might have been directly introduced to Europe, e.g. by airplane with flowers imported from regions where BTV is enzootic. This hypothesis was also considered when BTV-6 occurred in the district of Grafschaft Bentheim (Lower Saxony) on the border to the Netherlands, although the respective virus closely resembled an isolate used in an attenuated live vaccine (Eschbaumer et al. 2010a).

Despite intensive epidemiological investigations, the source of the introduction has never been unambiguously identified.

5.5.3 Course of the 2006–2009 Bluetongue Epidemic in Germany

Until the end of 2006, a total of 890 cases/outbreaks were reported from the German federal states of North Rhine-Westphalia, Hesse, Rhineland-Palatinate and Lower Saxony to the German Animal Disease Notification System (TierseuchenNachrichtenSystem, TSN; accessed 23/11/2011). Between January and April 2007, when no transmission was expected, 185 further outbreaks were reported (Table 5.1). It is

Table 5.1 Number of reported outbreaks 2006/2007 and affected species

Year	Month	Cattle	Sheep	Goats	Wildlife	Total
2006	August	35	4	–	1	40
2006	September	64	37	–	–	101
2006	October	295	170	–	4	469
2006	November	137	95	–	5	237
2006	December	37	3	–	3	43
2007	January	80	–	–	1	81
2007	February	52	–	–	–	52
2007	March	19	1	–	–	20
2007	April	31	1	–	–	32
Total		750	311	0	14	1,075

likely that at least the vast majority of infected animals detected in winter 2006/2007 had contracted BT in summer or autumn 2006.

By analysing the outbreak data as reported to the TSN database and comparing them to the number of animals kept on the affected farms, it became apparent that at least 67,080 cattle, 9,825 sheep and 56 goats were present on premises affected by BTV-8 between August 2006 and April 2007 (Table 5.3). Of these animals, 1,529 cattle (2.28%) and 592 sheep (6.03%) were found infected. Eighty-four cattle and 222 sheep died. The case-fatality rate was much higher in sheep (37.5%) than in cattle (5.5%). These calculations are based on the assumption that all BT cases were reported. Since the infections caused only mild disease or remained even clinically inapparent in some animals, in particular cattle, it is likely that there was a substantial level of underreporting. As a consequence, the case-fatality rate in cattle might be slightly overestimated (Conraths et al. 2009).

Apparently, BTV-8 overwintered in the region and flared up again in 2007 to spread over most of western Germany during summer and autumn 2007. The first outbreak after the end of the cold season was confirmed in June 2007 in the district Oberbergischer Kreis, North Rhine-Westphalia, when a sentinel animal (cattle) sampled in May 2007 tested positive (Hoffmann et al. 2008).

The infection also re-emerged in other European countries that had been affected in 2006. BT spread rapidly through Belgium, the Netherlands, Germany, France and Luxembourg and reached the Czech Republic, Denmark, Italy, Spain, Switzerland and the United Kingdom.

Between May 2007 and April 2008, more than 22,600 cases/outbreaks were reported from Germany (Table 5.2; Conraths et al. 2009; Gethmann et al. 2010). Due to the enlargement of the affected territory in 2007, the exposed population of animals kept in farms with BT cases rose to at least 1,501,994 cattle, 505,934 sheep and 3,736 goats. The number of diseased animals on these farms amounted to 33,839 cattle, 32,158 sheep and 227 goats (Table 5.3). While mortality remained at relatively low levels as in 2006, the case-fatality rate rose to 10.8% in cattle and 41.5% in sheep.

To take potential underreporting of BT cases into account, we also determined the number of animals for which the owners received financial aid from the German

Table 5.2 Number of reported outbreaks 2007/2008 and affected species

Year	Month	Cattle	Sheep	Goats	Wildlife	Total
2007	May	–	–	–	–	0
2007	June	2	–	–	–	2
2007	July	10	8	–	–	18
2007	August	979	1,253	6	3	2,241
2007	September	5,142	4,792	60	21	10,015
2007	October	3,916	1,621	42	29	5,608
2007	November	1,755	96	6	14	1,871
2007	December	836	20	1	14	871
2008	January	854	11	2	5	872
2008	February	572	9	4	1	586
2008	March	428	5	1	3	437
2008	April	122	1	1	3	127
Total		14,616	7,816	123	93	22,648

Table 5.3 Exposed, diseased and dead animals and morbidity, mortality and case-fatality rate of BT infections for Germany in 2006 and 2007

Outbreak season	May 2006–April 2007			May 2007–April 2008			May 2008–April 2009		
Species	Cattle	Sheep	Goats	Cattle	Sheep	Goats	Cattle	Sheep	Goats
Premises	758	319	13	14,756	7,910	257	2,956	278	11
Animals kept on affected farms	67,080	9,825	56	1,501,994	505,934	3,736	425,959	62,915	141
Diseased animals	1,445	370	0	30,175	18,821	173	5,358	429	4
Dead animals	84	222	0	3,664	13,337	54	94	238	1
Morbidity (%)[a]	2.15	3.77	0.00	2.01	3.72	4.63	1.26	0.68	2.84
Mortality (%)[b]	0.13	2.26	0.00	0.24	2.64	1.45	0.02	0.38	0.71
Case fatality (%)[c]	5.49	37.50	0.00	10.83	41.47	23.79	1.72	35.68	20.00

[a]Number of diseased animals/number of animals in affected farms
[b]Number of dead animals/number of animals in affected farms
[c]Number of dead animals/number of infected animals

animal disease compensation funds (Tierseuchenkassen). In total, 10,240 cattle, 33,233 sheep and 102 goats were compensated for in 2007. This indicates an overall mortality of 0.08% in cattle and 1.36% in sheep. By focussing on the core region (North Rhine-Westphalia), where a prevalence of up to 100% can be assumed (by comparison to the situation in Belgium; Gethmann et al. unpublished), the mortality was 0.51% in cattle and 13.19% in sheep.

While several member states declared vector-free periods according to Commission Regulation (EC) No. 1266/2007 of 26 October 2007 during the cold season, i.e. defined as a period where the risk of virus transmission is deemed extremely low or negligible, thus allowing for a temporary lift of some trade restrictions, transmission was shown in Schleswig-Holstein in February 2008 (Hoffmann et al. 2008). This indicates that vector transmission on a low level may have played a role in the

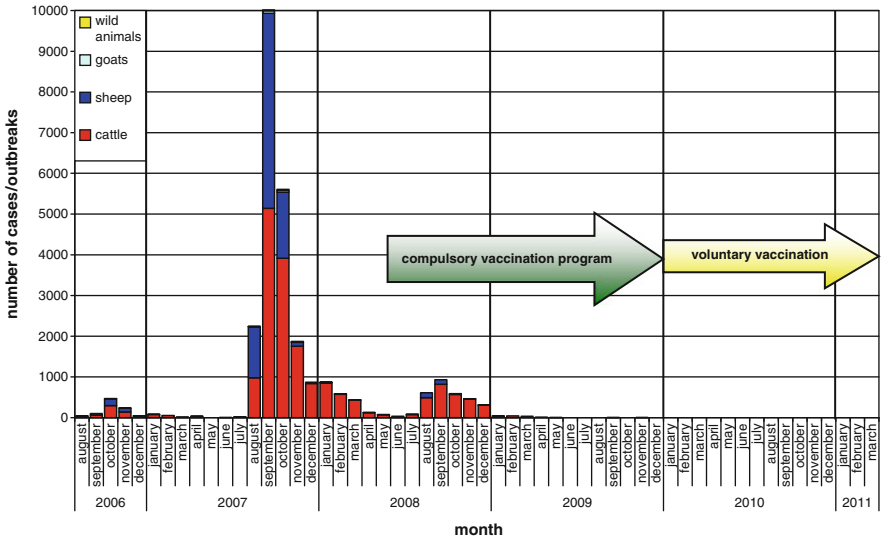

Fig. 5.7 Bluetongue disease, monthly incidence (*source*: TSN)

overwintering mechanism of BT in Germany and supported the view that declaration of a seasonally vector-free period was not appropriate for Germany.

In order to control BTV-8, to reduce the suffering of BT-infected animals and to mitigate the economic damage caused by the epizootic, it was decided to conduct a compulsory vaccination programme in Germany using inactivated vaccines which had not yet been licensed when the programme started (for details, see Chap. 5.6.2 Vaccination). The vaccination programme started in May and led to a massive decrease of new outbreaks in 2008 (Conraths et al. 2009) (Fig. 5.7, Table 5.4).

Between May and December 2008, a total of 3,083 new BTV-8 outbreaks plus 19 BTV-6 cases were reported (Table 5.4). They were mainly found in two regions in the north-west of Lower Saxony and Western parts of Baden-Württemberg. These cases can be explained by the relatively late onset of the immunization campaign because of initially limited supply of BTV-8 vaccines.

