Epidemiology of a Bluetongue outbreak in a sheep flock in Brazil

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Summary
In January 2013, an outbreak of Bluetongue (BT) affecting a Lacaune sheep flock occurred in Vassouras, Rio de Janeiro state, Brazil. From March to August 2013, blood samples collection and clinical examination were performed monthly, in order to monitor the epidemiological profile of Bluetongue virus (BTV) circulation and clinical disease in the flock. Agar gel immunodiffusion (AGID) and reverse transcription polymerase chain reaction (RT-PCR) targeting BTV segment 10 were used as diagnostic assays. Additionally, insect trapping was conducted in the farm from May to July 2013. The flock serological prevalence to BTV was 80% since the first month of monitoring, with a variation in the serological rate depending on the sheep age categories. The number of susceptible lambs increased with time, probably due to the decrease of passive immunity. Viral RNA was detected in blood samples, demonstrating viral circulation, prolonged viraemia, and potential source for virus transmission in the region, even in a dry and cool season. The presence of *Culicoides pusillus* and *Culicoides insignis* was confirmed in the farm. The emergence of this outbreak in a Brazilian endemic area for BTV emphasises an urgent need of animal surveillance and BTV epidemiological studies.

Analisi epidemiologica di un focolaio di Bluetongue in un gregge di pecore in Brasile

Parole chiave
Bluetongue,
Brasile,
Epidemia,
Epidemiologia,
Ovino,
Segno clinico,
Sierologia,
Sorveglianza.

Riassunto
Nel mese di gennaio 2013 un'epidemia di Bluetongue (BT) ha colpito un gregge di pecore di razza Lacaune nel comune di Vassouras, nello stato di Rio de Janeiro in Brasile. Nel periodo marzo-agosto 2013 sono stati raccolti campioni ematici ed effettuati esami clinici per monitorare il profilo epidemiologico della circolazione del virus della Bluetongue (BTV) e le conseguenze cliniche sul gregge. Per le diagnosi sono stati utilizzati l'immuno-diffusione in gel di agar (AGID) e la reazione a catena della polimerasi trascrittasi inversa (RT-PCR) che amplifica una regione del segmento 10 del genoma del BTV. Nell'azienda agricola sono state collocate trappole per insetti nel periodo maggio-luglio 2013. La prevalenza sierologica di BTV nel gregge è risultata pari a circa l'80% già dal primo mese di monitoraggio con una discreta variabilità relativa alle diverse fasce di età degli animali. Nel corso dello studio è stato riscontrato un aumento di agnelli sensibili all'infezione, probabilmente, dovuto alla diminuzione dell'immunità passiva. La presenza di RNA virale nei campioni di sangue ha permesso di valutare la durata della viremia e di dimostrare la circolazione del virus nella regione anche nella stagione secca e fredda. Nell'azienda è stata inoltre confermata la presenza di *Culicoides pusillus* e *Culicoides insignis*. L'emergere di questa epidemia in Brasile, in una zona endemica per BTV, accentua l'urgente necessità di avere piani di sorveglianza negli animali per approfondire le conoscenze epidemiologiche su BTV.

Introduction

Bluetongue (BT) is a viral vector-borne disease that affects domestic and wild ruminants, resulting in economic losses and obstacles to animal trade. Due to its economic impact, the World Organisation for Animal Health (OIE) lists BT as a terrestrial notifiable animal disease (OIE 2014). Its aetiological agent is Bluetongue virus (BTV), a non-enveloped, double-stranded segmented RNA virus, the prototype of the genus *Orbivirus*, family *Reoviridae*. So far, 27 BTV serotypes have been characterized (Maan *et al.* 2012, Zientara *et al.* 2014). The last characterised serotype (BTV-27) was identified in goats without clinical signs, in the Corsica Island, located in the Mediterranean Sea (Zientara *et al.* 2014). The virus is transmitted among ruminants by certain species of biting midges from the genus *Culicoides* (Diptera: Ceratopogonidae), which act as biological vector (Mellor *et al.* 2000). Transplacental and horizontal BTV transmission routes may occur during infections with certain serotypes. However, it seems that these transmission routes have a less epidemiological importance in virus spread (van der Sluijs *et al.* 2013, Batten *et al.* 2014).

Clinical disease is more frequently observed in sheep and varies in severity, which could be influenced by the serotype or strain of the infecting virus, ambient conditions, husbandry factors, as well as by the animal breed (Verwoerd and Erasmus 2004). Although BTV infection in cattle and goats is typically asymptomatic or subclinical, these species play an important role in BT epidemiology. These species may act as virus reservoir hosts, mainly cattle, due to the prolonged viraemia, which may last for more than 2 months (MacLachlan 2004).

