Bluetongue
in northern Europe

Edited by
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Preface

Bluetongue (BT), which is a notifiable disease listed by the World Organisation for Animal Health (OIE), is an infectious but non-contagious viral disease of a broad spectrum of domestic and wild ruminants. It is transmitted by midges, small insects, of the genus *Culicoides*. During its recent and unexpected emergence in northern Europe the causal pathogen, BT virus serotype 8 (BTV-8), has mainly infected sheep and cattle. For the first time, BTV-8 showed greater virulence in cattle, causing a severe clinical disease in this species. What is also striking about this occurence of BT is the fact that it was able to spread all over northern Europe, including the United Kingdom, in less than two years.

Preventing the spread of transboundary diseases is one of the OIE's main missions. This scientific monograph describing BT outbreaks in northern Europe, and in particular, the clinical picture observed in cattle and sheep, will be of great value to all those involved in animal health surveillance and control. It will be particularly useful in facilitating early detection of BT and differentiating this disease from other emerging diseases.

Epidemiological information on BT is available in the World Animal Health Information System (WAHIS) and the World Animal Health Database (WAHID) (accessible at www.oie.int/wahid). The OIE Reference Laboratory for BT in Teramo, Italy, has provided welcome support for the continuous updating of the database. At the European Union level, epidemiological information about BT surveillance is also available via the new EU-BTNET System (accessible at www.eubtnet.izs.it/btnet/).

My heartfelt thanks go Prof. Claude Saegerman, Dr Francisco Reviriego-Gordejo and Prof. Paul-Pierre Pastoret for coordinating and editing this monograph, which will undoubtedly be an important tool for veterinarians and veterinary public health authorities. I would also like to thank all the authors who contributed to this monograph, which is on a subject of great importance for the OIE and its Members. I also wish to express my gratitude to the staff of the OIE Publications and Administration Departments for their dedication. Finally, this publication would never have been possible without the strong support of many different organisations, including the Belgian Federal Agency for the Safety of the Food Chain (FASFC), and I extend my warmest thanks to each of them.

Bernard Vallat
Director General
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Many colleagues kindly placed additional photographic material at our disposal; we especially thank Jan Mast (Veterinary and Agrochemical Research Centre [VAR], Brussels, Belgium), and Philip Mellor (Institute for Animal Health [IAH] – Pirbright Laboratory, United Kingdom), Reginald De Deken and Maxime Madder (Institute of Tropical Medicine [ITM], Antwerp, Belgium), Sam Mansley (IAH, United Kingdom) and Richard Irvine (University of Glasgow, United Kingdom).

The European Commission Health and Consumer Protection Directorate-General (Brussels, Belgium), the University of Liege (Liege, Belgium), the Notre-Dame de la Paix University Faculties of Namur (Namur, Belgium), VAR, ITM FASFC, and the Belgian Federal Public Service of Public Health, Food Chain Security and Environment (Brussels, Belgium) have financed both the editing and printing of this monograph.

Maintaining the wealth of information on animal health is becoming increasingly dependent on the generous participation of a large number of national and international sponsors. We are very grateful to these sponsors for their concern for the promotion of animal health throughout the world and for their contribution to sustainable livestock development and, more generally, to the good of humanity.

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Bluetongue: General Introduction 1

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Until 2006, the geographical distribution of bluetongue virus extended between 50° latitude North and 35° latitude South (1).

Bluetongue (BT) was discovered in northern Europe* for the first time on the 14 August 2006. Germany, Belgium, the Netherlands and to a lesser extent Luxembourg and France were affected. Serotype 8 of the BTV, as well as the two indigenous vectors, *Culicoides dewulfi* and *C. obsoletus* complex, were identified. The disease spread rapidly and by 1 February 2007, 2122 clinical outbreaks of bluetongue had been reported. Uncommonly, the disease affected both cattle and sheep. After a period of remission, BT (due to the same serotype), reappeared in summer 2007 (2), which underlines the fact that BT could become enzootic in these regions.

The modified host range and the clinical picture of BT in Northern Europe, raise questions about the pathogenicity, the infection

* In this monograph, Northern Europe refers to areas bordering or in close proximity to the Baltic Sea and North Sea.

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dynamics within affected herds (outbreak, resurgence, and diffusion), and the development of an efficient system of early detection of emerging vector-borne diseases.

Due to the proximity of the University of Liege in Belgium to the epicentre of the BT epizootic in Northern Europe, a multidisciplinary team from the University's Faculty of Veterinary Medicine conducted a transversal and longitudinal clinical follow-up of a number of affected domestic ruminant herds. This follow-up was based on a standardised clinical report form (with photographs).

Clinical observations in cattle had been infrequent up to that point. It was therefore considered that a scientific monograph describing the episode of BT would be of great importance for veterinarians and health professionals, within the framework of early detection of BT and, indeed, of emerging diseases in general. If an opportunity for early detection is missed, a disease outbreak may go undetected until multiplication and transmission of the pathogen are so advanced that it becomes very difficult to control. Moreover, globalisation and climat change are two additional conditions for emergence. Sharing the experiences among the Member Countries and Territories of the World Organisation for Animal Health (OIE) helps to increase the awareness of veterinarians and health professionals, in order to improve the early detection of emerging diseases.

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Bluetongue: Virology, pathogenesis, and biology of the culicoides vector

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INTRODUCTION

Bluetongue (BT) is a non-contagious vector-borne disease. The causal virus (BTV) belongs to the *Orbivirus* genus of the family *Reoviridae*. The infection is usually inapparent in cattle, which can then act as a reservoir for the virus. However, some serotypes, such as serotype 8, which recently caused infection in northern Europe, exhibit greater virulence in cattle than in the past (20). Sheep are still the main host of the virus but the infection also occurs, though usually subclinically, in free-living ruminants, cattle and goats. Local breeds of sheep are usually more resistant than others to the viral infection. Cervidae can also be infected by a closely related orbivirus responsible for epizootic haemorrhagic disease. The insect vectors of BT are *Culicoides* midges (14, 15).

The BTV contains 2 capsids enclosing a core consisting of 10 segments of double-stranded RNA that encode 7 structural proteins (VP1 to VP7) and 4 non-structural proteins (NS1 to NS3, NS3A). The external capsid contains the VP5 and VP2 proteins, involved in virus neutralisation and

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responsible for serotype specificity. The internal capsid consists of the VP7 protein, which is the specific group antigen. The VP1 protein is present in the core of the virus and is the viral RNA polymerase. The genome is segmented and reassortment of segments can occur during co-infections. In addition, the viral genome has a high rate of mutations contributing to an antigenic drift. The genetic variability of BTV, such that 24 serotypes exist, is due to these characteristics. Among these 24 serotypes the serotypes 1, 2, 4, 9 and 16 were reported in Mediterranean Europe between 1998 and 2006 (15). Serotype 8 was responsible for the epizootic in northern Europe in 2006 and 2007 (21).

PATHOGENESIS

The virus persists in *Culicoides* during their lifespan. After a blood meal, the virus passes through the insect's intestinal wall and is distributed via the hemocoel to various tissues and then to the salivary glands, where it continues to replicate. It is subsequently excreted in the insect's saliva. Viral transmission is solely by insect bite. The vector reaches its maximum infective capacity 10 days after having absorbed blood from a viraemic animal.

After an animal has been infected by an insect bite, the BTV replicates in the regional lymph nodes. It disseminates to infect the vascular endothelium and macrophages as well as dendritic cells in various organs. In the blood, the virus is adsorbed at the surface of erythrocytes and platelets, whereas it replicates in monocytes and lymphoblasts. The infectious virus is enclosed in erythrocyte and lymphocyte plasmic membrane invaginations and therefore viraemia is persistent in the presence of neutralising antibodies.

In sheep, the average incubation period is 6 to 8 days (range: 2 to 18 days). The incubation period is suspected to be the same in cattle as in sheep.

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The pathogenesis can vary according to both the virus serotype and the ruminant species. There is a great difference in expression of the disease between cattle and sheep, which may be due to a different response of the endothelial cells following infection: in contrast to sheep, cattle usually develop only a subclinical infection with the exception of infection with serotype 8 as has occurred in northern Europe. In sheep, lesions in the endothelial cells of small blood vessels provoke vascular thrombosis and ischaemic necrosis of the affected tissue. These lesions result in buccal ulcers, inflammation of the coronary band, muscular necrosis, and extravasation leading to facial and pulmonary oedema and pleural and pericardial effusions (8, 9).

A cell-associated viraemia of long duration is characteristic of BT. The cell-free viraemia is transient. The high level of viral infection and long-lasting viraemia increase the risk of infection of culicoid vectors. Neutralising antibodies appear after 14 days but do not eliminate the virus, which is protected by its association with blood cells. At the beginning of the viraemia, the virus is associated with various blood cells. Subsequently, viraemia is nearly exclusively associated with blood erythrocytes. These cells do not however contain the necessary machinery for viral replication (8).

Bluetongue virus infection is not persistent. The duration of viraemia is in part associated with the lifespan of erythrocytes and therefore the viraemia is longer in cattle than in sheep. In experimental conditions, viraemia lasts for 14 to 45 days in sheep and up to 31 days in goats. Reverse transcription-polymerase chain reaction (RT-PCR) is used to detect the viral genome. With this technique the detection of viraemia takes much longer than the true viraemia. The duration of viraemia that is capable of infecting haematophagous vectors is about 60 days. It is likely much shorter in field conditions. In the majority of cases viraemia is less than 60 days in cattle and is therefore longer than in sheep.

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The infected bulls may excrete the virus in sperm and become carriers for a long period (9).

In addition to transmission by insect vectors, the BTV can be transmitted vertically *in utero*. Cases of abortion and foetal malformations due to BT occur sporadically in ruminants. Transplacental passage of virus produces variable clinical signs depending on the period of gestation when infection occurs. During the first third of the gestation period, embryonic and foetal deaths occur. Infection during the second third of pregnancy can provoke congenital abnormalities such as hydranencephaly and retinal dysplasia, which are due to destruction of neuron and glial cell precursors by the virus, before these cells migrate to different areas of the brain. During the last third of gestation, the foetus develops an immune response and eliminates the infection. Abortion is rare compared to congenital abnormalities. Some abortions are non-specific and are a direct, stress-related consequence of infection in the ewe (10).

Competent vectors include *Culicoides imicola* in Africa and Mediterranean Europe, *C. sonorensis* in North America, *C. insignis* and *pusillus* in South America, and *C. brevitarsis* in Australia (14). In Europe, *C. obsoletus* and *scoticus* have been identified in central Italy and *C. pulicaris* in Sicily. *C. dewulfi* is recognised as a vector in northern Europe (11).

Bluetongue occurs after introduction of infected sheep or vectors to a virus-free area where the vector is indigenous. Subclinical infection commonly occurs in cattle and goats and these species could serve as reservoirs for the infection. When the disease is enzootic, clinical signs are seen mainly in susceptible imported sheep. The geographical distribution of the virus depends on the presence of culicoid vectors and the disease is therefore seasonal and seen mainly in hot, humid areas, near stagnant pools of water. The discovery of other culicoid vectors is possible. In temperate regions, the disease occurs mainly at the end of

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summer or beginning of winter, whereas in subtropical areas it mainly occurs in spring or early summer but may also occur throughout the year (14).

In the absence of transovarial transmission of the virus in insects, other mechanisms have been suggested to explain the phenomenon of overwintering, i.e. virus survival over the winter period and during 9 to 12 months in the absence of adult vectors. Such a mechanism would be dependent on the establishment of chronic infections in sheep and cattle. In this context, γδ T lymphocytes are known to be associated with a persistent infection in sheep (19).

BLUETONGUE VECTORS: THE BITING MIDGES CULICOIDES

Midges of the *Culicoides* species are small (1-4 mm in length) biting dipterous insects belonging to the family Ceratopogonidae (Figure 1). They are found from the tropics to the tundra and from the sea level up to an altitude of 4,000 m. Their role as a vector of parasitic and viral diseases of humans and especially animals has long been recognised (Table I). In addition, *Culicoides* can, by their very abundance, have a very detrimental effect, due to the nuisance caused by bites from female midges. Their presence can thus hinder the economic growth of certain areas by blocking the agricultural activities and the development of tourism. Moreover, they have been implicated in the occurence of several viral epizootics, including two major viral animal diseases, African horse sickness and BT (5).

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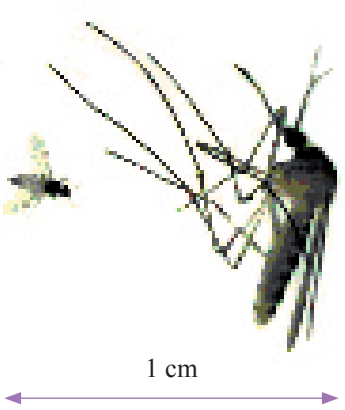

*Figure 1. Size comparison between a biting midge (*Culicoides scoticus*) (on the left) and a mosquito (*Culex sp.*) (on the right), both females*

In most *Culicoides* species, adult females are haematophagous; they take a blood meal approximately every 3 or 4 days (1) and they are mainly found near ground level, close to animals (17). Some species are anthropophilic (*C. obsoletus* and *C. impunctatus,* for example) while others prefer to feed on livestock (sheep, goats, cattle) or birds. The majority of biting midge species are active – and thus bite – in the twilight and at night; however, some midges bite preferentially in full daylight (*C. nubeculosus,* for example).

