IX. BLUETONGUE IN NON-DOMESTIC RUMINANTS: EXPERIENCES GAINED IN EAZA ZOOS DURING THE 2007 & 2008 BTV8 AND BTV1 EPIZOOTICS.

Stephanie Sanderson
Chester Zoo

Introduction

Until the introduction of Bluetongue virus serotype 8 (BTV8) in 2006, Bluetongue had not been a significant disease problem in Europe. The ensuing epizootic and subsequent incursion of BTV1 have caused widespread mortality and morbidity in livestock and been a major focus for international disease control in the EU. This chapter provides information pertaining to these European epizootics – focusing primarily on the clinical species susceptibilities of non-domestic ungulates and their response to vaccination. The data presented has been derived from the literature and two EAZWV endorsed Bluetongue web surveys of EAZA zoos covering the 2007 and 2008 disease seasons.

General disease information on Bluetongue can be found in disease fact sheet 7.

BTV8 in Europe: an atypical virus strain

Bluetongue is an insect borne disease caused by an orbivirus and affecting mainly domestic sheep breeds and occasionally cattle. It is transmitted by midges of a few select *Culicoides* species and its global distribution is largely defined by suitable climatological factors for these species. Bluetongue viruses have been found on all continents excepting Antarctica but the disease is generally only endemic in the tropics and subtropics (34° S to 53°N OIE terrestrial animal health code). (Hately 2009, MacLachlan 2009).

Until 2006, BTV was not considered to be a significant threat to Central and Northern European livestock as the resident Palaearctic midge species were not competent vectors. However the unexpected occurrence of an atypical BTV virus in Maastricht in 2006 has challenged established thinking on the behaviour of this disease.

Key differences from other BTV serotypes and strains include:

- Ability to use Palaearctic midges as vectors (*C.obsoletus* and *C.pulicaris*)
- Significant morbidity and mortality in cattle as well as sheep
- Fairly frequent vertical transmission in pregnant ruminants – a rare event in other BTV strains.

Other features of BTV epidemiology in temperate climates include a marked seasonality with clinical cases occurring almost exclusively between July and December and with recrudescence occurring the following summer. It is still unclear how the virus is maintained from year to year.
The source of this BTV8 strain is still unknown. Phylogenetic studies indicate the likely origin to be sub-Saharan Africa and it has been postulated that it could have been introduced either by an infected animal originating from Africa, imported infected vectors or as an illegally imported vaccine strain. (EFSA 2007)

Other BTV subtypes in Europe

A number of BT serotypes have been present in Southern Europe from 1900's onwards. The spread of different BT serotypes since 1998 is shown in figure 1. The distribution of serotypes in May 09 is shown in figure 2. BTV1 has been seen on numerous occasions in Southern Europe however it was not until 2008 that it spread northwards and is also now capable of spread via Palaearctic midges. Cases of BTV6 and BTV11 were also reported in the Netherlands and Belgium in 2008. These are postulated to have been introduced via illegal imports of modified live vaccine containing these serotypes from Southern Africa. At the time of writing it appears that these serotypes have not become established unlike BTV8 and BTV1. Increased surveillance has also uncovered a previously unknown orbivirus in Toggenberg goats which may indeed be a new serotype of BTV. Its clinical significance is currently unknown. (Chaignat et al. 2009)