At the end of 2008, the genome of BTV serotype 6 (BTV-6) was detected in the district of Grafschaft Bentheim in BTV-1-vaccinated animals. In November and December, outbreaks in a total of 19 cattle farms were reported (Eschbaumer et al. 2010a). None of the animals showed clinical symptoms. Similar cases had previously been reported in the Netherlands (presentation at SCFCAH, section animal health and animal welfare, 08 December 2008, http://ec.europa.eu/food/committees/regulatory/scfcah/animal_health/presentations_en.htm). Despite comprehensive epidemiological investigations, the source of infection could not be identified. It cannot be excluded that animals were illegally vaccinated with an imported modified-live vaccine and that spread vaccine virus may have reassorted (Saegerman and Pastoret 2009). In 2009, no further cases of BTV-6 were detected despite intensive monitoring.

Table 5.4 Number of reported outbreaks 2008/2009

Year	Month	Cattle	Sheep	Goats	Wildlife	Total
2008	May	70	3	–	–	73
2008	June	33	1	–	–	34
2008	July	73	13	1	–	87
2008	August	492	118	–	1	611
2008	September	819	111	2	–	932
2008	October	567	20	1	1	589
2008	November	461	3	–	–	464
2008	December	307	3	–	2	312
2009	January	39	2	–	–	41
2009	February	44	–	–	1	45
2009	March	34	1	–	–	35
2009	April	12	–	–	–	12
Total		2,951	275	4	5	3,235

Since May 2009, only 12 outbreaks of BTV-8 in 9 cattle herds and 3 sheep flocks were reported, the last one occurred in November 2009 (TSN; accessed 23/11/2011).

5.6 Control Measures

After the first occurrence of BTV-8, Germany carried out the measures according to "Council Directive 2000/75/EC of 20 November 2000 laying down specific provisions for the control and eradication of bluetongue" in combination with national legislation (Verordnung zum Schutz gegen die Blauzungenkrankheit, Verordnung zum Schutz vor der Verschleppung der Blauzungenkrankheit). The measures focussed on (1) outbreak investigations in combination with monitoring and surveillance, (2) establishing restriction zones (e.g. movement restrictions) and (3) treating affected animals, farms, etc., with insecticides. In 2006, zones with a radius of at least 20 and 150 km were established around each outbreak farm (Fig. 5.8). In October 2007, measures regarding control, monitoring, surveillance and restrictions on movements of certain animals of susceptible species in relation to bluetongue were specified in Commission Regulation (EC) No 1266/2007 of 26 October 2007.

5.6.1 Movement Restrictions

Historically, BT has been regarded as an "exotic" disease in Europe, although sporadic incursions were observed on Cyprus in the first half of the twentieth century and in the mainland of Europe since the 1950s (Wilson and Mellor 2009). As a consequence, a protection zone with a radius of at least 100 km around

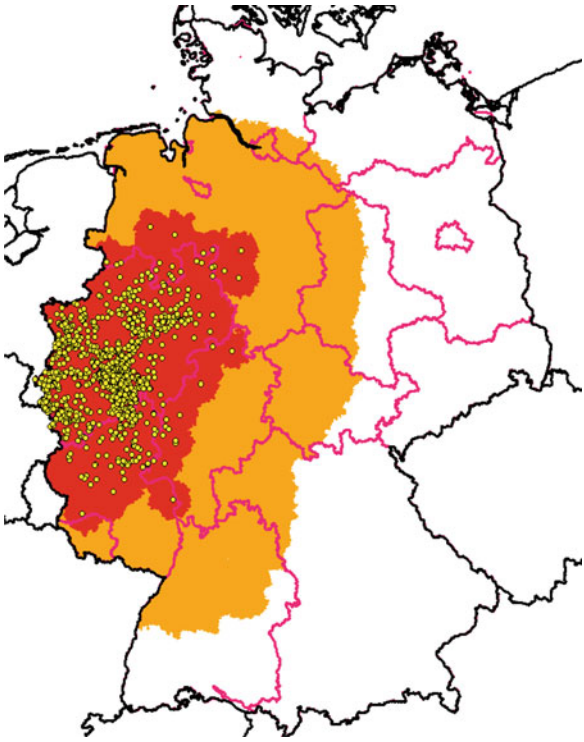

Fig. 5.8 Outbreaks and restriction zones in Germany by the end of April 2007

the infected holding and a surveillance zone with a depth of at least 50 km extending beyond the limits of the protection zone were provided for (Council Directive 2000/75/EC of 20 November 2000 laying down specific provisions for the control and eradication of bluetongue). An exit ban on animals and an epidemio-surveillance programme based on the monitoring of sentinel groups preferentially of bovine animals and of vector populations were imposed for both zones. While vaccination against BT can be allowed in the protection zone, animals must not be immunized against the disease in the surveillance zone. Trade within the same zone was allowed, but there were strict limitations for moving animals from one zone to another according to defined criteria (Commission Decision 2005/393/EC, replaced by Commission Regulation (EC) No 1266/2007), such as testing blood samples of the animals for BTV before movement, protecting them against vectors and treating them against insects prior to movement (Hateley 2009).

Since all control measures, including restrictions of animal transport, use of insecticides and indoor keeping of animals, had only limited effect during the BTV-8 epidemic that started in Belgium, Germany and the Netherlands in August 2006 (Mintiens et al. 2008b), vaccination of susceptible species with inactivated vaccines was included as the method of choice for BT control as soon as BTV-8-specific vaccines became available.

5.6.2 *Vaccination*

In general, vaccination is the only reliable means to protect animals from clinical bluetongue disease, while at the same time preventing the onward transmission of the virus. The two most common types of BTV vaccines are attenuated modified-live viruses and inactivated whole virus preparations with adjuvants. For modified-live vaccines, there is a delicate balance between achieving an acceptable reduction in virulence while at the same time maintaining the required level of immunogenicity (Alpar et al. 2009). In adverse circumstances, live vaccines can cause disease (Veronesi et al. 2005, 2010; Monaco et al. 2006), can be transmitted by vectors (Ferrari et al. 2005; Listes et al. 2009) and can exchange genetic information with field strains (Batten et al. 2008b; Maan et al. 2010). The repeated culture passages used for attenuation alter the tissue tropism of the virus; this can lead to teratogenic effects in pregnant animals (Kirkland and Hawkes 2004; Maclachlan et al. 2009). Hence, when BTV-8 was introduced to Europe in 2006, concerns over the safety of live vaccines prevented their use. The affected countries opted to wait until highly effective and safe inactivated vaccines became available in 2008 (Eschbaumer et al. 2009; Gethmann et al. 2009). A large-scale vaccination campaign all across Europe eventually brought the epizootic to a hold and it appears as if BTV-8 has been eradicated (Zientara et al. 2010). Since the end of mandatory vaccination in 2010, however, coverage is decreasing; even though the inactivated vaccines afford good long-term protection (Wäckerlin et al. 2010; Oura et al. 2012), replacement of stock will eventually return the animal population to its initial vulnerable state (Gethmann et al. 2010).

Infection with one serotype does not lead to cross-protective immunity, and neither does vaccination with a monovalent vaccine (Alpar et al. 2009; Bréard et al. 2011; Eschbaumer et al. 2011b). It has been shown experimentally, however, that sequential infection with several serotypes can give rise to neutralizing antibody against others (Jeggo et al. 1983). This suggests that broadly protective vaccines are possible, but none have been developed so far.

Another issue with currently available vaccines is the absence of a reliable strategy to differentiate infected from vaccinated animals (DIVA) (van Oirschot 1999; Mertens et al. 2009). Experimental vector vaccines (based on pox and herpes viruses) have shown some potential (Wade-Evans et al. 1996; Lobato et al. 1997; Boone et al. 2007; Calvo-Pinilla et al. 2009a; Franceschi et al. 2011). Since these vaccines only elicit an immune response to a subset of BTV proteins, the absent proteins can serve as negative markers in a DIVA strategy. The same goes for virus-like particles (Roy et al. 1994; Stewart et al. 2010), in vitro-assembled virus capsids without genome that do not evoke antibodies to non-structural proteins. Building on the reverse genetics system for BTV (Boyce et al. 2008), upcoming disabled infectious single-cycle vaccines (Matsuo et al. 2011) could combine the excellent immunogenicity of modified-live vaccines, the safety of inactivated vaccines and the DIVA capability of vector vaccines, if suitable companion tests are developed.