The BTV distribution in the world coincides with the presence of the competent insect vectors, which occurs approximately between latitudes 50°N and 35°S, including tropical, subtropical, and temperate regions of the globe (Coetzee *et al.* 2012). The disease is endemic in most parts of South America, Central America, and the Caribbean, probably due to the existence of appropriate climate conditions for vector maintenance. In South America, serotypes 1, 2, 4, 6, 10, 12, 13, 17, and 24 have been detected whilst clinical disease is a rare event (Clavijo *et al.* 2002, Legisa *et al.* 2013, Balaro *et al.* 2014, Viarouge *et al.* 2014). Outbreaks of BT in South America were only notified in Brazil, involving serotypes 4 or 12 (Clavijo *et al.* 2002, Antoniassi *et al.* 2010, Balaro *et al.* 2014).

In late January 2013, a BT outbreak affecting a dairy sheep flock in Rio de Janeiro, Brazil, caused by BTV-4, was reported. Apathy, anorexia, oral mucosal lesions, face oedema, lameness, abortion, and deaths were the clinical signs suggestive of the disease (Balaro *et al.* 2014). Considering that most part of Brazil is a BT endemic area and the rare opportunity to study the dynamics of a BT outbreak in the country, the affected flock was monitored for 6 months, from March to August 2013. The occurrence of clinical signs, serology, and BTV nucleic acid detection were studied in order to understand the infection progress and virus circulation. Insect trapping was also performed to verify the presence of the BTV vector.

Materials and methods

Flock and sampling

The flock belonged to a dairy sheep farm, located in Vassouras, Rio de Janeiro State – latitude of 22º 24' 14'' South and longitude of 43° 39' 46'' West. At that time, it was composed of 82 ewes, 6 rams, and 19 lambs, totalling 107 animals of the Lacaune breed. The animals were raised under 2 husbandry managements. The first one, characterized by an intensive system (stabled sheep), had 6 rams, 6 ewes, and 19 weaned lambs. In the second one, the animals were kept on pasture (grazing sheep), with 76 ewes. The farm had a sheep health control program with flock trimming every 6 months and vaccination against rabies and clostridiosis. Parasite control was done by egg count per gram of faeces (EPG) technique every 2 months, followed by deworming, if necessary.

The BT outbreak in the farm was first noticed in late January 2013 (Balaro *et al.* 2014), which coincided with the lambing period, from January to March. Two months after the beginning of the outbreak, from March until August, the flock was monitored. The animals were examined for BT clinical signs and blood samples were collected monthly into vacuum tubes in the presence and absence of ethylenediamine tetraacetic acid (EDTA). The serum was separated by centrifugation (2600 x g/10 min) and stored at -20ºC.

Clinical data and treatments

In the same period, from March to August, the sheep flock was also examined at least twice a month. Symptomatic pulmonary and gastrointestinal disorders, observed during the study, were treated with oxytetracycline (10 mg/kg) administered intramuscularly every 48 hours for 3 times. For oral mucosa lesions, a topical solution of hydrogen peroxide 3% plus a chlorhexidine ointment 1% was used twice a day until the lesions healed.

Serology

Serum samples were tested for antibodies against BTV using the agar gel immunodiffusion (AGID)

method (OIE 2014). The antigen was produced in the Laboratorio de Pesquisa em Virologia Animal, Escola de Veterinaria (UFMG), following the protocol provided by Costa and colleagues (Costa *et al.* 2006).

Bluetongue RNA detection

Total RNA was extracted from 500µL of EDTA-blood using the phenol-chloroform method (Sambrook and Russell 2001). The RNA was reverse-transcribed with Superscript III Reverse Transcriptase (Invitrogen™, Carlsbad, California, USA) using random hexamer primers, and BTV nucleic acid was detected by a semi-nested PCR targeting segment 10, as described by Favero (Favero 2009). The PCR products were analysed by 0.9% agarose gel electrophoresis (AGE) with ethidium bromide and visualised under UV light.

Insect collection

Insects were collected using CDC incandescent light traps, set from May to July 2013, between 6pm and 6am. The traps were set in the stable and in the pasture, close to the farm's dam, where the animals had access to water supply. Insects were stored in 70% ethanol and sent to the Laboratory of Diptera of the Oswaldo Cruz Foundation in Rio de Janeiro, Rio de Janeiro, Brazil, for morphological identification.