Male midges are generally flower-dwelling (6): they feed on nectar, sugar and pollen as well as on liquids from decomposing organic matter (3). The males are therefore more likely to be found at the tops of trees (4, 17). Midge larvae feed on a variety of organic matter or are predatory on nematodes, bacteria, protozoa and even on conspecific individuals (4).

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Table I. Human and animal species affected by certain species of biting midges (23)

CULICOIDES	Affected species						
	Humans	**Cattle**	**Sheep**	**Goats**	**Equids**	**Wildlife species**	**Birds**
C. imicola							
C. milnei							
C. nubeculosus							
C. obsoletus							
C. brevitarsis							
C. insignis							
C. fulvus							
C. actoni							
C. variipennis							
C. riethi							
C. impunctatus							
C. circumscriptus							
C. festivipennis							

Most of *Culicoides* species require a wet medium to reproduce and lay their eggs. Indeed, larval development is optimal in the semi-aquatic microhabitats, consisting mainly of warm, moist or wet substrates, rich in organic matter (edges of manure-polluted water sources, mud, water meadows, etc.) (6, 23). In general, the larvae are mainly found in the first 5 to 6 cm of the upper layer of the medium (22). The pupae are also found on the surface of the medium (mud or water) where the larval development takes place (23).

Adults usually mate in the immediate vicinity of cattle sheds, primarily near wet media or stagnant water. Indeed, they rarely actively move away from the site where they hatched (13). *Culicoides* spp. have a large nocturnal component in their activity. During the daytime, they usually rest in the shade on the underside of leaves or grasses (23).

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The survival, activity and dispersal of biting midges are strongly influenced by meteorological factors such as temperature, moisture and wind. Temperature is undoubtedly the main environmental factor influencing the behaviour and survival of these midges. Indeed, their activity is highest between 13°C and 35°C (2), even if these limits vary according to the species. For example, Losson *et al.* (7) observed *C. obsoletus* flights at minimum temperatures ranging between 6°C and 12°C in cattle sheds during the winter 2006-2007.

A high moisture level is also an important factor for the development and survival of the *Culicoides* (16). Indeed, the larvae are particularly sensitive to desiccation, which kills them quickly. Dryness is also unfavourable to aduls and they take refuge in the vegetation until a change in the weather enables them to resume their activity. They are also averse to rain, since it prevents their flights. These behaviours account for the fact that in temperate areas these vectors are particularly abundant towards the end of summer and the beginning of autumn.

Throughout the flight period, adult *Culicoides* move no more than a few hundred meters away from the site where the imagos emerged. Their active dispersal is thus very limited (13). Their passive dispersal by warm, humid winds blowing at low altitude (<2,000 m) at a mean speed of 10 to 40 km/h is a far more important factor and they can be carried over a distance of several hundred kilometres (2). This dispersal of insects to new areas could account for some of the BT epizootics of recent years, such as the one in Spain (12).

The density of adult *Culicoides* populations varies according to the season. Some species have a broader distribution during the year while others are encountered only during short periods. For example, the species *C. impunctatus* is observed from approximately late May to late September (18), while *C. obsoletus* and *C. scoticus* are earlier species with a longer flight period; they appear in mid-April and disappear at the beginning of November (17). In general, two generations of biting

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midges are prodeced each year, a large one in spring and a smaller one in summer (17).

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Bluetongue: Epidemiology in the European Union

3

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Bluetongue (BT) is a World Organisation for Animal Health (OIE) notifiable disease of considerable socio-economic concern and of major importance in the international trade of animals and animal products (6). Before 1998, BT was considered an exotic disease in Europe with just a few sporadic incursions (e.g. in Spain and Portugal from 1956 up to and including 1960) (16).

The aim of this chapter is to provide a synthesis of the epidemiology of BT in the European Union since its introduction in 1998. To this effect, a short overview is given of the epidemiological situation in Europe, followed by a brief description of the susceptible species, a discussion of the vectorial capacity and competence, and an outline of the modes of introduction and mechanisms of amplification.

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EPIDEMIOLOGICAL SITUATION IN EUROPE

BLUETONGUE VIRUS IN THE EUROPEAN UNION DURING THE PERIOD 1998-2005

Between 1998 and 2005 at least six BT virus (BTV) strains belonging to five serotypes (BTV-1, BTV-2, BTV-4, BTV-9 and BTV-16) were continuously present in parts of the Mediterranean Basin, including several Member States of the European Union (Table II and Fig. 1). This emergence of BT in parts of Europe never before affected has been attributed essentially to climate change and is linked both to the northern expansion of the major Old World vector *Culicoides imicola* (Kieffer), which is an Afro-Asiatic species, and to the involvement, for the first time, of novel indigenous European vector species of *Culicoides* within the *C. Obsoletus* and *C. Pulicaris* complexes (20). In the Mediterranean Basin two epidemiological systems seem to predominate. The first one is located in the eastern part of the Basin, where serotypes 1, 4, 9 and 16 have been identified. The BTV strains in this system originated in the Near, Middle or Far East. The vectors also appeared to include other *Culicoides* species in addition to *C. imicola*. This was deduced from the fact that the disease had penetrated into areas where *C. imicola* did not occur (the Balkans, for example) (19). The involvement of novel vectors was subsequently confirmed when the causative virus was isolated from mixed pools of two species, *C. obsoletus* (Meigen) and *C. scoticus* (Downes and Kettle), collected in central Italy (23) and from *C. pulicaris* (Linnaeus) in Sicily (5). The second epidemiological system concerns the western part of the Mediterranean Basin, where serotypes BTV-1, BTV-2, BTV-4 and BTV-16 have been identified and the main vector is *C. imicola*.

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Table II. Outbreaks of bluetongue in Europe in the period 1998-2005 (3, 4, 9, 19, 21)

Country or territory	Year of first outbreak	Serotype(s) of BTV	Main suspected or identified vector(s)
Albania	2002	9	*C. obsoletus, C. pulicaris*
Bosnia and Herzegovina	2002	9	N.D.
Bulgaria	1999	9	*C. obsoletus, C. pulicaris*
Croatia	2001	9, 16	*C. obsoletus, C. scoticus*
Cyprus	2003	16	*C. imicola, C. obsoletus*
Former Yugoslav Republic of Macedonia	2001	9	N.D.
France (Corsica)	2000	2, 4, 16 *	*C. imicola, C. pulicaris,C. obsoletus*
Greece	1998	1, 4, 9, 16	*C. imicola, C. obsoletus*
Italy	2000	1, 2, 4, 9, 16	*C. imicola, C. obsoletus, C. pulicaris*
Kosovo	2001	9	N.D.
Montenegro	2001	9	N.D.
Portugal	2004	2 #, 4	*C. imicola, C. obsoletus, C. pulicaris*
Serbia	2001	9	N.D.
Spain	2000	2	*C. imicola,C. obsoletus, C. pulicaris*
Turkey	1998	4, 9, 16	*C. imicola,C. obsoletus, C. pulicaris*

N.D.: no data recorded; * This was an insufficiently attenuated vaccine strain (22); # This strain was indistinguishable from Onderstepoort BTV-2 live attenuated vaccine strain (3)

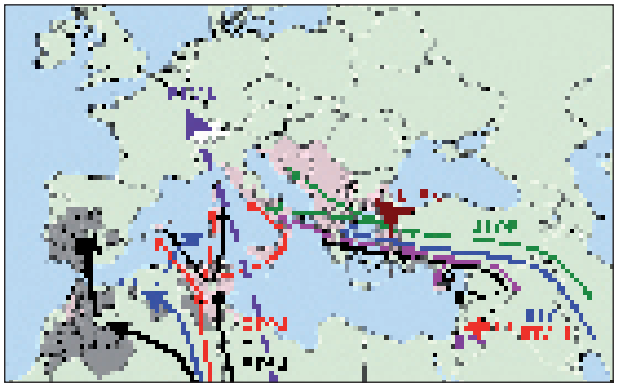

Figure 2. The molecular epidemiology of bluetongue since 1998: routes of introduction of different serotypes and individual virus strains (Mertens & Mellor, IAH-Pirbright)

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BLUETONGUE IN NORTHERN EUROPE FROM MID AUGUST 2006 TO LATE JULY 2007

Bluetongue was first identified in northern Europe in August 2006 following a heatwave and heavy rainfall. It can be defined as an emerging disease in this zone (27). Between the first notification (17 August 2006) and 1 February 2007 (7), 2122 BT cases were entered into the European Commission's Animal Disease Notification System (ADNS) (http://ec.europa.eu/food/animal/diseases/adns/index_en.htm) (Fig. 3). In this area, in 2006, a pool of 50 non-engorged, parous *C. dewulfi* (Goetghebuer) was found to be BTV PCR-positive in the Netherlands (14) and several pools of *C. obsoletus* complex (i.e. not identified down to species level) were also found to be BTV PCR-positive in Germany (13), (Fig. 4). Although isolation of live BTV was not attempted in either instance, this work, carried out in an area where *C. imicola* does not occur, confirms the earlier findings of Mellor and Pitzolis (1979), who isolated infectious BTV from non-engorged parous *C. obsoletus* in Cyprus and, shows that indigenous European *Culicoides* species can support a BT epizootic (18). As *C. obsoletus* complex midges and *C. dewulfi* occur widely across central and northern Europe this entire geographical area must now be considered at risk from BTV (11, 25). The focus of interest must now be to see if BTV is able to survive regularly between vector seasons in northern Europe and become endemic. The recrudescence of BTV-8 in northern France, the Netherlands, Belgium and Germany in 2007 suggests that this may well be the case (28). Unlike further south, where populations of the traditional vector *C. imicola* peak in the late summer and autumn which is when most BT cases occur, populations of the indigenous vectors peak earlier in the year. Whether this will be reflected in a change in the temporal occurrence of BT cases remains to be seen.

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During the period 2006 up to the 10 August 2007, nine EU Member States reported BT outbreaks comprising all of the serotypes reported in Europe since 1998 (Fig. 5).

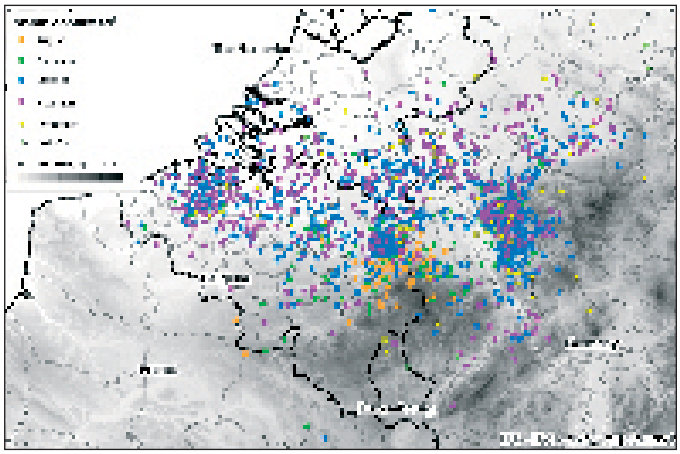

Figure 3. Monthly distribution of confirmed bluetongue outbreaks (serotype 8) in northern Europe between 17 August 2006 and 1 February 2007 (8)

Figure 4. A gravid female Culicoides dewulfi *collected from a location near bluetongue outbreaks in Belgium in 2006 (Photograph: Reginald De Deken & Maxime Madder, Institute of Tropical Medicine, Antwerp, Belgium)*

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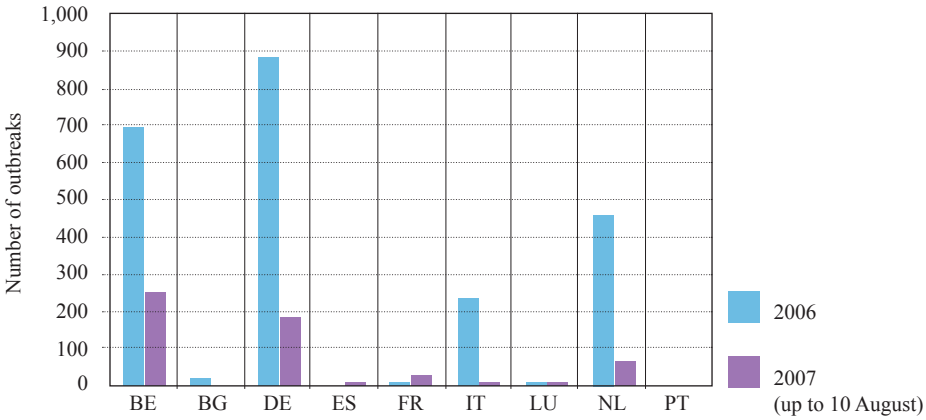

Figure 5. Bluetongue outbreaks in the European Union in 2006 and 2007 (1, 2)

BE, Belgium; BG, Bulgaria; DE, Germany; ES, Spain; FR, France; IT, Italy; LU, Luxembourg; NL, Netherlands; PT, Portugal.

SUSCEPTIBLE SPECIES

Bluetongue virus is transmitted between its ruminant hosts almost exclusively through the bites of the females of vector species of *Culicoides* biting midges (17). The global distribution of BTV is, therefore, restricted to those regions where these species of *Culicoides* occur, and its transmission period is limited to the times when adult vectors are active. Depending on the species, adult vector activity generally starts some time in spring. Activity is positively correlated with temperature, reaching a maximum when the temperature is between 28°C and 30°C, decreasing when the temperature drops and, in the case of the traditional Afro-Asiatic vector *C. imicola*, is probably non-existent at temperatures below 10°C (17, 20). BTV can infect a broad

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spectrum of domestic and wild ruminants. However, serious clinical signs have only been observed in certain breeds of sheep (improved breeds) and a few deer species (12, 24). Cattle and goats usually exhibit subclinical infections and therefore may serve as important and covert viral reservoirs for sheep (12).