After the first introduction of BTV-8 to Belgium, Germany and the Netherlands in 2006, the massive spread of BTV-8 in 2007, reports about a large number of diseased and dead animals, and the failure of other control measures, the commission and the member states decided to carry out an harmonized vaccination programme to control BT (EU 2008: Bluetongue: Commission offers co-funding for vaccination campaign, Press release, IP/08/51, Brussels, 16 January 2008). Since the available vaccines against BTV-8 had not been registered at this time and safety and efficacy assessments were rudimentary, a large-scale safety study in combination with an efficacy study was conducted before a compulsory vaccination campaign involving the administration of millions of doses of largely untested BTV-8 vaccine was started in Germany. Participation in the study was prerequisite for a temporary emergency authorization to be granted by German law (Tierseuchengesetz §17c). Three monovalent inactivated BTV8 vaccines, precisely BLUEVAC® 8 (CZ Veterinaria), BTVPUR® AlSap 8 (Merial), and Zulvac® 8 Ovis or Bovis, respectively (Fort Dodge), were tested and proved to be safe and efficacious (Gethmann et al. 2009; Eschbaumer et al. 2009; Wäckerlin et al. 2010). For the basic immunization, administration of two doses was necessary in cattle, while a single dose was deemed sufficient in sheep and goats.

The first batches of the vaccines were delivered in May 2008. Until the end of 2008, about 20 million doses were administered to cattle and 2.6 million doses to sheep. In 2009, 13 million doses were applied to cattle and 2.1 million doses to sheep, so that the vaccination coverage was over 80%. In 2009, a further vaccine, Bovilis® BTV8 (Intervet), was introduced in the vaccination programme. By the end of 2009, the German federal states decided by majority vote to switch from a compulsory vaccination programme to a voluntary programme, resulting in a decrease of the administered vaccine doses. Only 5 million doses in cattle and 0.6 million doses in sheep were reported to the national animal database (HI Tier).

It has been pointed out, however, that low vaccination coverage or the introduction of other serotypes could result in further, potentially severe outbreaks in the future (Szmaragd et al. 2010).

5.6.2.1 Claims of Potential Adverse Reactions

Although the application of BTV-8 vaccines might induce moderate, short-term local inflammatory reactions at the injection site and a transient rise in body temperature shortly after booster vaccination, the vaccines proved to be well tolerated by both cattle and sheep (Bruckner et al. 2009; Gethmann et al. 2009). However, despite the proof of the safety of the vaccines, farmers, especially in south-eastern Germany and Switzerland, claimed a wide range of adverse reactions during the compulsory vaccination programme in 2008/2009, including reduction in milk yield, increase of somatic cell count in milk, mastitis or alterations of milk quality, reduced fertility and abortions. Officially, a total of 616 adverse reactions were reported in Germany to the Federal Agency for Vaccines and Biomedicines,

thereof 547 in cattle (Hoffmann and Cußler 2009). In Switzerland, a total of 1,000 reports related to BTV-8 vaccination were received in 2009, the most frequently reported suspected adverse reactions being abortion, mastitis or alterations of milk quality (Müntener et al. 2010). However, in both countries evaluation of the data showed that plausible links between vaccination and the suspected adverse reactions could not be demonstrated (Probst et al. 2011; Tschuor et al. 2010). In any case, compared to the negative effect of BTV exposure on fertility, the possible side effect of vaccination seems to be rather small and therefore should not be an obstacle to vaccination (Nusinovicia et al. 2011).

5.6.3 Vector Evasion and Control

Vector evasion strategies and the use of repellents and insecticides alone are unlikely to lead to effective BT control (Mullens et al. 2001; EFSA 2007), although they may reduce vectorial capacity, i.e. reducing attack rates and the survival of adult midges (Mullens 1992). Vector evasion and control measures may thus be useful as auxiliary or mitigation measures which should be preferably applied in addition to vaccination against the relevant serotypes of BTV, the method of choice for the control of BT.

5.6.3.1 Vector Evasion Measures

It has been suggested that simple husbandry changes and practical midge control measures may help to diminish the risk of infection for susceptible animals, e.g. by housing livestock during times of maximum midge activity (from dusk to dawn) to reduce biting rates and thus transmission of BTV

Culicoides midges that carry BTV are believed to breed on animal dung and moist soil, either bare or covered in short grass. Identifying breeding grounds and breaking the breeding cycle may thus reduce the local midge population and hamper virus transmission.

It has been proposed that turning off taps, mending leaks and filling in or draining damp areas might also help to dry up breeding sites and that dung heaps or slurry pits should be covered or removed, and their perimeters regularly scraped to remove or destroy developing larvae of biting midges. Although it seems plausible that these measures might have an effect, published studies demonstrating their efficacy are lacking.

5.6.3.2 Use of Insecticides for Vector Control

Pyrethrum and synthetic pyrethroids are the most important compounds used against biting midges. They combine a repellent activity with toxic effects on

insects. Due to the high efficiency of transmission of BTV from the biting midge to the vertebrate host, the repellent effect is particularly relevant for preventing BT infections as it may protect hosts from vector bites. However, an extremely high efficacy of the repellent seems to be required to achieve a significant level of protection against BT infections. Since the repellent activity of pyrethroids decreases much quicker than their toxic effect, it is difficult to take advantage of the repellent activity without applying the compounds repeatedly in short intervals. Frequent application may however increase the risk of side effects and lead to an unacceptable impact on non-target insects such as bees, beetles, etc.

Other potentially suitable compound groups include macrocyclic lactones, organophosphates, carbamates, chloronicotinyls (e.g. imidacloprid) and phenylpyrazoles (fipronil).

The efficacy of various pyrethroids against *Culicoides* spp. has been examined in several studies. Depending on the specific product and its formulation, deltamethrin, permethrin, cyfluthrin and cypermethrin protected animals for 3–5 weeks (Mehlhorn et al. 2008a, b; Liebisch and Liebisch 2008; Liebisch et al. 2008a, b; Papadopoulos et al. 2009; Schmahl et al. 2008, 2009a–c; Mullens et al. 2010). It is important to note that the treated animals were only protected if a sufficient concentration of the compound near the predilection sites of biting midges was warranted.

Pour-On and Spot-On Formulations of Repellents and Insecticides

Pour-on and spot-on formulations of repellents and insecticides have been successfully used against biting midges. It should be noted that pour-on and spot-on treatments with permethrin and deltamethrin lead to a dorsoventral gradient of the compound concentration, also after correct application of the product, with the consequence of a reduced insecticidal effectiveness in the bioassay (Mullens et al. 2000, 2001; Liebisch et al. 2008a). Moreover, a field study conducted in Brandenburg, Germany, showed that a regular pour-on treatment of bulls in intervals of 6 weeks had neither an effect on the total number of biting midges caught in a UV trap nor on the number of blood-fed midges (Bauer et al. 2009).

Nets

Fine mesh nettings and fabrics impregnated with insecticide, in particular pyrethroids, have been proposed to protect stables or to reduce contact of livestock with potentially infected midges (Braverman 1989; Carpenter et al. 2008) and were recently evaluated under field conditions (Bauer et al. 2009; Skrock et al. 2010; Skrock 2011). When Meiswinkel et al. (2000) gauzed all windows of a stable with a fine mesh screening and kept the doors closed, a 14-fold reduction in the number of *Culicoides* entering the stable was achieved. This approach may be useful as it reduces the biting rate. It is also a cheap measure that is easy to implement and requires only little maintenance.

Ear Tags

Holbrook (1986) treated cattle with one fenvalerate ear tag per animal and exposed adult *C. variipennis* in the laboratory to hair clippings recovered from the animals on several days post treatment. The insecticidal activity on the biting midges lasted throughout the 70-day test period, with decreased efficacy following rainfall and after the 49th test day. The duration of the repellent and toxic effect of ear tags may be shorter than that of pour-on or spot-on formulations and depend on the number of ear tags used per animal. For permethrin, a toxic and repellent activity of up to 7 days was observed if a single ear tag was applied, while the effects of two ear tags lasted for up to 19 days (Liebisch et al. 2008b). The repellent effect of cypermethrin on *C. sonorensis* lasted for 3–5 weeks (Reeves et al. 2010), while Liebisch and Liebisch (2008) determined an insecticidal effect of 14 (one ear tag) to 21 days (two ear tags).

Dipping

While this method has been successfully applied in many regions of the southern hemisphere including African and South American countries as well as Australia, farms in central and northern Europe are rarely equipped with dips, and the required formulations of pyrethroids or organophosphates are hardly available or their use has been suspended due to their environmental impact (pyrethroids) or discouraged because of their toxicity to users (EFSA 2007).