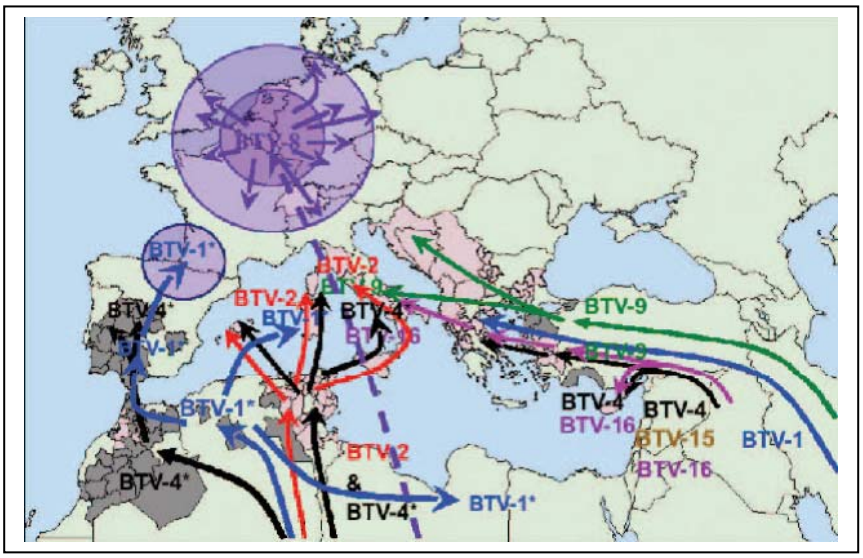
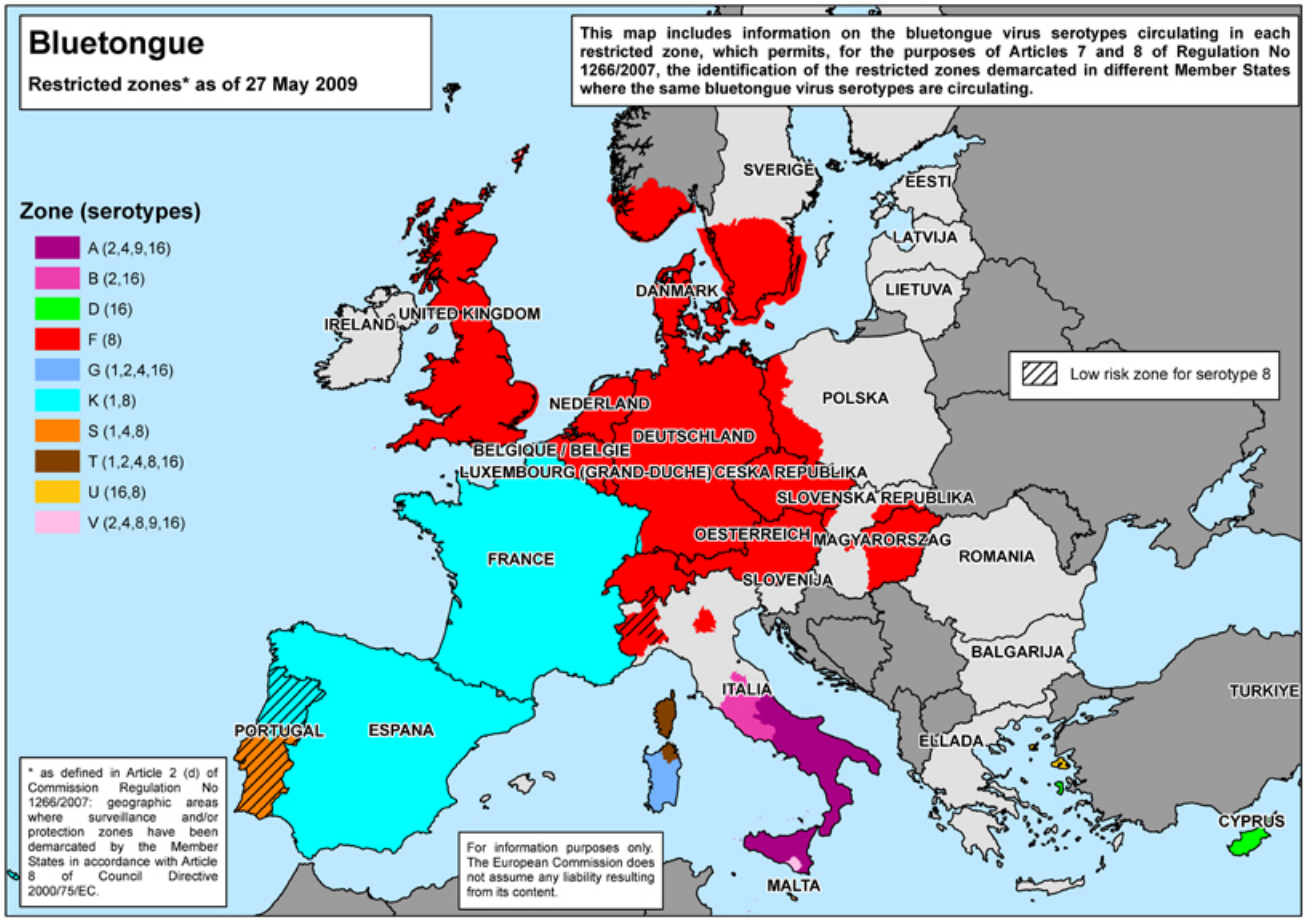


Fig 1. Spread of Bluetongue throughout Europe since 1998. Picture, Institute of Animal Health, Pirbright

Fig 2.