VECTORIAL CAPACITY AND VECTOR COMPETENCE

The risk of BTV infection is closely linked to the presence of adult vector *Culicoides* (17). Until recently, *C. imicola* was believed to be the only important vector of BTV in southern Europe, but it is now known that several newly recognised vector species are also involved, and others may be identified in the future.

The vector competence of an insect species and the vectorial capacity of an insect population are important parameters in this respect (10). Vector competence is the (innate) ability of a vector to acquire a pathogen, maintain it and successfully transmit it to a susceptible host (20). Vector competence may be determined in the laboratory by providing groups of insects of a particular species with blood meals containing appropriate concentrations of virus and assessing infection and transmission rates. Vector competence is expressed in terms of the proportion of feeding insects that support virus replication and can transmit the virus after a suitable incubation period. In situations where transmission is difficult to demonstrate because of the technical problems in re-feeding 'difficult' insects such as *Culicoides* it has become established practice to assume virus transmission capability, if the virus can be recovered from the insect's salivary glands. Vectorial capacity refers to the potential for virus transmission of an insect population and takes into account a range of insect, host and environmental variables including vector abundance,

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vector survival, biting and transmission rates, host preferences and host abundance under a range of external (e.g. bioclimatic) conditions. Vectorial capacity can be defined as the number of infective bites that an infected vector makes during its lifetime (i.e. two to four weeks in the case of vector *Culicoides*) (10, 26). The determination of the two parameters explained above is essential to enable an accurate estimation of vector transmission rates and so be in a position to make predictions on whether or not BTV will become established in an area. Such detailed studies inevitably demand significant financial and scientific resources and require a multi-disciplinary approach.

MODES OF INTRODUCTION AND MECHANISMS OF AMPLIFICATION

Introduction of BTV from one area into another can occur in four ways: through animal movements (domestic and wild ruminants) or the transport of animal product (semen, embryos); by infected vector *Culicoides* carried by various living (plants, animals) or inanimate (aircraft, ships) means; through the active flight of infected vector *Culicoides* (local propagation); and through the passive flight of infected vector *Culicoides* on the wind (responsible for long-distance dissemination). The number and distribution of susceptible hosts, the duration and titre of the BTV viraemia in the hosts, the vectorial capacity of the local vector population and the ambient temperature will determine whether the virus becomes established in a new area. In essence, establishment depends upon a sufficient number of vector *Culicoides*, infected by feeding upon local viraemic hosts, surviving long enough to ensure completion of the intrinsic incubation period (4 to 20 days, depending on the ambient temperature) and transmitting the virus by bite to new hosts (20). The extrinsic incubation period

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is the interval between the time of infection of a vector and when it first becomes capable of transmitting BTV to a new host (15). These requirements for BTV establishment have clearly been fulfilled in much of southern Europe, as BTV has survived there in many locations since the late 1990s. The widespread recrudescence of BTV-8 infections in northern France, and in Belgium, the Netherlands, Luxembourg and Germany and the radial extension of BTV-8 across Europe in 2007 suggest that, in a time of climate change, the requirements for BTV establishment may now also be fulfilled in many more northerly parts of Europe. Veterinary authorities and legislators throughout northern Europe should take notice of this situation.

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European Union policy on bluetongue prevention and control

4

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The European Union (EU) policy on bluetongue (BT) has evolved in the last ten years in parallel with the dynamics of the disease on the European continent. Prior to the late 1990s, only a few epidemics of BT had occurred in the Mediterranean basin after a long period of freedom from the disease. The situation changed dramatically when BT reappeared in Europe in 1998 and progressed beyond the 40th parallel considered at that time to be the northernmost limit for the disease. Since then the EU has had to deal with a new situation and Directive 2000/75/EEC, based on existing OIE standards, which partially mirror those applicable to African horse sickness, was the first EU reaction to the new epidemiological situation. This legislative text sets out the main lines of the EU policy on BT and presents a sufficient degree of harmonisation and flexibility to make it adaptable to specific local situations.

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The EU rules on BT lay down the measures that Member States should take if an outbreak of BT is suspected or confirmed. The immediate measures deal with protection from vector attacks and movement restrictions for susceptible animals. Restrictions are also applied to holdings located within a radius of 20 kilometres around the infected holding(s) and, in addition, protection (100 km radius) and surveillance zones (depth of at least 50 km beyond the limits of the protection zone and where vaccination is not applied) around the infected farm(s) must also be established. These restrictions are not lifted until the virus has been eradicated and the presence of the disease has been ruled out.

Depending on the epidemiological, geographical, ecological or meteorological circumstances, the competent authority of the Member States may adapt the measures or take further measures, in particular as regards movements of animals out of the restricted zones under certain conditions that are laid down in the implementing legislation.

A large part of EU territory is situated within the traditional bluetongue virus distribution areas (approximately between latitudes 50°N and 35°S), and the EU has consequently gained a high level of expertise and experience on BT in the past decade.

However, the global epidemiological situation of BT is changing and BT is now known to be spreading to the northern hemisphere, and the EU has experienced recent incursions of different BT virus serotypes. To adapt to these new challenges, the EU policy has been evolving and it now provides for a set of sustainable, proportionate and science-based principles that aim to control the disease, minimise its negative impacts and eradicate it under certain circumstances.

The EU policy is based on three pillars: surveillance and transparent exchange of epidemiological information, proportionate restrictions on movements, and vaccination.

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BLUETONGUE MONITORING AND SURVEILLANCE IN THE EUROPEAN UNION

To gain a better understanding of the epidemiological situation and of the risks associated with BT, and to establish proportionate measures, it is necessary to establish BT monitoring and surveillance programmes adapted to the risks.

Bluetongue monitoring programmes are implemented in restricted zones and aim to provide information on the dynamics of BT in a zone already subjected to restrictions. These programmes include at least a serological monitoring programme, consisting of an active annual programme of testing sentinel animals aimed at assessing the circulation of BT virus within the restricted zones, and an entomological monitoring programme, consisting of an active programme of vector catching using traps to determine the population dynamics and the over-wintering features of the vector (*Culicoides* species) in order to determine the seasonally vector-free period.

Bluetongue surveillance programmes are implemented outside restricted zones and are aimed at the early detection of virus circulation in a BT-free Member State or zone. These surveillance programmes include passive clinical surveillance to detect and investigate suspicions of BT by using an early warning system for reporting suspected cases, serological surveillance based on random or targeted serological testing of susceptible species populations to detect BT virus transmission, and entomological surveillance consisting of vector catching to gather information on the proven and potential vector species, their distribution and seasonal profiles.

In addition, an information system called BT-Net has been established to gather and exchange data on BT surveillance in the EU and in many neighbouring third countries. This system is a useful disease

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management tool that ensures the rapid exchange of information on the disease situation and surveillance data between Member States. It is an essential tool to modulate disease control measures and facilitate the safe trade of live ruminants, thereby reducing the losses caused by the disease.

MOVEMENTS OF ANIMALS WITHIN AND FROM RESTRICTED ZONES

Movements of animals within the same restricted zone (where the same BT virus serotype is circulating) are unrestricted.
However, animals in restricted zones are only allowed to move to BT-free zones if they meet certain well-defined requirements. Essentially, animals that have remained for 60 days protected from vector attacks or in a seasonally-free period are considered to be safe. Also animals that have been protected from vector attacks for 14 or 28 days and give negative PCR or ELISA tests results are considered to be safe. In addition, vaccinated animals or naturally immunised animals are considered to be safe and are therefore allowed to move to a BT-free zone.

VACCINATION AGAINST BLUETONGUE

Vaccination is the most efficient mitigation measure that can be implemented in an infected territory. The main purpose of vaccination is to avoid clinical cases and therefore to limit the losses incurred by farmers. Vaccination is also used to control the disease, and can be used to facilitate safe trade or even to eradicate the disease. However, vaccination presents certain drawbacks.

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Live attenuated (classic) vaccines are available for most serotypes. They are cheap, protect after a single inoculation, and prevent clinical disease. However, certain adverse effects (e.g. abortions due to underattenuation) have been reported. In addition, these vaccine viruses are spread by vectors in the same manner as the wild virus, and there is the potential for reversion to virulence and theoretical reassortment of genes with those of wild virus field strains.

Inactivated virus vaccines are safe and can be effective, but they are more expensive and re-vaccination is needed. Availability of these vaccines is currently limited to a few serotypes, but it is feasible to produce vaccines for new serotypes in large quantities.

The EU has supported the vaccination option whenever national authorities have chosen to adopt this policy, and it considers that the advantages of vaccination are maximised when a harmonised strategy is adopted by the affected Members States.

To summarise, the EU policy on BT is sustainable, proportionate, science-based and flexible enough to adapt to the global climate changes, while respecting the underlying principle that the decisions must minimise the impact on the economy and should be clearly understood and supported by stakeholders in order to be fully effective.

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Role of the World Organisation for Animal Health

5

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INTRODUCTION

Preventing the spread of disease through international trade in animals and animal products is one of the primary objectives of the World Organisation for Animal Health (OIE). This is accomplished by establishing international standards for a wide range of diseases. The OIE standards relating to bluetongue (BT) are published in the *Terrestrial Animal Health Code* (*Terrestrial Code*) and the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (*Terrestrial Manual*).

These standards include guidelines and recommendations on declaring a country or zone free from BT virus (BTV), requirements for seasonal freedom from BTV and recommendations for the safe importation of live animals, semen and embryos into a BTV-free country or zone, as well as specific surveillance guidelines for BT. There are also other general but related chapters in the *Terrestrial Code* and *Terrestrial Manual* complementing the chapter 2.2.13 (accessible at http://www.oie.int/eng/normes/mcode/en_chapitre_2.2.13.htm)
and appendix 3.8.10 (accessible at http://www.oie.int/eng/normes/mcode/en_chapitre_3.8.10.htm) specifically on BT.

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Developing BT standards that allow the safe trade of animals and animal products, has been very difficult as much of the world between the latitudes of approximately 53°N and 35°S is already infected or has the potential to be infected (1). This task is further complicated by the presence of 24 serotypes of BTV and many known and potential vectors with varying degrees of competence.

OIE *TERRESTRIAL ANIMAL HEALTH CODE* STANDARDS FOR BLUETONGUE

For the purposes of the *Terrestrial Code* (2), the infective period for bluetongue virus (BTV) is deemed to be 60 days and the global BTV distribution is currently considered to be between the latitudes of approximately 53°N and 35°S, though it is acknowledged that the disease is expanding in the northern hemisphere. In the absence of clinical disease in a country or zone between these latitudes, its BTV status should be determined by an ongoing surveillance programme (2). The programme may need to be adapted to target parts of the country or zone at a higher risk due to historical, geographical and climatic factors, ruminant population data and *Culicoides* ecology, or proximity to enzootic or incursional zones. All countries or zones adjacent to a country or zone not having free status should be subjected to similar surveillance. In such cases, surveillance should in general be carried out over a distance of at least 100 kilometres from the border with that country or zone.

The *Terrestrial Code* chapter on BT (chapter 2.2.13) provides for the application of a BTV seasonally free zone with the onset of the seasonally free period commencing the day following the last evidence of BTV transmission and of cessation of activity of adult *Culicoides* likely to be competent BTV vectors. The seasonally free period is

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considered to end at least 28 days before the earliest historically proven date of commencement of BTV activity.

The BT chapter also provides several approaches for moving ruminants and other BTV susceptible herbivores based on the epidemiology of BT and the period in the BT-free country or zone before shipment: if the period is 60 days or more, there are no restrictions; if this period is at least 28 days, a negative serological test for presence of antibody is required; if this period is at least 7 days, a test with negative results for agent identification is required. The *Terrestrial Code* further provides for animals to be vaccinated at least 60 days prior to shipment or certification that the animals either did not transit through an infected zone or were protected from competent *Culicoides* vectors. More or less the same requirements are applicable when importing from BTV seasonally free zones.

The requirements for importing susceptible animals from BT-infected countries or zones are basically similar for periods of 60, 28 or 14 days prior to shipment, with the additional requirement of protection from attack by *Culicoides* likely to be competent BT vectors. Alternative requirements are either that animals were vaccinated at least 60 days before shipment or that surveillance has been conducted for a similar period in accordance with *Terrestrial Code* Appendix 3.8.10.

The requirements for importing semen, embryos and oocytes from BT susceptible animals are also described and are based on the same approach depending on the status of the country or zone of origin.

SPECIFIC SURVEILLANCE GUIDELINES FOR BLUETONGUE

Appendix 3.8.10 of the *Terrestrial Code* provides specific guidelines on surveillance for BT, although it is acknowledged that the impact

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and epidemiology of BT differ widely in different regions of the world and it is therefore impossible to provide specific surveillance guidelines for all situations. OIE Member Countries and Territories should thus provide scientific data that explain the epidemiology of BT in the region concerned and adapt the surveillance strategies for defining their infection status (free, seasonally free or infected country/zone) to the local conditions. The Appendix provides a case definition for BTV infection; describes general conditions and methods for surveillance, different surveillance strategies for clinical, serological, virological and vector surveillance and information on how to interpret serological and virus detection tests.