Systemic Use of Insecticides

The use of injectable macrocyclic lactones against biting midges yielded variable results (Standfast et al. 1985; Holbrook 1994; Holbrook and Mullens 1994). Since these compounds have no repellent, but only a toxic effect on insects, they may only act by reducing the population density of biting midges due to their effect on adult stages and on the larvae of dung-inhabiting *Culicoides*, thus reducing the vectorial capacity in a limited area surrounding the treated animals (EFSA 2007). A direct effect on BT transmission by reducing the biting rate of the relevant vectors cannot be expected.

5.7 Economic Impact

The financial impact of BTV-8 in the Netherlands including production losses, diagnosis, treatment and disease control amounted to 32 million Euros in 2006, 164–175 million Euros in 2007 (Velthuis et al. 2010) and about 41 million Euros in 2008 (Velthuis 2011).

Table 5.5 Economic losses in euros caused by the bluetongue disease epidemic in 2006–2009

Year	2006	2007	2008	2009	2010
Direct costs	2,878,082	57,738,386	8,201,700	22,789	0
Indirect costs	27,741,160	31,601,248	80,734,580	34,554,815	10,990,442
Control measures including vaccination	127,445	2,708,616	55,580,720	34,453,858	10,940,442
Trade restrictions, testing	27,124,515	23,131,575	24,533,595	0	0
Administrative costs	189,200	2,242,152	320,265	957	0
Monitoring/surveillance	3,218,905		No data	No data	No data
Public costs for research and development, consultancy	300,000	300,000	300,000	100,000	50,000
Total costs	30,619,243	89,339,634	88,936,281	34,577,604	10,990,442

The Animal Health Service of North Rhine-Westphalia, Germany, calculated mean production losses of 197 € per cow for a farm with 25 cows (http://www.landwirtschaftskammer.de/landwirtschaft/tiergesundheit/rgd/index.htm).

These data were used as reference for estimating the financial impact of BTV-8 for Germany as a whole. Outbreak data from the German animal disease notification system, information on payments made by the German animal disease compensation funds (Tierseuchenkassen) to BT-affected farms and administrative data were also included in the analysis. According to these calculations, the financial losses BTV-8 caused in Germany amounted to approximately 31 million Euros in 2006, 90 million Euros in 2007, 89 million Euros in 2008, 35 million Euros in 2009 and 11 million Euros in 2010, i.e. a total loss of 254 million Euros so far (Table 5.5). It has to be taken into account, however, that some parameters (e.g. morbidity, trade costs) could not be calculated exactly but had to be estimated.

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References

Afshar A (1994) Bluetongue: laboratory diagnosis. Comp Immunol Microbiol Infect Dis 17:221–242

Alba A, Casal J, Domingo M (2004) Possible introduction of bluetongue into the Balearic Islands, Spain, in 2000, via air streams. Vet Rec 155:460–461

Alpar HO, Bramwell V, Veronesi E, Darpel K, Pastoret PP, Mertens P (2009) Bluetongue virus vaccines past and present. In: Mellor P, Baylis M, Mertens P (eds) Bluetongue, Biology of animal infections. Elsevier, London

Anderson J, Mertens PP, Herniman KA (1993) A competitive ELISA for the detection of anti-tubule antibodies using a monoclonal antibody against bluetongue virus non-structural protein NS1. J Virol Methods 43:167–175

Anthony S, Jones H, Darpel KE, Elliott H, Maan S, Samuel A, Mellor PS, Mertens PP (2007) A duplex RT-PCR assay for detection of genome segment 7 (VP7 gene) from 24 BTV serotypes. J Virol Methods 141:188–197

Aradaib IE, Schore CE, Cullor JS, Osburn BI (1998) A nested PCR for detection of North American isolates of bluetongue virus based on NS1 genome sequence analysis of BTV-17. Vet Microbiol 59:99–108

Backx A, Heutink CG, van Rooij EMA, van Rijn PA (2007) Clinical signs of bluetongue virus serotype 8 infection in sheep and goats. Vet Rec 161:591–592

Bandyopadhyay SK, Kataria RS, Tiwari AK (1998) Detection of bluetongue virus genome segment 6 sequences by RT-PCR. Indian J Exp Biol 36:1034–1037

Barratt-Boyes SM, Maclachlan NJ (1994) Dynamics of viral spread in bluetongue virus infected calves. Vet Microbiol 40:361–371

Barratt-Boyes SM, Rossitto PV, Stott JL, Maclachlan NJ (1992) Flow cytometric analysis of in vitro bluetongue virus infection of bovine blood mononuclear cells. J Gene Virol 73:1953–1960

Barratt-Boyes SM, Rossitto PV, Taylor BC, Ellis JA, Maclachlan NJ (1995) Response of the regional lymph node to bluetongue virus infection in calves. Vet Immunol Immunopathol 45:73–84

Barros SC, Cruz B, Luis TM, Ramos F, Fagulha T, Duarte M et al (2009) A DIVA system based on the detection of antibodies to non-structural protein 3 (NS3) of bluetongue virus. Vet Microbiol 137:252–259

Batten CA, Bachanek-Bankowska K, Bin-Tarif A, Kgosana L, Swain AJ, Corteyn M et al (2008a) Bluetongue virus: European Community inter-laboratory comparison tests to evaluate ELISA and RT-PCR detection methods. Vet Microbiol 129:80–88

Batten CA, Maan S, Shaw AE, Maan NS, Mertens PP (2008b) A European field strain of bluetongue virus derived from two parental vaccine strains by genome segment reassortment. Virus Res 137:56–63

Bauer B, Jandowsky A, Schein E, Mehlitz D, Clausen PH (2009) An appraisal of current and new techniques intended to protect bulls against Culicoides and other haematophagous nematocera: the case of Schmergow, Brandenburg, Germany. Parasitol Res 105:359–365

Boone JD, Balasuriya UB, Karaca K, Audonnet JC, Yao J, He L et al (2007) Recombinant canarypox virus vaccine co-expressing genes encoding the VP2 and VP5 outer capsid proteins of bluetongue virus induces high level protection in sheep. Vaccine 25:672–678

Boyce M, Celma CC, Roy P (2008) Development of reverse genetics systems for bluetongue virus: recovery of infectious virus from synthetic RNA transcripts. J Virol 82:8339–8348

Braverman Y (1989) Control of biting midges *Culicoides* (Diptera:*Ceratopogonidae*), vectors of bluetongue and inducers of sweet itch: a review. Isr J Vet Med 45:124–129

Bréard E, Belbis G, Hamers C, Moulin V, Lilin T, Moreau F et al (2011) Evaluation of humoral response and protective efficacy of two inactivated vaccines against bluetongue virus after vaccination of goats. Vaccine 29:2495–2502

Brewer AW, Maclachlan NJ (1992) Ultrastructural characterization of the interaction of bluetongue virus with bovine erythrocytes in vitro. Vet Pathol 29:356–359

Bruckner L, Fricker R, Hug M, Hotz R, Muntwyler J, Iten C, Griot C (2009) Impfung gegen die Blauzungenkrankheit: Verträglichkeit und Immunantwort in der Praxis. Schweiz Arch Tierheilk 151:101–108

Calvo-Pinilla E, Rodríguez-Calvo T, Sevilla N, Ortego J (2009a) Heterologous prime boost vaccination with DNA and recombinant modified vaccinia virus Ankara protects IFNAR(-/-) mice against lethal bluetongue infection. Vaccine 28:437–445

Calvo-Pinilla E, Rodríguez-Calvo T, Anguita J, Sevilla N, Ortego J (2009b) Establishment of a bluetongue virus infection model in mice that are deficient in the alpha/beta interferon receptor. PLoS One 4:e5171

Caracappa S, Torina A, Guercio A, Vitale F, Calabro A, Purpari G, Ferrantelli V, Vitale M, Mellor PS (2003) Identification of a novel bluetongue virus vector species of Culicoides in Sicily. Vet Rec 153:71–74

Carpenter S, Mellor PS, Torr SJ (2008) Control techniques for *Culicoides* biting midges and their application in the U.K. and northwestern Palaearctic. Med Vet Entomol 22:175–187

Chaignat V, Nitzsche S, Scharrer S, Feyer D, Schwermer H, Thur B (2010) Milk concentration improves Bluetongue antibody detection by use of an indirect ELISA. Vet Microbiol 143:179–183

Clavijo A, Heckert RA, Dulac GC, Afshar A (2000) Isolation and identification of bluetongue virus. J Virol Methods 87:13–23

Conraths FJ, Kramer M, Freuling C, Hoffmann B, Staubach C, Gethmann J, Teifke J, Mettenleiter TC, Beer M (2007) Blauzungenkrankheit in Deutschland: Klinik, Diagnostik und Epidemiologie. Prakt Tierarzt 88:9

Conraths FJ, Gethmann JM, Staubach C, Mettenleiter TC, Beer M, Hoffmann B (2009) Epidemiology of bluetongue virus serotype 8, Germany. Emerg Infect Dis 15:433–435