Species susceptibility to clinical infection

It is generally accepted that all ruminant species and some camelids are likely to be capable of supporting BTV infection. Clinical expression of the disease is however highly variable between viral serotypes, species, breeds and indeed environmental conditions and individual health status. Animals indigenous to endemic areas appear to be clinically resistant. (Verwoerd & Erasmus, 2004).

The pathology and pathogenesis of the disease is reviewed in detail by MacLachlan et al. and the clinical picture seen in domestic species is well documented. (MacLachlan et al. 2009). The classical clinical signs of fever, nasal discharge, dypsnea, cyanosis of the tongue, oral lesions and ulcers, oedema of the head and neck, lameness and hyperaemia of the coronary band are due to virus mediated vascular injury. These signs are most frequently seen in sheep where mortality rates can be up to 30% or higher. Cattle rarely show clinical disease (with the exception of the European BTV8 strain).(MacLachlan et al. 2009)

Little is published about clinical susceptibility of non-domestic species. In North America, white-tailed deer *(Odocoileus virginianus),* prong-horn antelope *(Antilocapra americana)* and desert bighorn sheep *(Ovis canadensis)* are known to develop severe disease similar to that described in domestic sheep. (Verwoerd & Erasmus, 2004). Abortion and death has also been seen in dogs injected with BT contaminated vaccine and in a European lynx fed infected meat (EFSA 2007; Jauniaux et al. 2008, MacLachlan 2009). Natural, asymptomatic infection of African carnivores has also been reported. With the incursion of BTV8 into Europe, European Zoos were in a unique position to contribute to knowledge on species susceptibility to disease as they hold a naïve individuals representing a wide taxonomic and geographic spectrum.

A survey of all 313 EAZA zoos was undertaken in January 2008 to collate data on clinical disease seen during the 2007 BTV season. 49 zoos had confirmed BTV8 cases within 20km and could be classified as at risk of infection. These 49 zoos held over 1000 susceptible individuals of 53 different species and 7 ruminant families indigenous to Europe, North and South American, Africa and Asia. Clinical disease was seen in 62 individuals (6% of the at risk population), spread between 13 zoos (27% of at risk collections). (Sanderson et al. 2008).

Mortality and morbidity rates and the clinical picture seen in each affected species is summarised inTable 1 (Sanderson et al. 2008). Bovidae are the most susceptible family of ruminants to clinical disease, with four species showing morbidity rates of greater than 20% and mortality rates of greater than 10%. The average case fatality rate for the affected Bovidae species was 69%. All the affected ruminant species in this study were indigenous to Europe, Asia or South America. Clinical signs in these species and are consistent with those recorded for BTV8 infection domestic livestock. (Elbers et al., 2007) and with those reported in yak (Mauroy et al. 2008;). It is noteworthy that despite over 200 African ruminants of 20 species being held by zoos in at risk areas, none of these were reported to have shown clinical signs of infection. This is consistent with observations in Africa that indigenous antelope do not develop clinical disease (Verwoerd & Erasmus, 2004).

Data was also gathered by European Zoos on species susceptibility to BTV1. There is yet insufficient data on BTV1 to draw any firm conclusions however experiences so far suggest a similar clinical picture and species susceptibility to BTV8.

Table 1: Morbidity, mortality, case fatality and clinical signs reported in ruminant species by zoos situated within 20km of confirmed BTV8 outbreaks during the period August 2006 - December 2007 (Sanderson et. al. 2008)