GENERAL PROVISIONS OF THE *TERRESTRIAL CODE* RELEVANT TO BLUETONGUE

Over and above the disease specific chapter and appendix for BT, the *Terrestrial Code* also describes the criteria for notification of the disease, guidelines for the evaluation of Veterinary Services to assess credibility of certification, aspects related to obligations and ethics in international trade, principles of zoning and guidelines to conduct an import risk analysis. These chapters and appendices should be consulted together with the disease specific chapter and appendix when assessing risks for import and disease control and mitigation measures.

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THE OIE *MANUAL OF DIAGNOSTIC TESTS AND VACCINES FOR TERRESTRIAL ANIMALS*

The *Terrestrial Manual* (3) is a companion volume to the *Terrestrial Code* and provides a uniform approach to the diagnosis of BT. The purpose is to facilitate international trade in animals and animal products by describing internationally agreed laboratory methods for diagnosis and requirements for the production and control of BT vaccines. The methods described also form the basis for effective BTV surveillance and monitoring.

CONCLUSIONS

In setting international standards for safe trade to prevent the spread of BT, the OIE not only fulfils its international mandate and obligations as recognised by the World Trade Organisation (4), but also facilitates trade negotiations by providing a scientific reference to ensure the continiuty of trade without unnecessary and unjustified trade restrictions.

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Clinical aspects of bluetongue in ruminants

6

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INTRODUCTION

Bluetongue virus (BTV) is able to infect a wide variety of domestic and wild ruminants. However, a severe clinical picture is generally only seen in certain ovine breeds and some species of the family Cervidae; cattle and goats suffer rather from a subclinical infection and represent a virus reservoir (6, 9). The clinical signs commonly reported include fever, salivation, nasal discharge, oedema (especially of the head), congestion and ulceration of the oral mucosa, weakness, depression, and sometimes cyanosis of the tongue (hence the name bluetongue) (4, 6, 8, 10).

The morbidity and mortality rates, and the case fatality rate (related to the severity of the clinical signs), depend on various factors, such as

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the breed and the age of the infected animals (older animals are more suseptible) as well as the serotype and strain involved (7).

A presumptive diagnosis of bluetongue (BT) relies on the ability of veterinary practitioners and farmers to recognise the principal alerting signs. Veterinary health professionals must therefore be kept well informed and standardised clinical examination procedure should be applied. Identifying suspected cases of BT means that the disease can be recognised at an early stage and the competent authorities notified. Among other advantages, notification enables the authorities to determine the activity zones of vectors, gain a clearer understanding of the epidemiology of the disease and thus implement the most effective control measures.

The main objective of this chapter is to describe the BT cases (serotype 8) seen during the epizootic that occured in northern Europe in summer 2006 and during the resurgence of the disease in summer 2007 (12, 13). The data presented in this chapter were recorded in a standardised manner by a multidisciplinary team from the Faculty of Veterinary Medicine of the University of Liege (3).

During its emergence in Europe, BT (BTV-8) mainly infected sheep and cattle, with a similar incidence in these two species (1, 2). During the reappearance of BT in summer 2007, the incidence rate in sheep was initially higher than that in cattle. Later in the year, however, cattle were infected at the same rate as sheep. Few clinical cases of BT were reported in goats, even though this species is also susceptible to the disease (5).

The clinical data described below concern cattle and sheep of herds where BT had been confirmed by laboratory tests (11). The clinical description of BT in yaks has been included in order to draw the attention of veterinarians and farmers to the fact that other domestic ruminant species may be affected.

To increase readability, clinical signs are grouped topographically for

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each of these species. This is in contrast to Chapter 11, where clinical signs are grouped according to the organ system involved. They are grouped into several categories: general, local (head, limbs, udder, skin, haircoat, wool) and reproductive clinical signs. A summary of clinical signs observed in cattle in 2006 and sheep examined in 2006 and 2007 is given in Table III. Table III also indicates the presence or absence of these clinical signs in two yaks (*Bos grunniens grunniens*) in 2006. The morbidity, mortality and fatality rates observed in sheep and cattle during the BT epizootic are shown in Table IV.

CLINICAL SIGNS IN CATTLE

The exact incubation period for BT in cattle is not known but is suspected to be close to that in sheep, namely 6 to 8 days. Most of the infected cattle examined were adult.

The description of clinical signs is based on observations made in 38 animals in 11 different herds (mostly dairy herds) located close to the epicentre of the epizootic (province of Liege, Belgium). From mid August 2006 (call from a veterinarian regarding a suspicion of photosensitisation), weekly follow-up visits were made during a six-week period to examine some of the affected animals.

GENERAL CLINICAL SIGNS

Hyperthermia was rarely observed (5 of 38 bovines tested). Hyperthermia may be slight and transitory, however, and therefore may have gone unnoticed. Hence, this clinical sign is not at all representative and is not considered to be a reliable sign.

A reduced milk yield was mentioned by half of the dairy farmers. Nevertheless, this clinical sign should only be considered definitively

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present on the basis of objective parameters such as milk yield records. Numerous factors are known to influence milk production in dairy cows (e.g. season, number of lactations, state of lactation, ambient temperature, nutrition, availability and temperature of drinking water). According to the farmers, this sign seemed to persist for a long time (several weeks) after the disappearance of other clinical signs.

Anorexia was rarely reported, while weight loss was more commonly reported. Weight loss may have been due to reduced appetite in the febrile phase or to oral lesions hindering the animal from ingesting food. Weight loss and/or reduced food intake were not measured precisely and were among the observations made by farmers and the referring veterinarians. Lastly, animals affected by BT were lethargic.

LOCAL CLINICAL SIGNS: HEAD

The earliest, most common and most persistent lesions were located on the muzzle and nostrils. Ulcerative to necrotic lesions covered the dorsal side of the muzzle up to the mucocutaneous junction, generally on the non-pigmented parts (Photo 1) but sometimes covering the whole muzzle (Photo 2). Similar lesions could often also be seen on the external wing of the nostrils (Photo 3). These lesions generally formed crusts. Some animals showed a nasal discharge, which could be seromucous or mucopurulent.

Shortly after the first lesions appeared on the muzzle, lesions could be found in the oral cavity. Ulcerations were commonly observed. Most of these lesions were located around the teeth on the lingual sides of the gums, and could extend up to 1 cm from the teeth (Photos 4 and 5). Ulceration was sometimes seen on the dental pad (Photo 5) and on the mucosa of cheeks and tongue. Cyanosis of the tongue (i.e. a blue tongue) was seldom reported.

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Hyper-salivation and regurgitation were sometimes observed.
Periocular dermatitis was commonly observed (Photo 6). This could be accompanied by crusts and epiphora and occured early in the course of the disease.
Lastly, a submandibular oedema was observed in one of the animals (Photo 7). This would appear to be a rare clinical sign in cattle suffering from BT.

LOCAL CLINICAL SIGNS: LIMBS

At the beginning of the disease, a swelling of the lower part of the limbs was noted. Swelling included the pastern and fetlock, but rarely extended beyond the canon (Photo 8). Bluetongue-affected animals sometimes seemed to hesitate when walking, and showed muscular stiffness, lameness or even a reluctance to move. In most severe cases, the animals were unable to rise and the outcome was generally fatal.

LOCAL CLINICAL SIGNS: UDDER

Fairly often, and in general after the appearance of head lesions, erythema of the udder was observed (Photo 9). Most commonly, ulcerative and necrotic lesions were present on the teats (Photo 10). These lesions caused difficulty in milking, were associated with a decreased intake of feed and might have been the cause of the decreased milk production. The impact of BT on the somatic cell count of the milk and the incidence of mastitis were not evaluated.

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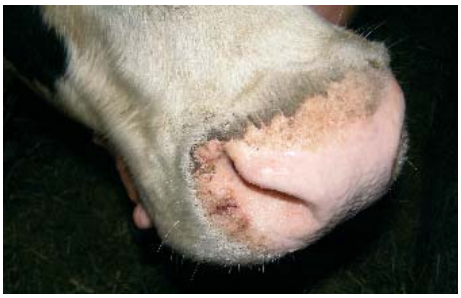

Photo 1. Mild ulcerative and necrotic lesions around the muzzle skin-mucosa junction (cattle).
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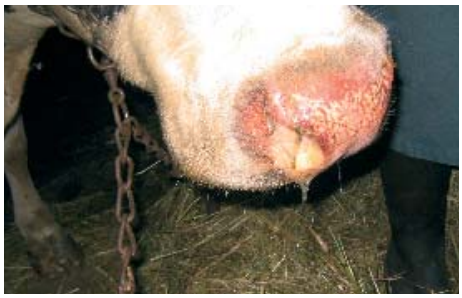

Photo 2. Severe ulcerative and necrotic lesions spread over the entire muzzle; seromucosal nasal discharge (cattle).
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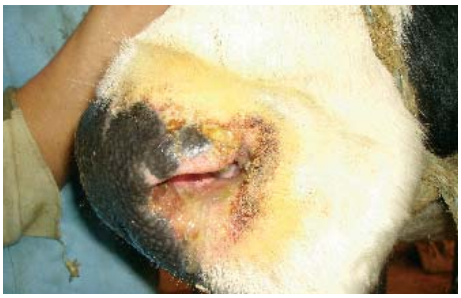

Photo 3. Ulcerative and necrotic lesions around nostrils (external wing of the nose) ; mucosal and purulent discharge (cattle).
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Photo 4. Gingival ulcerations about 1 cm behind incisor teeth (cattle).
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Photo 5. Ulcers on gums and incisor pad (cattle).
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Photo 6. Periocular dermatitis (cattle).
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Photo 7. Submandibular oedema (cattle).
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Photo 8. Fetlock and pastern oedema (cattle).
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LOCAL CLINICAL SIGNS: SKIN AND HAIRCOAT

Necrotic lesions were commonly observed on the non-pigmented skin of the backline and on the area close to the root of the tail are commonly observed but generally occured only two to three weeks after the appearance of the first clinical signs. In some cases these lesions (dry necrosis) led to the detachment of shreds of skin (Photo 11). These lesions were similar in appearance to those induced by photosensitisation, but occurred on animals that had been indoors and therefore out of direct sunlight.

REPRODUCTIVE CLINICAL SIGNS

In 2007, several suspicions of abortion, metritis and congenital abnormalities related to BT infection were reported in the field. However, the episode of BT occurred late in the calving season and our findings could have been biased.

EVOLUTION AND SIGNIFICANCE OF THE CLINICAL SIGNS

In the vast majority of cases, cattle survived the disease and their lesions regressed significantly within a 4 to 8 weeks. Breeding performances seemed to take the longest time to return to normal. In rare cases, the animals remained recumbent and their condition deteriorated until they died.

Even though no clinical sign seems to be pathognomonic for BT, the dominant clinical picture consists of ulcerative and necrotic lesions on the muzzle and nostrils and in the oral cavity, lameness and lesions on the teats in the adult animal.

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Photo 9. Teats and udder erythema (cattle).
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Photo 10. Ulcerative and necrotic lesions of the teats (cattle).
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Photo 11. Dry necrotic lesions leading to the detachment of skin shreds (cattle).
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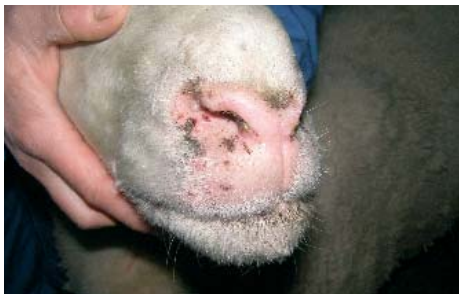

Photo 12. Ulcerative and necrotic lesions of lips, nostrils and muzzle (sheep).
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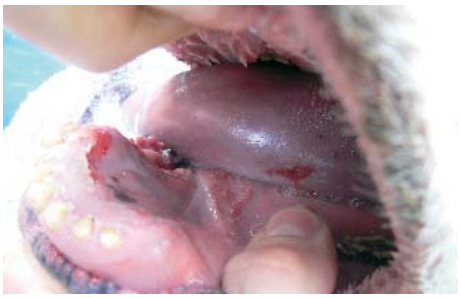

Photo 13. Ulcerative lesions in the mouth (sheep).
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Photo 14. Hypersalivation and face oedema (sheep).
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Photo 15. Regurgitation (sheep).
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Photo 16. Tongue cyanosis (sheep).
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CLINICAL SIGNS IN SHEEP

The incubation period in sheep is generally between 6 and 8 days (range: 2-18 days). Lesions in sheep are more oedematous and haemorrhagic than in cattle.

The following description of clinical signs is based on observations made in 39 sheep and one lamb from four flocks of mainly Texel breed located in the Liege and Namur Provinces of Belgium. A clinical follow-up of some of these animals was conducted on a weekly basis.

GENERAL CLINICAL SIGNS

A transient hyperthermia (up to 42°C) was observed more frequently in sheep than in cattle. Apathy and anorexia (no ingestion of food or water in the most severely affected animals) with a considerable weight loss also occured more often than in cattle. Diarrhoea was sometimes observed, essentially when the animals regain their normal feeding behaviour during the recovery period. In dairy sheep, a drop in milk production or even agalactia could sometimes be observed.