Results

Along the monitored period, which occurred 2 months after the beginning of the outbreak, specific and non-specific BTV clinical signs were observed. The most evident clinical signs were sloughing or break of the hooves, formation of a white line in the hoof wall, wool break, nasal discharge, and discrete face hyperaemia and oedema (Figure 1). Gastrointestinal and pulmonary disorders were observed during all the studied period. Whereas the BTV outbreak had occurred from late January until mid-March, late hoof lesions, characterized by the formation of lines in the hoof wall, or even sloughing and break of hooves, could be observed in some animals until August. Almost all the stabled and grazing sheep presented oral mucosal lesions, which decreased in the following months. Although the oral mucosal lesions had healed after topical treatment, they reappeared sometime later. Scrotum hyperaemia and sagging, accompanied by testicles dislocation, were observed in 1 of the rams. During the monitored period, 2 lambs died after recurrent pneumonia episodes. In general, the weaned lambs presented weight gain rate lower than expected, mouth lesions, and some recurrent episodes of diarrhoea and pneumonia.

Table I and Figure 2 show the serological data of the flock, obtained by AGID. Two months after the first BT clinical evidence, around 80% (86/107) of the flock were BTV seropositive. At a first analysis, 84% (69/82) of the ewes were seropositive and this rate increased to 91% (75/82) until the end of the study. Two out of the 6 rams were seropositive at the beginning of the monitoring, and only 1 of them seroconverted a month later. Two months after the parturition period, 79% (15/19) of lambs were still seropositive for BTV. However, this percentage drooped to 29% (5/17) in July (Figure 2A).

All 3-5 years old ewes (19/19) were already seropositive for BTV since the beginning of the monitoring. Of the 2-year old ewes, 92% (36/39) were also seropositive since March. Considering the 1-year old ewes, the BTV seropositive rate increased from 58% (14/24) in March to 75% (18/24) 4 months later. The rate of BTV seropositive female lambs (7/7) decreased along the monitoring period, until all of them became seronegative. However, one female lamb seroconverted 2 months later (Figure 2B).

The Seg-10 RT-PCR for BTV was performed on 45 blood samples collected in March (Table I). Of these samples, those of 9 ewes (9/30) and 2 lambs (2/15) were positive (Table II). Along the period of

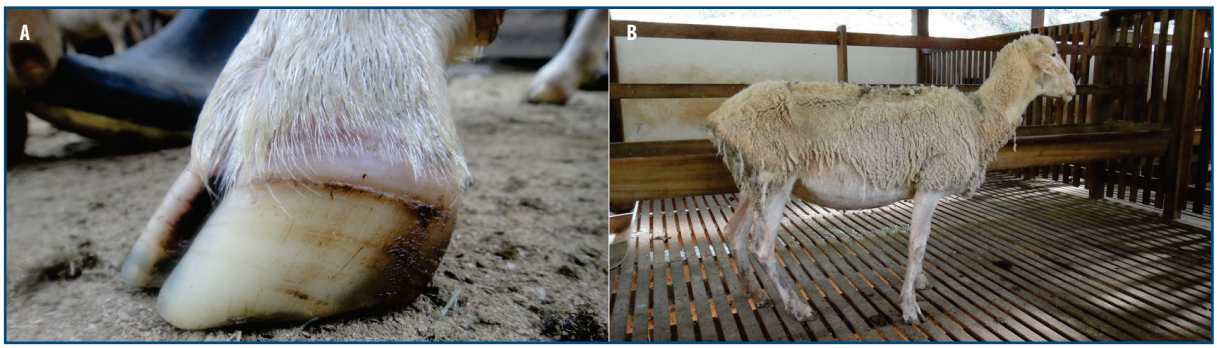

Figure 1. *Clinical signs observed in sheep, in the monitored flock.* **A.** Late sloughing of hooves and formation of a white line in the hoof wall. **B.** Sick ewe with wool break.

Table I. *Bluetongue virus Seg-10 RT-PCR and serological (AGID) results from the sheep blood samples collected from March until August 2013.*

	RT-PCR (Positive)		AGID (Reagent)	
Month	**N**	**%**	**N**	**%**
March	11/45	24.4%	86/107	80.4%
April	5/52	9.6%	89/106	84.0%
May	11/32	34.4%	88/107	82.2%
June	9/30	30.0%	83/105	79.0%
August	4/14	28.6%	83/105	79.0%

analysis, viral RNA was detected monthly in the flock and for more than 60 days in blood samples from the same animal (Tables I and II).