AFFECTED SPECIES	At risk individuals (BTV8 within 20km)	Clinically affected	Morbidity Rate*	Laboratory confirmation	Deaths	Mortality Rate**	Case Fatality Rate***	Clinical Signs Reported in Affected Animals.
	Abs. No.	Abs. No.	%	Abs. No.	Abs. No.	%	(%sick that died)	
AFFECTED BOVIDAE	**519**	**55**	**10.60**	**25**	**38**	**7.32**	**69.09**	
American bison *(Bison bison)*	30	10	33.33	3	5	16.67	50.00	Lethargy, fever, mouth ulcers, drooling, difficulty eating, conjunctivitis, corneal oedema, lameness, inflammation coronary band, sudden death.
European wisent *(Bison bonasus)*	20	8	40.00	4	4	20.00	50.00	Lethargy, fever, mouth ulcers, drooling, difficulty eating, conjunctivitis, corneal oedema, respiratory difficulty, lameness, inflammation coronary band, sudden death.
Yak *(Bos grunniens)*	35	6	17.14	6	6	17.14	100.00	Nasal discharge, conjunctivitis, corneal oedema, drooling, difficulty eating, respiratory difficulty, lameness, inflammation of coronary band, sudden death.
Blackbuck *(Antilope cervicapra)*	17	2	11.76	2	2	11.76	100.00	Sudden death
Sheep/mouflon *(Ovis aries)*	101	6	5.94	5	2	1.98	33.33	Lethargy, fever, swelling head and neck, mouth ulcers, drooling, difficulty eating, conjunctivitis, respiratory difficulty, lameness, inflammation coronary band, sudden death.
Goat *(Capra hircus)*	208	2	0.96	2	2	0.96	100.00	Lameness
Alpine ibex/Tur *(Capra ibex)*	34	2	5.88	2	2	5.88	100.00	Nasal discharge, sudden death.
Siberian ibex *(Capra sibirica)*	4	1	25.00	0	0	0.00	0.00	Swelling of head and neck.
Muskox *(Ovibos moschatus)*	5	1	20.00	1	0	0.00	0.00	Lethargy, fever, conjunctivitis, abortion.
AFFECTED CERVIDAE	**83**	**5**	**6.02**	**5**	**2**	**2.41**	**40.00**	
Fallow deer *(Dama dama)*	43	2	4.65	2	2	4.65	100.00	Mouth ulcers, difficulty eating, drooling, lameness, sudden death
AFFECTED CAMELIDAE	**40**	**2**	**5.00**	**2**	**2**	**5.00**	**100.00**	
Bactrian camel *(Camelus bactrianus)*	8	1	12.50	1	1	12.50	100.00	Sudden death
Alpaca *(Lama pacos)*	32	1	3.13	1	1	3.13	100.00	Sudden death

*Morbidity rate= number clinically affected / number at risk; **Mortality rate = number that die/number at risk; ***Case fatality = number that die / number clinically affected. Note: Morbidity and mortality rates for the 2006 BTV8 epidemic in domestic livestock were 20% and 5% for domestic sheep and 7% and 3% in domestic cattle (Elbers et al., 2006)

Control Strategies for BTV: legislative framework

Bluetongue is a disease of global importance. Its ability to cause death and debilitating disease across international borders has led to its inclusion in the World Organization for Animal Health (Office International des Epizooties) Terrestrial Animal Health Code which in turn has implications for trade. In EU Member States, control and eradication provisions are laid out in **Council Directive 2000/75/EC**. Measures include vector control, restriction to movements of live ruminants from affected areas to non-infected regions where the vector is present and the use of vaccines. Of these, vaccination is the mainstay of control in areas where BTV has become established.

The OIE guidelines on movement controls, diagnostic methods and vaccine production can be found at: **http://oiebtnet.izs.it/btlabnet/**.

Further information on EU control measures are laid out in **http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_en.htm**

In addition, individual European Countries have their own legislation and detailed disease control plans (e.g. **http://www.defra.gov.uk/animalh/diseases/notifiable/bluetongue/about/index.htm**).

Vaccination

Mass vaccination has been identified by the European Commission as the most efficient veterinary measure in combating bluetongue. Mass emergency vaccination campaigns can be used to achieve the following objectives (European Commission, 2008/655/EC, Savini et al. 2007):

1. prevention of clinical disease
2. limiting regional spread of BT
3. allowing regional/country eradication
4. safe movement of animals between affected and free zones.

A variety of vaccines have been developed of three different types: modified live vaccines (MLV), inactivated vaccines (either whole killed virus preparations or virus like particle produced from recombinant bacluovirus) and recombinant vaccinia, capripoxvirus or canarypox virus vectored vaccines. (EFSA 2007). Only vaccine types currently approved under EC approved national disease programmes (MLV and killed whole virus preparations) will be discussed further here.