Darpel KE, Batten CA, Veronesi E, Shaw AE, Anthony S, Bachanek-Bankowska K, Kgosana L, Bin-Tarif A, Carpenter S, Müller-Doblies UU, Takamatsu HH, Mellor PS, Mertens PRC, Oura CAL (2007) Clinical signs and pathology shown by British sheep and cattle infected with bluetongue virus serotype 8 derived from the 2006 outbreak in northern Europe. Vet Rec 161:253–261

Darpel K, Monaghan P, Anthony S, Takamatsu H, Mertens P (2009) Bluetongue virus in the mammalian host and the induced immune response. In: Mellor P, Baylis M, Mertens P (eds) Bluetongue. Elsevier, London

de Koeijer A, Boender G, Nodelijk G, Staubach C, Meroc E, Elbers A (2011) Quantitative analysis of transmission parameters for bluetongue virus serotype 8 in Western Europe in 2006. Vet Res 42:53

DeMaula CD, Jutila MA, Wilson DW, Maclachlan NJ (2001) Infection kinetics, prostacyclin release and cytokine-mediated modulation of the mechanism of cell death during bluetongue virus infection of cultured ovine and bovine pulmonary artery and lung microvascular endothelial cells. J Gen Virol 82:787–794

DeMaula CD, Leutenegger CM, Bonneau KR, Maclachlan NJ (2002a) The role of endothelial cell-derived inflammatory and vasoactive mediators in the pathogenesis of bluetongue. Virology 296:330–337

DeMaula CD, Leutenegger CM, Jutila MA, Maclachlan NJ (2002b) Bluetongue virus-induced activation of primary bovine lung microvascular endothelial cells. Vet Immunol Immunopathol 86:147–157

Drew CP, Gardner IA, Mayo CE, Matsuo E, Roy P, Maclachlan NJ (2010a) Bluetongue virus infection alters the impedance of monolayers of bovine endothelial cells as a result of cell death. Vet Immunol Immunopathol 136:108–115

Drew CP, Heller MC, Mayo C, Watson JL, Maclachlan NJ (2010b) Bluetongue virus infection activates bovine monocyte-derived macrophages and pulmonary artery endothelial cells. Vet Immunol Immunopathol 136:292–296

Ducheyne E, De Deken R, Bécu S, Codina B, Nomikou K, Mangana-Vougiaki O, Georgiev G, Purse BV, Hendickx G (2007) Quantifying the wind dispersal of Culicoides species in Greece and Bulgaria. Geospat Health 1:177–189

Ducheyne E, Lange M, Van der Stede Y, Meroc E, Durand B, Hendrickx G (2011) A stochastic predictive model for the natural spread of bluetongue. Prev Vet Med 99:48–59

EFSA (2007) Scientific Report of the Scientific Panel on Animal Health and Welfare on request from the Commission (EFSA-Q-2006-311) and EFSA Selfmandate (EFSA-Q-2007-063) on bluetongue. EFSA J 479:1–29; 480:1–20

Erasmus BJ (1990) Bluetongue virus. In: Horzinek MC (ed) Virus infections of vertebrates, Dinter Z, Morein B (eds) Virus infections of ruminants, vol 3. Elsevier, Amsterdam, pp 227–237

Eschbaumer M, Hoffmann B, König P, Teifke J, Gethmann J, Conraths FJ, Probst C, Mettenleiter TC, Beer M (2009) Efficacy of three inactivated vaccines against bluetongue virus serotype 8 in sheep. Vaccine 27:4169–4175

Eschbaumer M, Hoffmann B, Moss A, Savini G, Leone A, König P, Zemke J, Conraths F, Beer M (2010a) Emergence of bluetongue virus serotype 6 in Europe – German field data and experimental infection of cattle. Vet Microbiol 43:189–195

Eschbaumer M, Wäckerlin R, Rudolf M, Keller M, König P, Zemke J, Hoffmann B, Beer M (2010b) Infectious blood or culture-grown virus: a comparison of bluetongue virus challenge models. Vet Microbiol 146:150–154

Eschbaumer M, Schulz C, Wäckerlin R, Gauly M, Beer M, Hoffmann B (2011a) Limitations of sandwich ELISAs for bluetongue virus antibody detection. Vet Rec 168:643

Eschbaumer M, Wäckerlin R, Savini G, Zientara S, Sailleau C, Bréard E et al (2011b) Contamination in bluetongue virus challenge experiments. Vaccine 29:4299–4301

Faes C, Aerts M, Litière S, Méroc E, Van der Stede Y, Mintiens K (2011) Estimating herd prevalence on the basis of aggregate testing of animals. J R Stat Soc A 174:155–174

Ferrari G, De Liberato C, Scavia G, Lorenzetti R, Zini M, Farina F et al (2005) Active circulation of bluetongue vaccine virus serotype-2 among unvaccinated cattle in central Italy. Prev Vet Med 68:103–113

Franceschi V, Capocefalo A, Calvo-Pinilla E, Redaelli M, Mucignat-Caretta C, Mertens P et al (2011) Immunization of knock-out alpha/beta interferon receptor mice against lethal bluetongue infection with a BoHV-4-based vector expressing BTV-8 VP2 antigen. Vaccine 29:3074–3082

Gerdes GH (2004) A South African overview of the virus, vectors, surveillance and unique features of bluetongue. Vet Ital 40:39–42

Gethmann J, Hüttner K, Heyne H, Probst C, Ziller M, Beer M, Hoffmann B, Mettenleiter TC, Conraths FJ (2009) Comparative safety study of three inactivated BTV-8 vaccines in sheep and cattle under field conditions. Vaccine 27:4118–4126

Gethmann J, Hoffmann B, Probst C, Beer M, Conraths FJ, Mettenleiter TC (2010) Drei Jahre Blauzungenkrankheit Serotyp 8 in Deutschland – ein Überblick. Tierärztl Umschau 65:4–12

Gloster J, Mellor PS, Manning AJ, Webster HN, Hort MC (2007) Assessing the risk of windborne spread of bluetongue in the 2006 outbreak of disease in northern Europe. Vet Rec 160:54–56

Goldsmit L, Barzilai E (1985) Isolation and propagation of bluetongue virus in embryonating chicken eggs. Prog Clin Biol Res 178:307–318

Gould AR, Hyatt AD, Eaton BT, White JR, Hooper PT, Blacksell SD, LeBlanc-Smith PM (1989) Current techniques in rapid bluetongue virus diagnosis. Aust Vet J 66:450–454

Hamblin C (2004) Bluetongue virus antigen and antibody detection, and the application of laboratory diagnostic techniques. Vet Ital 40:538–545

Hateley G (2009) Bluetongue in northern Europe: the story so far. In Pract 31:202

Hemati B, Contreras V, Urien C, Bonneau M, Takamatsu HH, Mertens PP, Breard E, Sailleau C, Zientara S, Schwartz-Cornil I (2009) Bluetongue virus targets conventional dendritic cells in skin lymph. J Virol 83:8789–8799

Hendrickx G (2008) The spread of bluetongue in Europe. Small Ruminant Res 86:34–39

Higuchi R, Fockler C, Dollinger G, Watson R (1993) Kinetic PCR analysis: real-time monitoring of DNA amplification reactions. Biotechnology 11:1026–1030

Hoffmann A, Cußler K (2009) Impfkampagne zur Bekämpfung der Blauzungenkrankheit. D Tierärzteblatt 2:166–168

Hoffmann B, Sasserath M, Thalheim S, Bunzenthal C, Strebelow G, Beer M (2008) Bluetongue virus serotype 8 reemergence in Germany, 2007 and 2008. Emerg Infect Dis 14:1421–1423

Hoffmann B, Beer M, Reid SM, Mertens P, Oura CA, van Rijn PA, Slomka MJ, Banks J, Brown IH, Alexander DJ, King DP (2009a) A review of RT-PCR technologies used in veterinary virology and disease control: sensitive and specific diagnosis of five livestock diseases notifiable to the World Organisation for Animal Health. Vet Microbiol 139:1–23

Hoffmann B, Eschbaumer M, Beer M (2009b) Real-time quantitative reverse transcription-PCR assays specifically detecting bluetongue virus serotypes 1, 6, and 8. J Clin Microbiol 47:2992–2994

Hoffmann B, Bauer B, Bauer C, Bätza HJ, Beer M, Clausen PH, Geier M, Gethmann JM, Kiel E, Liebisch G, Liebisch A, Mehlhorn H, Schaub GA, Werner D, Conraths FJ (2009c) Monitoring of putative vectors of bluetongue virus serotype 8, Germany. Emerg Infect Dis 15:1481–1484