LOCAL CLINICAL SIGNS: HEAD

In general, the clinical signs observed in sheep were similar to those observed in cattle, namely ulcerative and necrotic lesions on the lips, nostrils and muzzle (Photo 12), ulcers in the oral cavity (Photo 13) and hypersalivation (Photo 14). Regurgitation occurred more commonly in sheep than in cattle (Photo 15). Contrary to cattle, cyanosis of the tongue was observed several times in sheep (Photo 16). Oedema of the periocular region (Photo 17) and/or the face (Photo 18) was observed

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more frequently than in cattle; sublingual oedema, however, was less frequent (Photo 19). Periocular dermatitis was also observed in sheep.

LOCAL CLINICAL SIGNS: LIMBS

Lameness, oedema of limbs, congestion of the coronary bands as well as stiffness of the limbs together with amyotrophia were commonly encountered. These signs occurred more often and were more severe than those in cattle.

LOCAL CLINICAL SIGNS: UDDER

As described in cattle, erythema of the skin of the udder was observed together with ulcerative lesions of the teats (Photo 20). This sign was less commoly detected in non-dairy ewes, most likely due to missing observations (extensive husbandry and wool hiding the lesions).

LOCAL CLINICAL SIGNS: SKIN AND WOOL

There was sometimes a focal loss of wool, together with dermatitis lesions (crusts). These lesions are not easy to identify in unshorn sheep. Photosensitisation-like lesions such as those seen on the backline in some cattle have not been described in sheep. However, some lesions of this type were noted on the ears of some sheep. A loss of wool, possibly as a consequence of hyperthermia, was sometimes observed during the recovery period.

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Photo 17. Periocular oedema (early clinical sign) (sheep).
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Photo 18. Face oedema in a lamb ; mucosal and purulent nasal discharge (sheep).
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Photo 19. Subglossal oedema (sheep).
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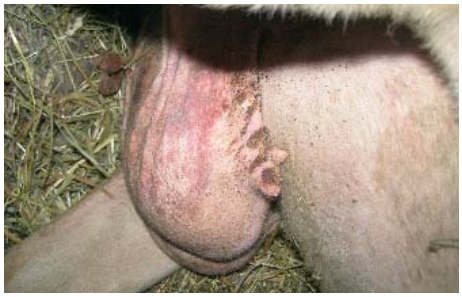

Photo 20. Erythema of udder skin (sheep).
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Photo 21. Hypersalivation, severe cyanosis of the tongue (Yak; *Bos grunniens grunniens*).
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Photo 22. Ulcers of lingual, palate and gingival mucosae (mostly behind incisor teeth) (Yak; *Bos grunniens grunniens*).
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REPRODUCTIVE CLINICAL SIGNS

To this day it is impossible to say whether abortions observed during the outbreak of BT epizootic were caused by the disease. In those rams in which semen quality was evaluated four to six weeks after the onset of the clinical signs, microscopic and macroscopic modifications were observed (watery semen with absence of living spermatozoa).

EVOLUTION AND SIGNIFICANCE OF THE CLINICAL SIGNS

In general, clinical signs observed in sheep are more debilitating than those in cattle. Other pathologies (pneumonia, diarrhoea, contagious ecthyma and intercurrent diseases such as parasite infestation and footrot) commonly complicated the clinical picture of BT. In cases where the disease was fatal, the interval between the first clinical signs and death was 8 to 12 days. This may be shorter in improved breeds (1 to 4 days). These animals often died due to complications (e.g. of the respiratory tract). Surviving animals presented a long recovery period (beginning 2 weeks after disease onset). After-effects may be observed after successful recovery, and consist of reduced growth (growth delay), poorer meat and wool quality and infertility in the case of rams. Subacute forms of bluetongue in sheep are very rarely observed in Europe.
The predominant signs in sheep are ulcerative and necrotic lesions of the muzzle, nostrils and/or the oral cavity, weight loss and lameness.

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CLINICAL SIGNS IN OTHER RUMINANTS: EXAMPLE OF THE YAK (*BOS GRUNNIENS GRUNNIENS*)

Two female yaks living in captivity in a BT-infected breeding yard were examined clinically and *post mortem*. Bluetongue was confirmed in these animals based on laboratory tests.

GENERAL CLINICAL SIGNS

Both animals exhibited an arched back and severe lethargy. Weight loss was noted, as well a fall in milk production in one lactating animal.

LOCAL CLINICAL SIGNS: HEAD

As in cattle, the clinical signs most commonly observed in the yaks were in the region of the eyes, the nostrils and the oral cavity. Both animals showed conjunctivitis, periocular erythema and epiphora. Slight ulcerative and necrotic lesions were visible around their nostrils. Hypersalivation, pronounced cyanosis and sublingual oedema were diagnosed in one of the two females (Photo 21). Numerous ulcers were found on the lingual mucosa, the palate and the gums, principally caudal to the incisors (Photo 22).

LOCAL CLINICAL SIGNS: LIMBS

Both animals were obviously reluctant to move and were frequently lying down. The arched posture of the back may indicate a lesion of the locomotor system, although there was no evidence of a myositis lesion

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on *post mortem* examination. A slight oedema of the limbs was visible around the pastern.

LOCAL CLINICAL SIGNS: UDDER

Dermatitis with papules and crusts was observed on the udder of one of the two animals.

LOCAL CLINICAL SIGNS: SKIN AND HAIRCOAT

One of the two animals showed acute dermatitis located on the inner thighs.

PROGRESSION AND SIGNIFICANCE OF THE CLINICAL SIGNS

The progression of the clinical signs and the deterioration of the animals were quite rapid, leading to death seven days after the onset of reduced feed intake. Generally speaking, the clinical signs were very pronounced in both animals. This was likely due to their poor condition and the difficulty of administrating supportive treatment.

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+++: Clinical signs very frequently present

++: Clinical signs frequently present;

+: Clinical signs sometimes present

(+): Clinical signs rarely present

– : Clinical signs absent;

?: Data not recorded;

!: Suspicion

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Table III. Clinical signs of bluetongue (virus serotype 8) in cattle and sheep, and in yaks living in captivity, examined by the Faculty of Veterinary Medicine of the University of Liege during the outbreak in Northern Europe in 2006 and the resurgence of the disease in 2007

Clinical signs	Cattle (n = 38)	Sheep (n = 40)	Yaks (n = 2)
General clinical signs			
Hyperthermia	(+)	+	?
Depression	+	++	present
Anorexia, loss of appetite	(+)	++	present
Decrease in milk production	+++	++	present
Weight loss	++	+++	present
Localised clinical signs: head			
Muzzle: ulcerations, necrosis, crusts (pus)	+++	+++	present
Nostrils: ulcerations, crusts	+++	+++	present
Nose: mucous discharge and /or pus	+	+	-
Mouth: cyanosis of the tongue	+	(+)	present
Mouth: ulcers on the tongue	+	+	present
Mouth: ulcers on gum, dental pad, cheeks	++	++	present
Mouth: hypersalivation	++	++	present
Mouth: regurgitation	(+)	+	?
Eyes: periocular erythema, crusts, epiphora	++	+	present
Oedema of submandibular head, lips, eyelids, periorbital region, ears	(+)	++	-
Local clinical signs: limbs			
Lower limbs (canon, fetlock, pastern): oedema	+	+	-
Muscular weakness, stiffness of the limbs	++	+++	-
Lameness, reluctance to move, recumbency	++	+++	present
Congestion of the coronary bands	+	+	-
Local clinical signs: udder			
Erythema, oedema	++	++	present
Ulcerative lesions and necrosis on the teats	++	++	-
Local clinical signs: skin, haircoat, wool			
Photosensitisation-like lesions (unpigmented skin)	++	+	-
	++	-	-
Cutaneous necrosis	-	++	-
Loss of hair/wool			
Clinical reproductive signs			
Abortions	!	+	?
Metritis	!	?	?
Infertility	?	?	?
Altered semen quality in males	(+)	(+)	?

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Table IV. Mortality, morbidity and case fatality rates observed in cattle and sheep during the bluetongue epizootic (virus serotype 8), in Northern Europe in summer/autumn 2006 (2)*

Parameter	Sheep	Cattle
Morbidity rate		
Mean	20%	6.8%
Minimum – Maximum	0% - 100%	0% - 100%
Other	≤ 25% in 80% of the herds	≤ 10% in 87% of the herds
Mortality rate		
Mean	5%	0.3%
Minimum – Maximum	0% - 100%	0% - 30%
Other	≤ 20% in 93% of the herds	≤ 5% in 99% of the herds
Case fatality rate		
Minimum – Maximum	0% - 100%	0% - 100%
Other	50% in 23% of the herds	50% in 6% of the herds

* Rates were higher in the 2007 than in the 2006 (probably twofold to threefold).

CONCLUSIONS

The outbreak of BT (serotype 8) that occurred in northern Europe during summer 2006 came as a surprise to many veterinarians and farmers, because this vector-borne disease was unexpected in these regions and because the disease occurred in cattle as well as sheep.

The clinical signs observed were similar to those described in the literature, except that cyanosis on the tongue was only rarely observed. Ulcerative and necrotic lesions on the muzzle and nostrils and in the oral cavity, ulcerative lesions on the udder and teats, photosensitisation-like lesions, lameness and deterioration of body condition are some of the alerting signs of BT in a region where the disease is endemic. The above-mentionned clinical signs should therefore be monitored in a

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standardised manner (see Chapter 11), as soon as the climatic conditions allow the vector activity to become active.

Given the diversity of the clinical signs and the fact that none of them is pathognomonic for BT, laboratory tests have to be performed for a confirmatory diagnosis (see Chapter 8 for differential diagnosis). The practitioner should alert the veterinary inspector in the event of any suspicion of BT (BT is a notifiable disease) and should arrange for laboratory tests in order to confirm or rule out the clinical suspicion (see Chapter 9 for laboratory tests). The appropriate samples should be sent to a reference laboratory.

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Bluetongue: Gross lesions 7

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The whole pathology of bluetongue is related to vascular endothelial damage, resulting in increased capillary permeability and fragility, disseminated intravascular coagulation, and eventually tissue necrosis. Associated lesions are oedema, congestion, haemorrhage, infarction and inflammation (2).

GROSS LESIONS OF BLUETONGUE IN SHEEP

Gross lesions of BT in sheep are highly variable. Externally, they include serous nasal discharge becoming, in some cases, mucopurulent with dry crusts on the nostrils, congestive to ulcerative muzzle, froth around the mouth and swelling of the lips, nose, face, intermandibular area, eyelids and sometimes ears (2, 3, 6). Skin hyperaemia can extend to the whole body, including axillary and inguinal areas. Crusts and excoriation can develop with time but the most prominent change in the skin is the congestion of the coronary bands of the hooves and of interdigital areas. This reddening may be accompanied by petechiae or ecchymoses extending down to the horn (2, 6). Congestion, oedema, petechiae or ecchymoses may be observed on mucous membranes of the oral cavity with excoriations in areas prone to mechanical abrasion, namely the lips, the tongue, the dental pad, cheeks opposite the molar teeth and sometimes the hard palate. Erosions or ulcerations may be

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covered by grey necrotic tissue. Occasionally, the tongue may be swollen, congestive or cyanotic ('bluetongue'). In the reticulum and rumen there may be hyperaemia and occasional erosions on papillae and laminae. The oesophagus may show similar lesions (2, 6). Lesions of the respiratory tract include congestion, oedema, haemorrhages and sometimes cyanosis of the nasal mucosa, larynx and pharynx. There may be tracheal congestion. The lungs may be hyperaemic with severe alveolar and interstitial oedema, froth in the tracheobronchial tree and, sometimes, excess of fluid in the thoracic cavity. The pericardial sac may show petechiae and excess of fluid (Photo 23) (1, 2, 3, 6).

A typical lesion, almost pathognomonic, is a variable sized haemorrhage in the tunica media of the pulmonary artery (Photo 24), near the heart. Similar haemorrhages may occur in the aorta and other large arteries. Subepicardial and subendothelial petechiae and ecchymoses are common, particularly in the left ventricle (1, 2, 3, 6).

Focal grey-white areas of necrosis and haemorrhagic foci may be present in the myocardium, often in papillary muscles. Grey-white areas of degeneration and necrosis as well as haemorrhagic patches can be observed in skeletal muscles. The subcutis and intermuscular fasciae are infiltrated with yellow gelatinous exudates, haemorrhages and contusions (2, 6). There is also congestion and haemorrhages are found in most tissues, in particular in lymph nodes, tonsils, kidneys and spleen (1, 2).

GROSS LESIONS OF BLUETONGUE IN CATTLE

Necropsy lesions of BT in cattle are not very different from those observed in sheep. The most prominent lesions involve the skin, the mouth and the hooves (2, 4, 5, 6). Skin lesions are characterised by severe oedema with thick pleats apparent in the cervical and dorsal

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thoracic areas. Dry crusty exudates with matted or spiky hair may be seen over these areas. The crusts are secondary to vesicles and ulcers. The external nares may show erosions covered with sloughing crusts. In the mouth, vesicular lesions evolving to ulcers sometimes covered with greyish necrotic exudates are observed on the oral mucosa and dental pad (Photo 25), but the tongue is rarely affected. Similar lesions may occur on teats. On the hooves, hyperaemia is observed around the coronary bands, sometimes with fissures. However, all lesions described in sheep may also be present in cattle (1, 2, 4, 5, 6). BTV infection during pregnancy may cause severe cerebral abnormalities in foetuses including hydrocephaly, porencephaly, focal encephalitis and retinal dysplasia in both sheep and cattle. Arthrogryposis, mouth and maxillae abnormalities are also reported (2, 5, 6).

To conclude, gross lesions of BT may be similar in sheep and cattle. However, field observations reveal that, in both species, infected animals exhibit an extremely variable arrays and intensity of macroscopic lesions.