MLV have been used for over 40 years in endemic bluetongue areas (Verwoerd D. W. and Erasmus, B. J. 2004).They are quick to produce (8-10wks), highly immunogenic and can confer long lasting protection after a single dose. Using live virus has significant disadvantages as there is potential for under attenuation causing symptomatic disease, milk drop and foetal pathology, and for infection of the vector population leading to local spread and potential for reassortment with field strains leading to new serotypes. For these reasons, inactivated vaccines are preferred even though they take longer (6-8mths) to develop, are more costly and require regular boosters in order to maintain efficacy. (EFSA 2007; OIE 2008)

There is little or no cross protection between different serotypes of Bluetongue, hence vaccines are produced specifically in response to circulating BTV serotypes and strains. All of the vaccines currently available in the EU for the control of BTV1 and BTV8 are inactivated vaccines using saponin and aluminium hydroxide as adjuvants. (Table 2)

Table 2: BTV 1 & 8 Vaccines licensed for use in 2008.

Manufacturer	BTV8 Vaccine Trade Names	BTV1 Vaccine Trade Names
Intervet	Bovilis BTV8	
CZ Veterinaria	Bluevac 8	Bluevac 1
Fort Dodge	Zulvac8 Bovis Zulvac8 Ovis	Zulvac1 Bovis Zulvac1 Ovis
Merial	BTVPUR AlSap 8	
Virbac		SYVAZUL 1

Safety

Trial and field experience has found these vaccines to be safe in domestic species (EMEA 2009, Gethman et al. 2009, Eschbaumer et al 2009). An overview of field experience following administration of over 60million doses of BTV8 vaccine in 12 countries was undertaken by the European Medicines Agency (EMEA 2009). Mass vaccination campaigns often necessitate deviations from normal procedure. Large groups of animals are brought together, less attention is paid to their individual health status, needle hygiene is less good and government instructions may deviate from those of the manufacturers (eg minimum age of vaccination, target species, duration of immunity). In addition, compensation schemes in some countries may lead to over reporting of certain adverse reactions. Despite these factors, adverse reactions were seen in less that 1 in 10,000 animal. Those recorded are typical for other inactivated vaccines and include local reactions and non-severe general reactions such as pyrexia (fever) and lethargy.

A survey of all 313 EAZA zoos was undertaken in February 2009 to collate data on vaccination in non-domestic species. Over 2000 individuals of 57 species in 47 institutions in 9 European countries were vaccinated for BTV8 using 5 of the products on the market during 2008. Adverse reactions occurred at a rate of 0.5% with half of these being local reactions and 40% being abortions. The slightly higher rate could well be due to the relatively small sample size and also because the species studied are not used to handling and are likely to have been more stressed than their domesticated counterparts. Nonetheless, the abortion rate is still well below that considered acceptable for vaccines. (EAMEA 2009)

Efficacy

Vaccine efficacy can be assed both by response to virus challenge (both clinical and levels of viraemia) and serological response induced by immunisation. (Savini et al. 2008). Whilst experimental virus challenge under laboratory conditions provides the most accurate measures of efficacy, are the mainstay of vaccine testing and are required for vaccine licensing (European Parliament 2001), field experiences are also provide a useful data. The licensed vaccines have been shown to be efficacious in domestic animals (Eschbauer et al. 2009, Gethmann et al. 2009) The 2008 European Zoo Survey (Sanderson et al. 2009) found that of the 37 bovidae (cattle, sheep, goat and antelope sp) and girrafidae tested post vaccination, 100% seroconverted post vaccination as did 87% of the 40 South American camelids tested. Of the 9 cervidae (deer sp) represented only 50% seroconverted. No vaccinated animals succumbed to clinical disease post vaccination, despite virus circulating in the area. These data suggest that the inactivated BTV8 vaccines are efficacious in bovidae, girrafidae and to a lesser extent camelids. The sample size in the cervidae is too small to draw any firm conclusions and further work needs doing to evaluate efficacy in these species.

Conclusions

BTV poses a significant risk of mortality and morbidity in naïve non-domestic ruminants. The clinical picture is similar to that seen in domestic livestock with species indigenous to temperate areas of Europe, Asia and the Americas being most severely affected. Species indigenous to Africa, the putative source of BTV8, were clinically unaffected. This suggests that there is a genetic resistance to particular BTV serotypes.

Inactivated BTV8 and BTV1 vaccines have been used in many European zoos both on a voluntary basis as part of national control measures. Adverse reactions were rare and in line with those seen in the domestic species for which they are licensed. Vaccination produced a reliable immune response and no animals showed clinical evidence of infection post immunisation despite the presence of circulating virus in the region. These vaccines would appear to be safe in non-domestic ruminants and efficacious in the bovidae and camelidae. Further work is required to evaluate their efficacy in cervidae.