Hofmann MA, Renzullo S, Mader M, Chaignat V, Worwa G, Thuer B (2008) Genetic characterization of Toggenburg orbivirus, a new bluetongue virus, from goats, Switzerland. Emerg Infect Dis 14:1855–1861

Holbrook FR (1986) Exposure of *Culicoides variipennis* (Diptera: Ceratopogonidae) to hair clippings to evaluate insecticide-impregnated ear tags in cattle. J Econ Entomol 79:1127–1129

Holbrook FR (1994) Survival, fecundity, and egg fertility of Culicoides variipennis (Diptera: Ceratopogonidae) fed on calves inoculated with ivermectin. J Am Mosq Control Assoc 10:7–9

Holbrook FR, Mullens BA (1994) Effects of ivermectin on survival, fecundity, and egg fertility in Culicoides variipennis (Diptera:Ceratopogonidae). J Am Mosq Control Assoc 10:70–73

Hourrigan JL, Klingsporn AL (1975) Classification of ruminants, semen and ova for freedom from bluetongue virus. Aust Vet J 51:211–212

Howell PG (1960) A preliminary antigenic classification of strains of bluetongue virus. Onderstepoort J Vet Res 28:357–363

Hübschle OJB (1980) Bluetongue virus hemagglutination and its inhibition by specific sera. Arch Virol 64:133–140

Jeggo MH, Gumm ID, Taylor WP (1983) Clinical and serological response of sheep to serial challenge with different bluetongue virus types. Res Vet Sci 34:205–211

Jimenez-Clavero MA, Aguero M, San Miguel E, Mayoral T, Lopez MC, Ruano MJ, Romero E, Monaco F, Polci A, Savini G, Gomez-Tejedor C (2006) High throughput detection of bluetongue virus by a new real-time fluorogenic reverse transcription-polymerase chain reaction: application on clinical samples from current Mediterranean outbreaks. J Vet Diagn Invest 18:7–17

Jochim MM, Jones SC (1983) Identification of bluetongue and epizootic hemorrhagic disease viruses by immunofluorescence with monoclonal antibodies. Proc Am Assoc Vet Lab Diagn 26:277–286

Katz J, Alstad D, Gustafson G, Evermann J (1994) Diagnostic analysis of the prolonged bluetongue virus RNA presence found in the blood of naturally infected cattle and experimentally infected sheep. J Vet Diagn Invest 6:139–142

Kirkland PD, Hawkes RA (2004) A comparison of laboratory and 'wild' strains of bluetongue virus – is there a difference and does it matter? Vet Ital 40:448–455

Kramps JA, van Maanen K, Mars MH, Popma JK, van Rijn PA (2008) Validation of a commercial ELISA for the detection of bluetongue virus (BTV)-specific antibodies in individual milk samples of Dutch dairy cows. Vet Microbiol 130:80–87

Laman JD, van den Eertwegh AJ, Deen C, Vermeulen N, Boersma WJ, Claassen E (1991) Synthetic peptide conjugates with horseradish peroxidase and beta-galactosidase for use in epitope-specific immunocytochemistry and ELISA. J Immunol Methods 145:1–10

Liebisch G, Liebisch A (2008) [Efficacy of Flectron-eartags (cypermethrin) for control of midges (Culicoides) as the vectors of bluetongue virus in cattle: field studies and biossays]. Dtsch Tierarztl Wochenschr 115:220–230

Liebisch G, Krieger K, Heine S, Thienel S, Hinrichs P, Liebisch A (2008a) BAYOFLY Pour on R (Cyfluthrin) bei der Abwehr und Bekämpfung von Gnitzen (Ceratopogonidae: Culicoides) den Überträgern des Bluetongue Virus: Felduntersuchungen und Bioassay mit Milchkühen und Jungrindern. Prakt Tierarzt 89:411–426

Liebisch A, Liebisch G, Heine S, Thienel S, Hintichs P (2008b) Wirksamkeit von Auriplak Ohrclips (Permethrin) gegen Gnitzen (Culicoides) als Überträger des Bluetongue Virus bei Rindern. Prakt Tierarzt 89:128–141

Linden A, Grégoire F, Nahayo A, Hanrez D, Mousset B, Massart L, De Leeuw I, Vandemeulebroucke E, Vandenbussche F, De Clercq K (2010) Bluetongue in wild deer, Belgium, 2005–2008. Emerg Infect Dis 16:833–836

Listes E, Monaco F, Labrović A, Paladini C, Leone A, Di Gialleonardo L et al (2009) First evidence of bluetongue virus serotype 16 in Croatia. Vet Microbiol 138:92–97

Lobato ZI, Coupar BE, Gray CP, Lunt R, Andrew ME (1997) Antibody responses and protective immunity to recombinant vaccinia virus-expressed bluetongue virus antigens. Vet Immunol Immunopathol 59:293–309

Maan S, Maan NS, van Rijn PA, van Gennip RG, Sanders A, Wright IM et al (2010) Full genome characterisation of bluetongue virus serotype 6 from the Netherlands 2008 and comparison to other field and vaccine strains. PLoS One 5(4):e10323

Maan S, Maan NS, Nomikou K, Batten C, Antony F, Belaganahalli MN et al (2011a) Novel bluetongue virus serotype from Kuwait. Emerg Inf Dis 17:886–889

Maan S, Maan NS, Nomikou K, Veronesi E, Bachanek-Bankowska K, Belaganahalli MN, Attoui H, Mertens PP (2011b) Complete genome characterisation of a novel 26th bluetongue virus serotype from Kuwait. PLoS One 6:e26147

Maclachlan NJ (2004) Bluetongue: pathogenesis and duration of viraemia. Vet Ital 40:462–467

Maclachlan NJ (2011) Bluetongue: history, global epidemiology, and pathogenesis. Prev Vet Med 102:107–111

Maclachlan NJ, Barratt-Boyes SM, Brewer AW, Stott JL (1992) Bluetongue virus infection of cattle. In: Walton TE, Osburn BI (eds) Bluetongue, African horse sickness, and related orbiviruses. CRC Press, Boca Raton, pp 725–736

Maclachlan NJ, Jagels G, Rossitto PV, Moore PF, Heidner HW (1990) The pathogenesis of experimental bluetongue virus infection of calves. Vet Pathol 27:223–229

Maclachlan NJ, Drew CP, Darpel KE, Worwa G (2009) The pathology and pathogenesis of bluetongue. J Comp Pathol 141:1–16

Mahrt CR, Osburn BI (1986) Experimental bluetongue virus infection of sheep; effect of vaccination: pathologic, immunofluorescent, and ultrastructural studies. Am J Vet Res 47:1198–1203

Mars MH, van Maanen C, Vellema P, Kramps JA, van Rijn PA (2010) Evaluation of an indirect ELISA for detection of antibodies in bulk milk against bluetongue virus infections in the Netherlands. Vet Mcrobiol 46:209–214

Matsuo E, Celma CC, Boyce M, Viarouge C, Sailleau C, Dubois E et al (2011) Generation of replication-defective virus-based vaccines that confer full protection in sheep against virulent bluetongue virus challenge. J Virol 85:10213–10221

McColl KA, Gould AR (1991) Detection and characterization of bluetongue virus using the polymerase chain reaction. Virus Res 21:19–34

Mecham JO (1993) Detection of bluetongue virus from blood of infected sheep by use of an antigen-capture enzyme-linked immunosorbent assay after amplification of the virus in cell culture. Am J Vet Res 54:370–372

Mecham JO, Wilson WC (2004) Antigen capture competitive enzyme-linked immunosorbent assays using baculovirus-expressed antigens for diagnosis of bluetongue virus and epizootic hemorrhagic disease virus. J Clin Microbiol 42:518–523

Mehlhorn H, Walldorf V, Klimpel S, Jahn B, Jaeger F, Eschweiler J, Hoffmann B, Beer M (2007) First occurrence of *Culicoides obsoletus*-transmitted bluetongue virus epidemic in Central Europe. Parasitol Res 101:219–228

Mehlhorn H, Schmahl G, D'Haese J, Schumacher B (2008a) Butox 7.5 pour on: a deltamethrin treatment of sheep and cattle: pilot study of killing effects on *Culicoides* species (Ceratopogonidae). Parasitol Res 102:515–518

Mehlhorn H, Schmahl G, Schumacher B, D'Haese J, Walldorf V, Klimpel S (2008b) Effects of Bayofly on specimens of *Culicoides* species when incubated in hair taken from the feet of previously treated cattle and sheep. Parasitol Res 102:519–522

Mehlhorn H, Walldorf V, Klimpel S, Schmahl G, Al-Quraishy S, Walldorf U, Mehlhorn B, Bätza HJ (2009a) Entomological survey on vectors of transmission parameters for bluetongue virus serotype 8 in Western Europe in 2006. Vet Res 42:53