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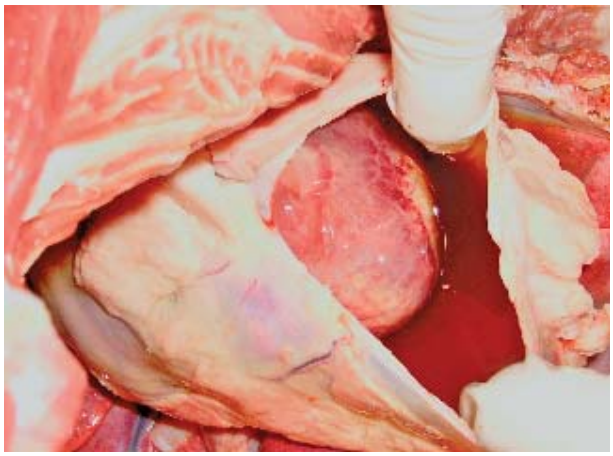

Photo 23. Hydro-pericardium (sheep)
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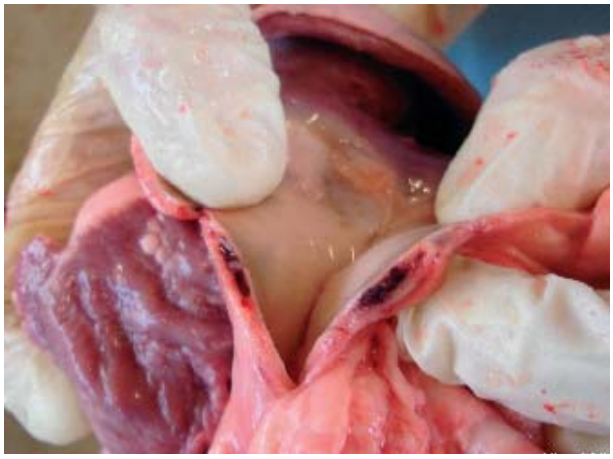

Photo 24. Haemorrhage at the origin of the pulmonary artery, media layer (sheep)
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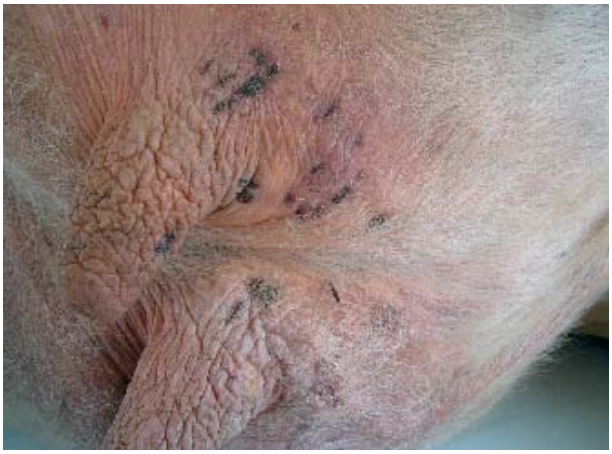

Photo 25. Ulcers of udder skin (cattle)
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Differential diagnosis of bluetongue 8

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There are a number of conditions with clinical syndromes similar to bluetongue (BT) in cattle and sheep. Their differentiation depends not only upon the clinical signs but also on their epidemiological characteristics, including morbidity, mortality, infectivity and seasonality. Only the most important clinical syndromes are presented in this chapter (Tables V and VI).

DIFFERENTIAL DIAGNOSIS IN CATTLE

For cattle, these clinical syndromes include mucosal disease (MD), malignant catarrhal fever (MCF), infectious bovine rhinotracheitis (IBR), foot and mouth disease (FMD), vesicular stomatitis, rinderpest and Rift Valley fever (RVF). Furthermore, conditions that lead to lameness, stomatitis or photosensitisation-type lesions must be taken into account when clinical signs suggestive of BT are present (see Chapter 6).

Animals affected by MD, caused by *Bovine viral diarrhoea virus*, can generally be differentiated by the presence of diarrhoea and interdigital ulceration (Photo 26), with marked depression and a decreased appetite. In the current BT epizootic, interdigital ulceration has seldom been

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observed in cattle and diarrhoea has been a rare clinical sign. Mucosal disease tends to be sporadic with low morbidity and almost 100% mortality, although a chronic form of the disease, characterised by milder clinical signs, does occur. *Bovine viral diarrhoea virus* occurs in most of Europe, with the exception of Norway where it was recently eradicated (6).

Malignant catarrhal fever can be differentiated largely by the presence of corneal opacity (Photo 27), bilateral lymph node enlargement, a high and persistent fever and, as with MD, a more severe depression than that reported in BT. It tends to be sporadic with a high mortality, although outbreaks occur occasionally (7). It occurs in most countries in Europe (9).

Infectious bovine rhinotracheitis can be differentiated from BT by the absence of oral and cutaneous lesions and the predominance of respiratory tract signs such as abundant nasal discharge (Photo 3), stridor and coughing (28). Morbidity can be high but mortality is relatively low. Austria, Denmark, Finland, Norway, Sweden and Switzerland have eradicated IBR and there are national eradication programmes in several other European countries.

Foot and mouth disease (FMD) cases tend to display vesicles on the coronary band and in the interdigital space (2), which are generally absent from BT cases (Photo 29). The type of oral lesions characteristic of FMD tend to be vesicular and therefore slightly different from the lesions typical of BT. Due to its highly contagious nature, high morbidity is normally seen with FMD. An outbreak of FMD occurred in the United Kingdom in the summer 2007 and the disease is regarded as enzootic in the Anatolia region of Turkey.

Vesicular stomatitis is clinically similar to FMD but its occurrence has not been reported in Europe for several decades. Rinderpest is clinically similar to MD, but digital lesions tend not to occur and affected animals develop profuse diarrhoea and die rapidly after the onset of fever (8). It is now nearly extinct wordwide. It has not been reported in Europe for

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many years, the last outbreak having occurred in Turkey in 1996 (9). Rift valley fever tends to cause more severe clinical disease in young animals and abortions are frequent (3); this disease is a zoonosis and has never been reported in Europe.

The veterinary clinician should also bear in mind that fewer clinical signs may be present in milder cases of BT in cattle. In such cases, a different group of disease conditions should also be considered. Erosive oral lesions in cattle can be caused by bovine papular stomatitis virus, several fungi (mycotic stomatitis), *Fusobacterium necrophorum* and occasionally the use of certain feeds (e.g. caustic soda-treated wheat). Pseudocowpox virus and herpes mammilitis viruses can lead to erosive teat lesions. The cutaneous lesions along the dorsal midline of cattle that are reported to occur later in the course of BT should be differentiated from photosensitisation, in which skin lesions frequently constitute the only single clinical sign. Moreover, in the case of BT, these skin lesions do not occur exclusively in animals kept outdoors at pasture.

DIFFERENTIAL DIAGNOSIS IN SHEEP

For sheep, differential diagnoses of BT include contagious ecthyma, pneumonia, sheep pox, FMD, vesicular stomatitis, peste des petits ruminants (PPR), rinderpest, RVF, oestrosis and ulcerative dermatosis.

Contagious ecthyma can generally be differentiated by the more proliferative nature of its lesions on the lips (Photo 30), the absence of oculonasal discharge and the absence of pyrexia. It tends to occur at lambing time, which may not coincide with a period when the vector of BT is active. Morbidity can be high, but mortality is low. It has a worldwide distribution and is a zoonosis (4).

Foot and mouth disease in sheep is clinically similar to the disease in cattle but milder, therefore, the main differentiating clinical sign from BT is the absence of the oedematous appearance of the head (2).

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Sheep pox can be differentiated mostly by the absence of lameness and by the infrequency of the oedematous changes that are characteristic of BT. Both morbidity and mortality can be high. Sheep pox has occurred in Greece in 2007 (9) and also in Turkey.

Peste des petits ruminants (PPR) can be differentiated by the absence of oedematous changes to the head and lameness, both signs that are characteristic of BT. Moreover, PPR is characterised by profuse diarrhoea and high mortality, which are not features of BT, though diarrhoea can occur. This disease still occurs in Turkey.

Clinical RVF can occur in sheep of all ages, but is most severe in young lambs. It can be differentiated from BT by the presence of haemorrhagic diarrhoea, higher frequency of abortions and a high morbidity rate (3).

Vesicular stomatitis in sheep tends to cause milder clinical signs than in cattle and the absence of oedematous lesions (present in BT) is probably the most useful differentiating clinical sign.

Pneumonia of varied aetiology can appear similar to BT due to the presentation of pyrexia, tachypnoea and nasal discharge; however, the absence of lameness and of erosive and oedematous lesions should enable it to be differentiated from BT.

Nasal bot fly (*Oestrus ovis)* infection can have a similar geographical distribution and seasonality and cause similar clinical signs to BT and should therefore be considered in the differential diagnosis (1). It is probably best differentiated from BT by the absence of lameness and oedematous lesions to the head.

It should be borne in mind that in less severe cases of BT in sheep few clinical signs may be observed. In such cases a different group of disease conditions should be considered. Erosive oral lesions in sheep can also be caused by *Fusobacterium necrophorum*. Oedema in the head area can be due to fascioliasis, gastrointestinal parasitism (e.g. acute haemonchosis), malignant oedema due to infection with various members of the genus *Clostridium* or paratuberculosis. Lameness can be caused by footrot, contagious ovine digital dermatitis or arthritic

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lesions. Cutaneous lesions should be differentiated from primary or secondary photosensitisation.

In addition, differential diagnoses for goats showing clinical signs of BT include contagious ecthyma, FMD, goat pox, PPR and rinderpest.

It is important to note that in rare cases, BT and one of the aforementioned conditions may occur together. This shows the importance of a complete clinical examination (see Chapter 11) and of compulsory confirmation of diagnosis by laboratory techniques. Moreover, not all clinical signs may be present in an individual animal, but among the members of an affected herd or flock, many of the characteristic signs may be observed.

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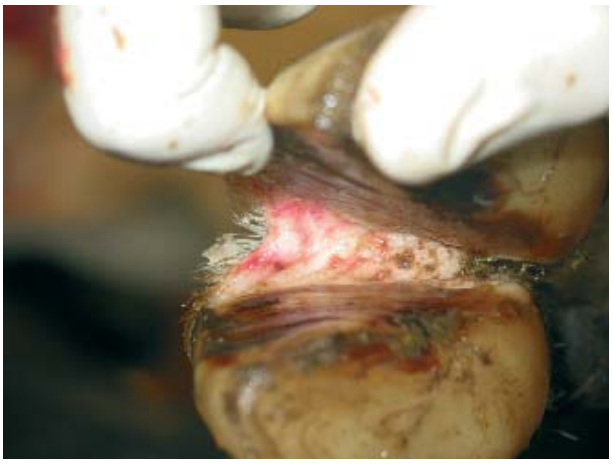

Photo 26. Acute interdigital ulcer in a cow with mucosal disease (BVD-MD)
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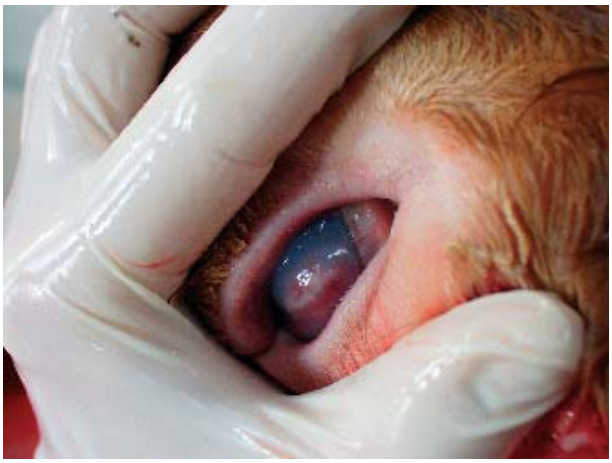

Photo 27. Corneal oedema in a case of malignant catarrhal fever (cattle).
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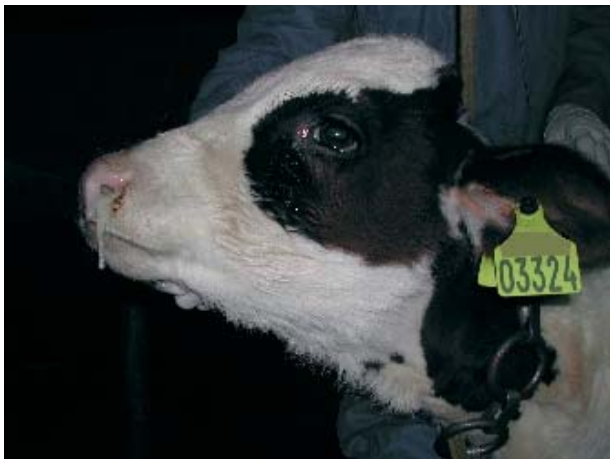

Photo 27. Bilateral purulent nasal discharge and lacrymation in a calf suffering from bovine infectious rhinotracheitis
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Photo 29. Ruptured blisters in the interdigital space in a cow with foot and mouth disease
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United-Kingdom

Photo 30. Proliferative lesions on the lips of a sheep
with contagious ecthyma
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Table V. Differential diagnosis of bluetongue in cattle (main clinical syndromes)