Further work is underway within the zoo community to expand our knowledge on vaccine efficacy and duration of immunity in non-domestic species. Data is also being collected on species susceptibilities to other BTV serotypes as they appear.

References

Chaignat, V., Worwa, G., Scherrer, N., Hilbe, M., Ehrensperger, F., Batten, C., Cortyen, M., Hofmann, M., Thuer, B., (2009). Toggenburg Orbivirus, a new bluetongue virus: Initial detection, first observations in field and experimental infection of goats and sheep. Veterinary Microbiology 138, 11-19.

Elbers, A.R.W , K. Mintiens , C. Staubach , G. Gerbier , E. Meroc , H.M. Ekker , F.J. Conraths , A.N. van der Spek , A. Backx (2007) Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe; Nature and severity of disease in sheep and cattle. In EFSA scientific report: Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe

http://www.efsa.europa.eu/EFSA/1178620925100/efsa_locale-1178620753812_Bluetongue.htm

EMEA CVMP.(2009)An overview of field safety data from the EU for bluetongue virus vaccines serotype 8 emerging from the 2008 national vaccination campaigns. London: EMEA/CVMP/652019/2008.

Eschbaumer, M., Hoffmann, B., Konig, P., Teifke, J.P., Gethmann, J.M., Conraths, F.J., Probst, C., Mettenleiter, T.C., Beer, M., (2009). Efficacy of three inactivated vaccines against bluetongue virus serotype 8 in sheep. Vaccine 27, 4169-4175.

European Commission, 2008/655/EC: Commission Decision of 24 July 2008 approving the emergency vaccination plans against bluetongue of certain Member States and fixing the level of the Community's financial contribution for 2007 and 2008, *Off J Eur Union* **L214/66** (August).

The European Parliament and the Council of the European Union (2001). Directive 2001/82/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to veterinary medicinal products. Off J Eur Commun;L311/1(November).

European Food Standards Agency (EFSA) (2007) Report of the Scientific Panel on Animal Health an Welfare (AHAW) on the EFSA Selfmandate on bluetongue origin and occurrence. EFSA Journal 479,1-29; 480, 1-20

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

Hateley, G., 2009. Bluetongue in northern Europe: the story so far. In Practice 31, 202-+.

Jauniaux, T.P., De Clercq, K.E., Cassart, D.E., Kennedy, S., Vandenbussche, F.E., Vandemeulebroucke, E.L., Vanbinst, T.M., Verheyden, B.I., Goris, N.E., Coignoul, F.L., (2008). Bluetongue in Eurasian lynx. Emerging Infectious Diseases 14, 1496-1498.

Mauroy, A., Guyot, H., De Clercq, K., Cassart, D., Thiry, E., Saegerman, C., (2008). Bluetongue in captive yaks. Emerging Infectious Diseases 14, 675-676.

Maclachlan, N.J., Drew, C.P., Darpel, K.E., Worwa, G., (2009). The Pathology and Pathogenesis of Bluetongue. Journal of Comparative Pathology 141, 1-16.

Office International des Epizooties (OIE). Bluetongue. In: Manual of diagnostic tests and vaccines for terrestrial animals. Paris: OIE; 2008.

Sanderson, S., Garn, K., Kaandorp, J. (2008) Species Susceptibility to Bluetongue in European Zoos during the Bluetongue Virus Subtype 8 (BTV 8) Epizootic Aug 2006-Dec 2007. Proc. European Association of Zoo and Wildlife Veterinarians.

Sanderson, S., Edwards, S.J., Setzkorn, C and Baylis M. (2009) Survey of Species Susceptibility to Bleutongue virus and Bluetongue Vaccine Usage in European Zoos During 2008. Proc. International Conference on Diseases of Zoo and Wild Animals. 1. 2.

Savini, G., MacLaclalan, N.J., Sanchez-Vinaino, J.M., Zientara, S., (2008). Vaccines against bluetongue in Europe. Comparative Immunology Microbiology and Infectious Diseases 31, 101-120.

Verwoerd D. W. and Erasmus, B. J. (2004) in: Infectious Diseases of Livestock. Eds Coetzer J.A.W & Tustin R.C. Oxford University Press pp 1201-1220