Mehlhorn H, Walldorf V, Klimpel S, Schaub G, Kiel E, Focke R, Liebisch G, Liebisch A, Werner D, Bauer C, Clausen H, Bauer B, Geier M, Hörbrand T, Bätza HJ, Conraths FJ, Hoffmann B, Beer M (2009b) Bluetongue disease in Germany (2007-2008): monitoring of entomological aspects. Parasitol Res 105:313–319

Meiswinkel R, Baylis M, Labuschagne K (2000) Stabling and the protection of horses from Culicoides bolitinos (Diptera: Ceratopogonidae), a recently identified vector of African horse sickness. Bull Entomol Res 90:509–515

Meiswinkel R, Van Rijn P, Leijs P, Goffredo M (2007) Potential new *Culicoides* vector of bluetongue virus in northern Europe. Vet Rec 161:564

Mellor PS, Wittmann EJ (2002) Bluetongue virus in the Mediterranean Basin 1998–2001. Vet J 164:20–37

Mertens PP, Maan NS, Prasad G, Samuel AR, Shaw AE, Potgieter AC, Anthony SJ, Maan S (2007) Design of primers and use of RT-PCR assays for typing European bluetongue virus isolates: differentiation of field and vaccine strains. J Gen Virol 88:2811–2823

Mertens PPC, Maan S, Batten C, Darpel KE, Shaw A, Maan NS et al (2009) Bluetongue virus diagnosis. In: Mellor P, Baylis M, Mertens P (eds) Bluetongue, Biology of animal infections. Elsevier, London

Mintiens K, Méroc E, Mellor PS, Staubach C, Gerbier G, Elbers AR et al (2008a) Possible routes of introduction of bluetongue virus serotype 8 into the epicentre of the 2006 epidemic in north-western Europe. Prev Vet Med 87:131–144

Mintiens K, Méroc E, Faes C, Abrahantes JC, Hendrickx G, Staubach C, Gerbier G, Elbers ARW, Aerts M, De Clercq K (2008b) Impact of human interventions on the spread of bluetongue virus serotype 8 during the 2006 epidemic in north-western Europe. Prev Vet Med 87:145–161

Monaco F, Cammà C, Serini S, Savini G (2006) Differentiation between field and vaccine strain of bluetongue virus serotype 16. Vet Microbiol 116:45–52

Mullens BA (1992) Integrated management of Culicoides variipennis: a problem of applied ecology. In: Walton TE, Osburn BI (eds) Bluetongue, African horse sickness and related orbiviruses. Proceedings of the second international symposium on bluetongue, Paris, 17–21 June 1991. CRC, Boca Raton, pp 193–196

Mullens BA, Velten RK, Gerry AC, Braverman Y, Endris RG (2000) Feeding and survival of Culicoides sonorensis on cattle treated with permethrin or pirimiphos-methyl. Med Vet Entomol 14:313–320

Mullens BA, Gerry AC, Velten RK (2001) Failure of a permethrin treatment regime to protect cattle against bluetongue virus. J Med Entomol 38:760–762

Mullens BA, Gerry AC, Monteys VS, Pinna M, González A (2010) Field studies on Culicoides (Diptera: Ceratopogonidae) activity and response to deltamethrin applications to sheep in northeastern Spain. J Med Entomol 47:106–110

Müntener CR, Bruckner L, Stürer A, Althaus FR, Caduff-Janosa P (2010) Vigilance der Tierarzneimittel: Gemeldete unerwünschte Wirkungen im Jahr 2009. Schweiz Arch Tierheilk 152:575–583

Napp S, Gubbins S, Calistri P, Allepuz A, Alba A, García-Bocanegra I, Giovannini A, Casal J (2011) Quantitative assessment of the probability of bluetongue virus overwintering by horizontal transmission: application to Germany. Vet Res 42:4

Nunamaker RA, Ellis JA, Wigington JG, Maclachlan NJ (1992) The detection of intracellular bluetongue virus particles within ovine erythrocytes. Comp Biochem Physiol 101A:471–476

Nusinovicia S, Seegersa H, Joly A, Beaudeaua F, Fourichon C (2011) A side effect of decreased fertility associated with vaccination against bluetongue virus serotype 8 in Holstein dairy cows. Prev Vet Med 101:42–50

Orru G, Santis PD, Solinas F, Savini G, Piras V, Caporale V (2004) Differentiation of Italian field and South African vaccine strains of bluetongue virus serotype 2 using real-time PCR. J Virol Methods 122:37–43

Orru G, Ferrando ML, Meloni M, Liciardi M, Savini G, De Santis P (2006) Rapid detection and quantification of bluetongue virus (BTV) using a molecular beacon fluorescent probe assay. J Virol Methods 137:34–42

Oura CA, Wood JL, Sanders AJ, Bin-Tarif A, Henstock M, Edwards L et al (2009) Seroconversion, neutralising antibodies and protection in bluetongue serotype 8 vaccinated sheep. Vaccine 27:7326–7330

Oura CA, Edwards L, Batten CA (2012) Evaluation of the humoral immune response in adult dairy cattle three years after vaccination with a bluetongue serotype 8 inactivated vaccine. Vaccine 30(2):112–115. doi:10.1016/j.vaccine.2011.10.100

Palacios G, Cowled C, Bussetti AV, Savji N, Weir R, Wick I, Travassos da Rosa A, Calisher CH, Tesh RB, Boyle D, Lipkin WI (2011) Rapid molecular strategy for orbivirus detection and characterization. J Clin Microbiol 49:2314–2317

Papadopoulos E, Bartram D, Carpenter S, Mellor P, Wall R (2009) Efficacy of alphacypermethrin applied to cattle and sheep against the biting midge Culicoides nubeculosus. Vet Parasitol 163:110–114

Parsonson IM (1992) Overview of bluetongue virus infection of sheep. In: Walton TE, Osburn BI (eds) Bluetongue, African horse sickness and related orbiviruses. Proceedings of the second international symposium on bluetongue, Paris, 17–21 June 1991. CRC, Boca Raton, pp 713–724

Pierce CM, Balasuriya UB, Maclachlan NJ (1998) Phylogenetic analysis of the S10 gene of field and laboratory strains of bluetongue virus from the United States. Virus Res 55:15–27

Pini A (1976) Study on the pathogenesis of bluetongue: replication of the virus in the organs of infected sheep. Onderstepoort J Vet Res 43:159–164

Probst C, Gethmann JM, Höreth-Böntgen D, Cussler K, Conraths FJ (2011) Lack of evidence for claims of farmers in south-eastern Germany regarding adverse reactions caused by BTV-8 vaccines. Berl Münch Tierärztl Wschr 124:282–287

Reeves WK, Lloyd JE, Stobart R, Stith C, Miller MM, Bennett KE, Johnson G. (2010) Control of *Culicoides sonorensis* (Diptera: Ceratopogonidae) blood feeding on sheep with long-lasting repellent pesticides. J Am Mosq Control Assoc 26:302–305

Ritter D, Roy P (1988) Genetic relationships of bluetongue virus serotypes isolates from different parts of the world. Virus Res 11:33–47

Roy P, Bishop DH, LeBlois H, Erasmus BJ (1994) Long-lasting protection of sheep against bluetongue challenge after vaccination with virus-like particles: evidence for homologous and partial heterologous protection. Vaccine 12:805–811

Ruckerbauer GM, Gray DP, Girard A, Bannister GL, Boulanger P (1967) Studies on bluetongue and detection of the virus in infected materials by immunofluorescence. Can J Comp Med 31:175–180

Saegerman C, Pastoret PP (2009) Bluetongue in northern Europe: appearance of new serotypes within an enzootic ground. Bull Off Int Epiz 1:35–39

Saegerman C, Mellor P, Uyttenhoef A, Hanon J-B, Kirschvink N et al (2010) The most likely time and place of introduction of BTV8 into Belgian ruminants. PLoS One 5:e9405. doi:10.1371/journal.pone.0009405

Savini G, Goffredo M, Monacol F, Di Gennaro A, de Santis P, Meiswinkel R, Caporale V (2004) The isolation of bluetongue virus from field populations of the Obsoletus Complex in central Italy. Vet Ital 40:286–291

Schmahl G, Walldorf V, Klimpel S, Al-Quraishy S, Mehlhorn H (2008) Efficacy of Oxyfly on Culicoides species – the vectors of bluetongue virus – and other insects. Parasitol Res 103:1101–1103

Schmahl G, Klimpel S, Walldorf V, Al-Quraishy S, Schumacher B, Jatzlau A, Mehlhorn H (2009a) Pilot study on deltamethrin treatment (Butox 7.5, Versatrine) of cattle and sheep against midges (*Culicoides* species, Ceratopogonidae). Parasitol Res 104:809–813