Signs	BT	FMD	RP	VS
General clinical signs				
Hyperthermia	(+)	++	++	++
Drop in milk yield	+++	+++	++	++
Weight loss	++	+++	++	++
Oedema of the head	(+)	-	-	-
Cutaneous clinical signs				
Lesions (ulcers, crusts) on the muzzle, lips and nose	+++	+	++	+
Lesions (ulcers, crusts) on the teats	++	++	+	++
Conjunctivitis, lacrimation	++	-	++	-
Lesions of dry necrosis of the skin	++	-	-	-
Locomotory clinical signs				
Lameness	++	+++	-	+++
Limb swelling	+	-	-	-
Digestive clinical signs				
Anorexia	(+)	+++	++	+++
Lesions (ulcers) of the buccal mucosa/tongue	++	+++	++	+++
Excess salivation	++	+++	+	+++
Diarrhoea	(+)	+	++	+
Respiratory clinical signs				
Nasal discharge	+	+	+++	+
Dyspnoea	-	(+)	(+)	(+)
Neurological clinical signs				
Dullness	+	++	++	++
Generalised weakness, paresis, paralysis	-	++	+	++
Reproductive clinical signs				
Abortions	+	++	+	+

+++	Clinical signs very frequently present
++	Clinical signs frequently present
+	Clinical signs sometimes present
(+)	Clinical signs rarely present
–	Clinical signs absent
BT	bluetongue
FMD	foot and mouth disease

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	BPS	BVD MD	IBR	MCF	RVF	PS
	(+)	+++	++	+++	++	-
	(+)	++	++	+++	+	++
	(+)	+++	++	+++	+	++
	-	-	-	-	-	-
	-	++	++	+++	-	++
	-	+	-	+	-	++
	-	++	+++	+++	-	-
	-	+	-	++	-	+++
	-	++	-	++	-	-
	-	(+)	-	(+)	-	-
	(+)	+++	++	+++	++	++
	+++	+++	-	+++	-	-
	++	++	++	++	++	-
	-	+++	(+)	++	++	-
	-	++	+++	+++	+	-
	-	+	++	(+)	-	-
	(+)	++	++	+++	++	+
	-	++	+	+	+	+
	-	++	++	++	+++	(+)

RP	rinderpest
VS	vesicular stomatitis
BPS	bovine papular stomatitis
BVD MD	bovine viral diarrhoea – mucosal disease
IBR	infectious bovine rhinotracheitis
MCF	malignant catarrhal fever
RVF	Rift Valley fever
PS	photosensitisation

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Table VI. Differential diagnosis of bluetongue in sheep (main clinical syndromes)

Signs	BT	FMD	RP	VS	
General clinical signs					
Hyperthermia	+	+	++	+	
Drop in milk yield	++	+	+	+	
Weight loss	+++	+	+	+	
Oedema of the head	++	-	-	-	
Cutaneous clinical signs					
Lesions (ulcers, crusts) on the muzzle, lips and nose	+++	-	-	-	
Lesions (ulcers, crusts) on the teats	++	+	-	+	
Conjunctivitis, lacrimation	+	-	-	-	
Lesions of dry necrosis of the skin	-	-	-	-	
Locomotory clinical signs					
Lameness	+++	+++	-	+++	
Limb swelling	+	+	-	+	
Digestive clinical signs					
Anorexia	++	++	++	++	
Lesions (ulcers) of the buccal mucosa/tongue	++	+	-	+	
Excess salivation	++	+	-	+	
Diarrhoea	(+)	-	+	-	
Respiratory clinical signs					
Nasal discharge	+	-	-	-	
Dyspnoea	+	-	-	-	
Neurological clinical signs					
Dullness	++	+	+	+	
Generalised weakness, paresis, paralysis	-	++	-	++	
Reproductive clinical signs					
Abortions	+	++	-	++	

+++	Clinical signs very frequently present
++	Clinical signs frequently present
+	Clinical signs sometimes present
(+)	Clinical signs rarely present
–	Clinical signs absent
BT	bluetongue;
FMD	foot and mouth disease;
RP	rinderpest;

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s)

	CE	P	PPR	SP	PGE+F	PS	FR
	-	+++	++	++	-	-	+
	+	+++	+	++	++	+	++
	+	+++	+	+	+++	+	++
	+	-	-	(+)	++	+	-
	+++	+	+	+++	-	+	-
	+++	-	-	-	-	+	-
	+	+	+	++	-	-	-
	+	-	-	+	-	+	-
	(+)	-	-	-	-	+	+++
	-	-	-	-	-	-	+
	++	+++	++	++	-	+	+
	+	-	++	+	-	(+)	-
	+	+	+	++	-	-	-
	-	(+)	+	-	++	-	-
	-	+++	++	++	-	-	-
	-	+++	+	+	-	-	-
	(+)	++	++	++	+	+	-
	(+)	+	+	+	+	-	++
	-	+	+	+	+	-	-

VS: vesicular stomatitis
CE: contagious ecthyma
P: pneumonia
PPR: peste des petits ruminants
SP: sheep pox
PGE + F: parasitic gastro-enteritis + fluke
PS: photosensitisation
FR: foot rot

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Bluetongue: Laboratory diagnosis 9

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The bluetongue (BT) serogroup includes 24 serotypes, which may be further divided into different topotypes depending on the geographical origin (11). The genome of BT virus (BTV) consists of 10 double-stranded RNA molecules (32), coding for 7 structural proteins and 4 non-structural proteins. VP2 and VP5 are two variable proteins located in the outer capsid of the virion determining the antigenic variability of the virus and the serotype (3, 8, 10, 14, 33). VP7 is the major immunodominant serogroup antigen (12, 15, 24) and is used widely for the identification of the bluetongue serogroup by serological assays (13). VP1 is the viral RNA-dependent RNA polymerase that is found in the subcore of the virion (25). Four non-structural proteins, NS1, NS2, NS3 and NS3A have been identified (20, 26).

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BLUETONGUE VIRUS DETECTION

BTV identification can be carried out by virus isolation, enzyme-linked immunosorbent assay (ELISA) and reverse transcription combined with polymerase chain reaction (RT-PCR). Virus isolation requires a long time before the results are available and it is especially labour-intensive (38). Usually, intravascular injection into embryonated chicken eggs or intracranial injection in sheep or baby mice is required before passage on cell culture (6). Direct isolation on cell culture is less sensitive and it can fail to identify weak positive samples (2, 7).

Detection of the viral genome by RT-PCR is a convenient and rapid method for the identification of BTV (for a review, see Ref. 38). Several RT-PCR protocols detecting segments 2, 3, 6, 7 or 10 have been developed in the last 20 years (1, 2, 17, 19, 34, 35, 37). These tests are acknowledged by the World Organisation for Animal Health (OIE), which recommends one nested PCR amplifying segment 5 of BTV (30). RT-PCR often has a higher sensitivity than virus isolation and it may result in a positive test result in animals several weeks after they have ceased to be infectious (18). The conventional RT-PCR methods require agarose gel electrophoresis, which limits the number of samples that can be tested in a day. Over the past three years, quantitative real-time RT-PCR (RT-qPCR) assays have been developed for the detection of certain vaccine and/or field strains of BTV (5, 16, 21, 22, 23, 36). Since the detection of PCR products is based on fluorescence intensities, it considerably enhances the throughput of the test and it reduces the risk of contamination. The development of a universal assay has long been hampered by the high diversity of BTV. Recently, however, two universal RT-qPCR assays have been described that allow the detection of all BT serotypes (30, 27).

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BIOLOGICAL SAMPLES

Blood samples must be collected with ethylamine diamine tetra-acetic acid (EDTA) as anti-coagulant. The use of heparin as anti-coagulent is not recommended as it may interfere with the RT-PCR. Tissues must be examined immediately or stored at -80°C until they are used for nucleic acid extraction. A storage buffer such as RNAlater™ can be used to avoid degradation of the RNA.

VIRUS ISOLATION ON EMBRYONATED CHICKEN EGGS AND CELL CULTURE

Virus isolation can be undertaken on embryonated chicken eggs (ECE) followed by one to three passages in cell culture as described by Bréard *et al.* (4). Groups of 5 ECE are inoculated intravenously with 100 µl of a tenfold dilution of lysed red blood cells from suspected field samples. The ECE are incubated for 5 days at 35°C and examined daily using a cold candling lamp. Embryos that die between 2 and 5 days post-infection are homogenised individually in 10 volumes of Eagles medium (Invitrogen, Carlsbad, CA, USA). The homogenates are clarified by centrifugation at 10,000 *g* for 10 min at 4°C and inoculated onto baby hamster kidney (BHK)-21 cells (4). If a tenfold dilution series is made of the sample then the final virus titre can be calculated using Karber's method.

The virus can also be isolated on BHK-21, African green monkey kidney (Vero) or insect cells in culture. As virus isolation on cell cultures is often less successful than in ECE it is advisable to start from ECE homogenates. After washing monolayers of cell cultures grown in a flat bottom 24-well plate, a volume of 300 µl of homogenate is added. Cell monolayers are monitored for the appearance of a cytopathogenic effect

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(CPE) for 5 days at 37°C in 5% CO_2 with humidity. If no CPE appears, a second passage is made in cell culture after freezing and thawing.
The virus can be identified using a virus neutralisation test. The BTV to be determined is grown on cell cultures and titrated. Flat-bottomed microtitre plates are used and a volume of 50 µl of a well-defined serotype-specific antiserum at a predetermined dilution, known to be capable of neutralising the virus, is mixed with an equal volume of 100 $CCID_{50}$ (50% cell culture infective dose) of the virus to be identified. After incubation for 1h at 37°C, a volume of 100 µl of 10^4 cells is added to each well. Using a microscope, the wells are checked for the presence of a CPE microscopically after incubation for 3-5 days at 37°C. Wells containing cells only or cells and antiserum should show no CPE. In contrast, wells containing non-neutralised virus should show a CPE.

NUCLEIC ACIDS EXTRACTION, DENATURATION AND DETECTION

Several protocols are possible as outlined above. The following protocol (as described by (30, 31) is given as an example. Blood samples are subjected to the following pre-treatment before RNA extraction: 250 µl of red blood cells are washed with PBS and lysed with 1 ml diethylpyrocarbonate (DEPC) water. After centrifugation for 10 min at 10,000 *g*, the supernatant is discarded and the pellet is resuspended in 250 µl DEPC water. Total RNA is extracted from 250 µl lysed red blood cells or 250 µl supernatant from infected BHK-21 cells with 750 µl Trizol-LS reagent (Gibco-BRL), according to the method recommended by the manufacturer. The precipitated RNA is suspended in 30 µl DEPC water. Prior to RT-PCR, the RNA samples are denaturated by heating for 3 min at 95°C with 10% dimethylsulfoxide (DMSO, Sigma). Methylmercuric hydroxide, which is recommended by the OIE as a denaturing agent, is highly toxic and no longer commercially available in several countries.

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RT-QPCR SPECIFIC FOR SEGMENTS 1 AND 5 OF BLUETONGUE VIRUS

■ Primers and probes

The first quantitative real-time PCR (RT-qPCR_S1) amplifies a 357 base region of BTV segment 1. The second RT-qPCR assay (RT-qPCR_S5) generates an amplicon of 75 bases at the 5' end of segment 5. These regions are chosen because the alignment of sequences available in public databases with the software ClustalW (29) showed that they both contain sufficiently conserved sequences to design PCR primers and TaqMan probes hybridising to strains of the 24 serotypes.

The primers and the probe specific for segment 5 contain some degenerated bases, which ensure a wide recognition of the various bluetongue strains. The 3' end of the probe specific for the segment 1 is conjugated to the minor groove binder (MGB), which increases its hybridisation temperature. The probe specific for segment 5 includes 6 locked nucleic acid (LNA) residues (28), which also increases the hybridisation temperature (Table VII). LNA residues are preferred to MGB in the segment 5-specific probe because they can be incorporated at any location and therefore confer more flexibility when designing the probe.

■ Fluorogenic RT-qPCR specific for segment 1

The RT-qPCR_S1 has been developed and validated as a single-step procedure combining the reverse transcription and quantitative PCR reactions. The reactions are prepared using TaqMan EZ RT-PCR Core Reagents (Applied Biosystems) in a 25µl final volume containing 1x EZ Buffer, 5 mM Mn++, 300 µM of each dNTP, 2.5 units rTth Polymerase, 0.25 unit UNG, 300 nM of each primer (BTV_S1_F_2-23 and BTV_S1_R_343-325, Eurogentec), 200 nM of a TaqMan probe conjugated to FAM at the 5'end and to MGB at the 3' end (BTV_S1_P_25-37, Applied

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Biosystems) and 5 µl of RNA. Real-time RT-qPCR amplification/ detection is carried out on a thermocycler Applied Biosystems 7900 using the following programme: a first cycle of 2 min at 50°C, 30 min at 60°C and 5 min at 95°C is followed by 40 cycles of 20 sec at 94°C and 1min at 60°C.

■ **Fluorogenic RT-qPCR specific for segment 5**

The RT-qPCR_S5 has been developed and validated as a two-steps procedure with separate reverse transcription and quantitative PCR reactions. Reverse transcription is carried out using the TaqMan Reverse Transcription Reagents (Applied Biosystems). Each 10 µl reaction contains 2.5 µM of random hexamers, 5.5 mM $MgCl_2$, 0.5 mM of each dNTP, 0.4 IU RNAse inhibitor, 1.25 IU MultiScribe Reverse Transcriptase and 2 µl of RNA. The mixture is incubated for 10 min at 25°C, 30 min at 48°C and 5 min at 95°C in a GeneAmp 9600 thermocycler (Perkin Elmer). The 20 µl real-time qPCR mixtures consisted of 10.0 µl 2× concentrated TaqMan fast universal PCR master mix (Applied Biosystems), 375 nM (beta-actin) or 500 nM (bluetongue) of each primer, 250 nM of the TaqMan probe conjugated to FAM at the 5' end and to TAMRA at the 3'end and 5 µl cDNA. Cycling conditions were as follows: 1 cycle at 95°C for 20 sec, 45 cycles for 1 sec at 95°C and 20 sec at 60°C and carried out on an Applied Biosystems 7900 thermocycler.

The diagnostic sensitivity and specificity of this test are estimated to be respectively 99.5% (95% CI: 99.0-100.0) and 98.5% (95% CI: 97.1-100.0), based on a Bayesian analysis of field samples from animals with unknown infectious status during the BTV 8 epidemic in Belgium and checked with the cELISA and the RT-qPCR (31).

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CONVENTIONAL RT-PCR SPECIFIC FOR BLUETONGUE SEGMENT 9

This serogroup-specific conventional RT-PCR often used in laboratories, targets segment 9 of BTV (30). The RT-PCR reactions are prepared with the One step RT-PCR Kit (QIAGEN) and they contain 1x Qiagen buffer, 400 µM of each dNTP, 0.6 µM of each primer (S9P: GTTAAAAAATCGCATATG and S9M: CTACGTCAAGAAGGTAC), 1µl of enzyme mix and 2.5 µl of RNA per 25 µl of reaction. The samples are incubated for 30 min at 45°C and 15 min at 94°C before an amplification of 40 cycles comprising 30 sec at 94°C, 30 sec at 54°C, 1 min at 72°C and a final extension of the PCR product for 10 min at 72°C. The PCR products are analysed on 2% agarose gels stained with 1µg/ml ethidium bromide.

FLUOROGENIC RT-QPCR FOR THE DETECTION AND QUANTITATION OF THE BETA-ACTIN mRNA

A quantitative real-time RT-PCR specific for beta-actin mRNA has been designed to measure the level of an endogenous control (30). The primers and the probe sequences are given in Table VII. The two-step RT-qPCR is carried out according to the two-step protocols described for RT-qPCR specific for BTV segment 5, but with 300 nM of each primer (ACT_F_1005-1029 and ACT_R_1135-1114, Eurogentec) and 200 nM of the probe ACT_P_1081-1105 (Eurogentec).

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PREPARATION OF THE SYNTHETIC RNA CONTROLS

Synthetic RNA controls for the BTV segments 1 and 5 qPCR and for the beta-actin qPCR can be prepared by inserting the PCR products, obtained with the primers described in Table1, into a pCRII-TOPO vector by TA-cloning (Invitrogen). The recombinant plasmids are purified with the Pure Yield Plasmid Midiprep System (Promega) and linearised with endonuclease SpeI (Roche). RNA is then synthesised *in vitro* with the Riboprobe System T7 (Promega). The template DNA is digested with RNAse-free DNAse (Promega) and the RNA quantified by spectrophotometry.

Table VII. Primers and probes

PCR target / name	Primer/probe name	Sequence (5' - 3')
BTV segment 1 / RT-qPCR_BTV_S1	*BTV_S1_F_2-23*	TTAAAATGCAATGGTCGCAATC
	BTV_S1_R_343-325	TCCGGATCAAGTTCACTCC
	BTV_S1_P_25-37	FAM-CCGTGCAAGGTGC-MGB
BTV segment 5 / RT-qPCR_BTV_S5	*BTV_S5_F_1-19*	GGCAACYACCAAACATGGA
	BTV_S5_R_76-57	AAAGTYCTCGTGGCATTWGC
	BTV_S5_P_49-27	FAM-CYCCA**C**TG**A**TRTT**G**TA**T**T**T**T**C**TCAA-TAMRA
beta actin / RT-qPCR_ACT	*ACT_F_1005-1029*	CAGCACAATGAAGATCAAGATCATC
	ACT_R_1135-1114	CGGACTCATCGTACTCCTGCTT
	ACT_P_1081-1105	FAM-TCGCTGTCCACCTTCCAGCAGATGT-TAMRA

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BLUETONGUE VIRUS ANTIBODY DETECTION BY ELISA

Anti-BTV antibodies can be detected using home-made or commercial ELISAs. The following protocol is an example of a competitive ELISA: 50 µl of the serum samples and kit controls are diluted in 50 µl of dilution buffer and added to VP7 pre-coated microplates. Besides kit controls delivered by the manufacturer it is advisable to include a two-fold dilution series of an anti-BTV antibody positive reference serum as working standard in each assay in order to monitor the performance of the cELISA in time, as described for foot and mouth disease by Goris and De Clercq (2005) (9). Following 45 min incubation at room temperature, 100 µl of an anti-VP7-peroxidase conjugate is added to bind to the remaining free VP7 epitopes. After 30 min incubation at room temperature, microplates are washed 3 times prior to the addition of 100 µl of substrate solution, 3, 3', 5, 5'-tetramethylbenzidine (TMB). The colour reaction is stopped after 15 min and the absorbance measured spectrophotometrically at 450 nm. Results are expressed as percentage inhibition or percentage negativity (PN) compared respectively to the positive or negative control and transferred to a positive, doubtful or negative result according to the cut-off settings.

The diagnostic sensitivity and specificity of this test are estimated to be respectively 87.8% (95% CI: 85.1-91.1) and 98.2% (95% CI: 96.3-99.6), respectively, based on a Bayesian analysis of field samples from animals with unknown infection status during the BTV 8 epidemic in Belgium and checked with the cELISA and the RT-qPCR (31).

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Conclusion: Lesson learning on bluetongue

10

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Bluetongue (BT) is a notifiable disease of considerable socio-economic concern and of major importance in the international trade of animals and animal products. Before 1998, BT was considered an exotic disease in Europe. Between 1998 and 2005 at least six BT virus strains belonging to five serotypes (BTV-1, BTV-2, BTV-4, BTV-9 and BTV-16) have been continuously present in the Mediterranean Basin. Since August 2006, BTV-8 has caused an unexpected and severe epizootic of BT in northern Europe. The widespread recrudescence and extension of BTV-8 infections in northern Europe during 2007 suggest that the requirements for BTV to become established may now have been fulfilled in this area.

A detailed knowledge of the pathology and biology of the *Culicoides* species that occur in northern Europe is of prime importance in order to understand their behaviour and to improve the control of BT. In terms of vector biology, not enough is yet known about *Culicoides* (e.g. daytime activity, overwintering, reservoir for virus, control).

Bluetongue in northern Europe is a new challenge for veterinarians and the first step is still the clinical examination of suspected cases. There

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are a number of disease conditions with clinical syndromes similar to BT in cattle and sheep. Their differentiation depends not only upon the clinical signs but also on their epidemiological characteristics, including morbidity, mortality, infectivity and seasonality. Given the diversity of the clinical signs and the fact that none of them is pathognomic for BT, laboratory tests have to be performed to confirm the clinical diagnosis.

Preventing the spread of disease through international trade is one of the primary objectives of the World Organisation for Animal Health (OIE). This is accomplished by establishing International Standards on a wide range of animal diseases. In the case of BT, these standards are published in the *Terrestrial Animal Health Code* and the *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*. In addition, the European Union policy on BT has evolved over the last ten years in parallel with the dynamics of the disease on the European continent, the experience gained and the increased scientific knowledge available.

With regard to prophylaxis, the best strategic option to control clinical BT outbreaks in the European enzootic areas of Europe may well be to vaccinate susceptible animals in order to protect against the infection, after careful consideration of the advantages and disadvantages of the available vaccines.

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Bluetongue in ruminants: a standardised clinical report form for the use in different species

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A form for the clinical evaluation of bluetongue was initially established by the Belgian Federal Agency for Safety on the Food Chain based on existing publications:

- *Vademecum Fièvre Catarrhale Ovine (Bluetongue) à l'usage de vétérinaires sanitaires* (bluetongue vademecum for veterinary inspectors) published by CIRAD (http://bluetongue.cirad.fr/Vademecum/IndexVademecum.php);
- OIE technical disease card on bluetongue (http://www.oie.int/eng/maladies/fiches/a_A090.htm) and
- The clinical disease sheets produced by the AVIS Consortium (Institute for Animal Health; Food and Agriculture Organisation of the United Nations; World Organisation for Animal Health; and Telos ALEFF Ltd). (http://www.fao.org/ag/aga/agah/empres/gemp/avis/A090-bt/mod0/0230-clinical-disease.html).

The final version of the form incorporates improvements made during visits made to farms infected with bluetongue virus (serotype 8).

The form is divided into subgroups of clinical signs, such as general

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clinical signs, digestive clinical signs, cutaneous clinical signs, locomotor clinical signs (musculoskeletal), neurological clinical signs, and reproductive and respiratory clinical signs. Other information, such as a description of the animal, and the date of the first appearance of the clinical signs and their duration, are also listed. The final version of the standardised clinical form for an animal examination in different species is shown hereafter.

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BLUETONGUE STANDARDISED CLINICAL REPORT FORM FOR THE USE IN DIFFERENT SPECIES

1. General Information

Identification number of the herd	
Identification number of the animal	
Animal species (C: for cattle; S: for sheep; G: for goats)	
If other species, please specify	
Breed	
Sex (M: male; F: female)	
Date of birth (dd/mm/yy)	
Date of last calving (dd/mm/yy)	
Stage of pregnancy (0 = if not pregnant; if pregnant = specify number of months)	
Date of clinical examination (dd/mm/yy)	
Name of the veterinary practionner	

Mark the correct answer :
YES = present; NO = not present
? = not known ; NA = not applicable

2. General clinical signs

	YES	NO	?	NA
Hyperthermia	❑	❑	❑	❑
Decreased milk production	❑	❑	❑	❑
Wasting, emaciation, weight loss	❑	❑	❑	❑
Tiredness	❑	❑	❑	❑
Oedema of head, ears, submandibular region, or periorbital region	❑	❑	❑	❑
Hypertrophied lymph nodes	❑	❑	❑	❑

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3. Clinical signs: skin and annedages	YES	NO	?	NA
Lesions of the muzzle, and appendages (congestion, ulcers or necrosis)	❑	❑	❑	❑
Conjunctivitis, lacrimation, periocular dermatitis	❑	❑	❑	❑
Photosensitisation-like lesions	❑	❑	❑	❑
Presence of petechias, contusions, ecchymoses	❑	❑	❑	❑
Erythema, inflammation of the skin, crusts	❑	❑	❑	❑
Cyanosis of the skin or limbs	❑	❑	❑	❑
Skin lesion of the udder, teats or vulva	❑	❑	❑	❑
Scrotal skin lesions	❑	❑	❑	❑
Wool loss (sheep)	❑	❑	❑	❑

4. Clinical loco-motor signs (musculoskeletal)				
Prostration or inability to rise	❑	❑	❑	❑
Reluctance to move or limited movement	❑	❑	❑	❑
Lameness, stiffness of forelimbs	❑	❑	❑	❑
Lameness, stiffness of hind limbs	❑	❑	❑	❑
Oedema of coronary bands	❑	❑	❑	❑
Swelling of pastern, fetlock, cannon, carpal or hock joint	❑	❑	❑	❑
Pododermatitis	❑	❑	❑	❑
Contracture of forelimbs	❑	❑	❑	❑
Contracture of hind limbs	❑	❑	❑	❑
Arched back	❑	❑	❑	❑
Amyotrophy	❑	❑	❑	❑
Torticollis or bent neck	❑	❑	❑	❑

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5. Digestive clinical signs

Loss of appetite	❑	❑	❑	❑
Anorexia	❑	❑	❑	❑
Difficulties in picking up food	❑	❑	❑	❑
Regurgitation	❑	❑	❑	❑
Congestion, erythema of the oral mucosa	❑	❑	❑	❑
Ulcerative lesions of the oral mucosa, excoriations	❑	❑	❑	❑
Salivation, drooling, foaming at the mouth	❑	❑	❑	❑
Oedema and/or protrusion of the tongue	❑	❑	❑	❑
Cyanosis of the tongue	❑	❑	❑	❑
Haemorrhagic stools	❑	❑	❑	❑
Diarrhoea	❑	❑	❑	❑

6. Respiratory clinical signs

Ulcerative lesions of the nasal mucosa	❑	❑	❑	❑
Purulent nasal discharge	❑	❑	❑	❑
Mucous, serous, watery nasal discharge	❑	❑	❑	❑
Halitosis or foetid breath	❑	❑	❑	❑
Dyspnoea, mouth breathing, stridor	❑	❑	❑	❑

7. Neurological clinical signs

Apathy, lethargy	❑	❑	❑	❑
Generalised weakness, paresis or paralysis	❑	❑	❑	❑

8. Reproductive clinical signs

Anoestrus	❑	❑	❑	❑
Abortion or premature calving	❑	❑	❑	❑
Stillbirth	❑	❑	❑	❑
Abnormalities of newborn	❑	❑	❑	❑

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9. Duration and course of the disease

Date of the first clinical signs
(dd/mm/yy; if unknown enter '?')

Comments on the course of the disease within the herd/flock

10. Post-mortem (PM)

Has a PM examination been performed? ❑ ❑ ❑ ❑

If 'yes', please attach a copy of the PM record(s) (with the animal's identification mentioned)

11. Concomitant pathologies:

12. Other comments:

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