Schmahl G, Klimpel S, Walldorf V, Schumacher B, Jatzlau A, Al-Quraishy S, Mehlhorn H (2009b) Effects of permethrin (Flypor) and fenvalerate (Acadrex60, Arkofly) on *Culicoides* species-the vector of bluetongue virus. Parasitol Res 104:815–820

Schmahl G, Mehlhorn H, Abdel-Ghaffar F, Al-Rasheid K, Schumacher B, Jatzlau A, Pohle H (2009c) Does rain reduce the efficacy of Butox 7.5 pour on (deltamethrin) against biting midges (*Culicoides* specimens)? Parasitol Res 105:1763–1765

Shaw A, Monaghan P, Alpar HO, Anthony S, Darpel KE, Batten CA, Carpenter S, Jones H, Oura CAL, King DP, Elliott H, Mellor PS, Mertens PPC (2007) Development and initial evaluation

of a realtime RT-PCR assay to detect bluetongue virus genome segment 1. J Virol Methods 145:115–126

Shone DK, Haig DA, McKercher DG (1956) The use of tissue culture propagated bluetongue virus for complement fixation studies on sheep sera. Onderstepoort J Vet Res 27:179–182

Skrock O (2011) Zur Wirksamkeit unterschiedlicher Netzprototypen zum Schutz von Milchvieh und Kälbern vor Überträgern (Gnitzen, Culicoides spp.) der Blauzungenkrankheit und anderen Lästlingsinsekten. Dissertation, Freie Universität Berlin

Skrock O, Manti B, Bauer B, Rohrmann KMA, Geericke N, Mehlitz D, Peters K-J, Frenzel K, Westerkamp A, Clausen P-H (2010) The effectiveness of insecticide-treated nets for the protection of cattle from biting midges and insect pests on dairy cattle. In: Joint meeting of the German Societies for Parasitology and Protozoology at Düsseldorf University, 17–20 Mar 2010. Düsseldorf University Press, Düsseldorf, p 179, ISBN:978-3-940671-37-0

Standfast HA, Muller MJ, Wilson DD (1985) Mortality of *Culicoides brevitarsis* fed on cattle treated with ivermectin. Prog Clin Biol Res 178:611–616

Stewart M, Bhatia Y, Athmaran TN, Noad R, Gastaldi C, Dubois E et al (2010) Validation of a novel approach for the rapid production of immunogenic virus-like particles for bluetongue virus. Vaccine 28:3047–3054

Szmaragd C, Wilson AJ, Carpenter S, Wood JLN, Mellor PS, Gubbins S (2010) The spread of bluetongue virus serotype 8 in Great Britain and its control by vaccination. PLoS One 5:e9353. doi:10.1371/journal.pone.0009353

Tabachnick WJ (1996) *Culicoides variipennis* and bluetongue-virus epidemiology in the United States. Annu Rev Entomol 41:23–43

Toussaint JF, Sailleau C, Mast J, Houdart P, Czaplicki G, Demeestere L, VandenBussche F, van Dessel W, Goris N, Bréard E, Bounaadja L, Thiry E, Zientara S, de Clercq K (2007) Bluetongue in Belgium, 2006. Emerg Infect Dis 13:614–616

Tschuor AC, Kaufmann T, Strabel D, Hässig M (2010) Abklärung von Aborten und anderen tiergesundheitlichen Problemen beim Rind im Zusammenhang mit der Blauzungenvirus-Impfung 2009. Schweiz Arch Tierheilk 152:501–506

Umeshappa CS, Singh KP, Nanjundappa RH, Pandey AB (2010) Apoptosis and immunosuppression in sheep infected with bluetongue virus serotype-23. Vet Microbiol 144:310–318

Unger RE, Chuang RY, Chuang LF, Doi RH, Osburn BI (1988) Comparison of dot-blot and Northern blot hybridization in the determination of genetic relatedness of United States bluetongue virus serotypes. J Virol Methods 22:273–282

van der Walt NT (1980) A hemagglutination and hemagglutination inhibition test for bluetongue virus. Onderstepoort J Vet Res 47:113–117

van Oirschot JT (1999) Diva vaccines that reduce virus transmission. J Biotechnol 73:195–205

Vanbinst T, Vandenbussche F, Vandemeulebroucke E, De Leeuw I, Deblauwe I, De Deken G, Madder M, Haubruge E, Losson B, De Clercq K (2009) Bluetongue virus detection by real-time RT-PCR in Culicoides captured during the 2006 epizootic in Belgium and development of an internal control. Transbound Emerg Dis 56:170–177

Vandemeulebroucke E, De Clercq K, Van der Stede Y, Vandenbussche F (2010) A proposed validation method for automated nucleic acid extraction and RT-qPCR analysis: an example using bluetongue virus. J Virol Methods 165:76–82

Vandenbussche F, Vandemeulebroucke E, De Clercq K (2010) Simultaneous detection of bluetongue virus RNA, internal control GAPDH mRNA, and external control synthetic RNA by multiplex real-time PCR. Methods Mol Biol 630:97–108

Velthuis AG, Saatkamp HW, Mourits MC, de Koeijer AA, Elbers AR (2010) Financial consequences of the Dutch bluetongue serotype 8 epidemics of 2006 and 2007. Prev Vet Med 93:294–304

Velthuis AG, Mourits MC, Saatkamp HW, de Koeijer AA, Elbers AR (2011) Financial evaluation of different vaccination strategies for controlling the bluetongue virus serotype 8 epidemic in The Netherlands in 2008. PLoS One 6:e19612

Veronesi E, Hamblin C, Mellor PS (2005) Live attenuated bluetongue vaccine viruses in Dorset Poll sheep, before and after passage in vector midges (Diptera: *Ceratopogonidae*). Vaccine 23:5509–5516

Veronesi E, Darpel KE, Hamblin C, Carpenter S, Takamatsu HH, Anthony SJ et al (2010) Viraemia and clinical disease in Dorset Poll sheep following vaccination with live attenuated bluetongue virus vaccines serotypes 16 and 4. Vaccine 28:1397–1403

Wäckerlin R, Eschbaumer M, König P, Hoffmann B, Beer M (2010) Evaluation of humoral response and protective efficacy of three inactivated vaccines against bluetongue virus serotype 8 one year after vaccination of sheep and cattle. Vaccine 28:4348–4355

Wade-Evans AM, Mertens PPC, Bostock CJ (1990) Development of the polymerase chain reaction for the detection of bluetongue virus in tissue samples. J Virol Methods 30:15–24

Wade-Evans AM, Romero CH, Mellor P, Takamatsu H, Anderson J, Thevasagayam J et al (1996) Expression of the major core structural protein (VP7) of bluetongue virus, by a recombinant capripox virus, provides partial protection of sheep against a virulent heterotypic bluetongue virus challenge. Virology 220:227–231

Willgert K, Schroedle B, Schwermer H (2011) Spatial analysis of bluetongue cases and vaccination of Swiss cattle in 2008 and 2009. Geospat Health 5:227–237

Wilson WC, Chase CCL (1993) Nested and multiplex polymerase chain reactions for the identification of bluetongue virus infection in the biting midge, *Culicoides variipennis*. J Virol Methods 45:39–47

Wilson AJ, Mellor PS (2009) Bluetongue in Europe: past, present and future. Phil Trans R Soc B 364:2669–2681

Wilson WC, Stallknecht DE, Mecham JO (2004) Field–deployable realtime polymerase chain reaction detection of bluetongue and epizootic haemorrhagic disease viral ribonucleic acid. Vet Ital 40:587–593

Wilson A, Darpel K, Mellor PS (2008) Where does bluetongue virus sleep in the winter? PLoS Biol 6:e210

Wittwer CT, Herrmann MG, Moss AA, Rasmussen RP (1997) Continuous fluorescence monitoring of rapid cycle DNA amplification. Biotechniques 22(1):130–131

Yang J, Hua Q, Chen H, Lv J, Chen B, Ruan Z (2010a) A rapid assay for detecting antibody against bluetongue virus with a latex agglutination test using recombinant VP7 antigen. J Vet Diagn Invest 22:242–244

Yang J, Hua Q, Chen H, Lv J, Qin Z, Jin M et al (2010b) Development and evaluation of an immunochromatographic strip for the detection of serum antibodies against bluetongue virus. J Virol Methods 163:68–73

Zientara S, Bréard E, Sailleau C (2004) Bluetongue diagnosis by reverse transcriptase-polymerase chain reaction. Vet Ital 40:531–537

Zientara S, Maclachlan NJ, Calistri P, Sanchez-Vizcaino JM, Savini G (2010) Bluetongue vaccination in Europe. Expert Rev Vaccines 9:989–991