In all the insect traps, from May until July, biting midges from the genus *Culicoides* were collected. A total of 41 specimens of *Culicoides* belonging to 6 species were identified. *Culicoides pusillus* was the most abundant species (29 females, 1 male). Two females of *Culicoides insignis* were also identified in the studied area.

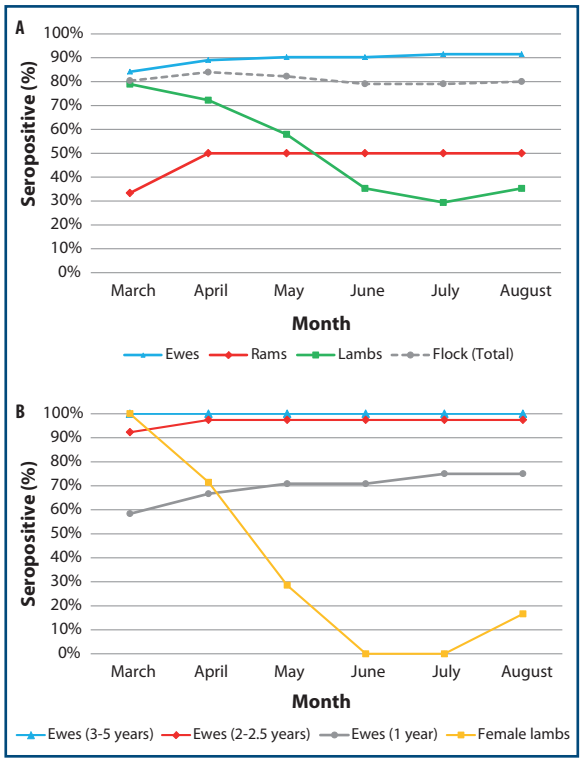

Figure 2. *Serological results.* Percentage of seropositive sheep by month. **A.** Serological data of the total flock and subdivided by categories: ewes, lambs, rams. **B.** Ewes and female lambs serological results. The ewes were separated by age.

Discussion

Bluetongue, as an infectious vector-borne disease, is distributed worldwide in the same area of certain species of biting midges from the genus *Culicoides*. Most part of Brazil is situated in the tropical zone, which has favourable climatic and biome conditions for presence and maintenance of many *Culicoides* species.

Bluetongue virus was isolated from *C. pusillus* and *C. insignis* in Central America and in the Caribbean (Mo *et al.* 1994). In Brazil, *C. insignis* and/or *C. pusillus* are present in all regions, at least in 16 of the 26 Brazilian states, including the state of Rio de Janeiro (M.L. Felippe-Bauer, personal communication). In this study, both *C. pusillus* and *C. insignis* were identified in traps collections, indicating that these species may be involved in BTV transmission in the farm. The identification of these *Culicoides* species in the affected area draws attention and highlights the need for further studies to confirm the importance of these midges in BTV transmission in the region.

Along the study, 2 ewes showed typical BT clinical signs, such as facial oedema, lesions in the oral mucosa, and lameness, observed in early April. Two more recurrent cases occurred in late May. Other adult sheep as well as lambs showed non-specific clinical signs, such as lesions in the oral mucosa, hyperaemia of limbs and face. However, these clinical signs were less intense than those observed at the beginning of the outbreak, as reported by Balaro and colleagues (Balaro *et al.* 2014).

The diarrhoea and pneumonia cases observed during the study were probably associated with opportunistic agents. In addition, the ewes showing

Table II. *Bluetongue virus Seg-10 RT-PCR results according to the sheep age categories from March to August 2013.*

Month	Ewes (1 year)		Ewes (2 - 2,5 year)		Ewes (3-5 years)		Female lambs		Male lambs		Total	
	N	%	N	%	N	%	N	%	N	%	N	%
March	2/8	25.0%	7/17	41.2%	0/5	0.0%	0/6	0.0%	2/9	22.2%	11/45	24.4%
April	1/15	6.7%	0/17	0.0%	0/4	0.0%	1/6	16.7%	3/10	30.0%	5/52	9.6%
May	4/11	36.4%	1/2	50.0%	-	-	4/7	57.1%	2/12	16.7%	11/32	34.4%
June	4/10	40.0%	0/2	0.0%	0/1	0.0%	1/6	16.7%	4/11	36.4%	9/30	30.0%
August	0/1	0.0%	0/1	0.0%	0/1	0.0%	0/6	0.0%	4/5	80.0%	4/14	28.6%

BT clinical signs also showed a low response to applied antibiotic therapy, with a longer convalescence period.

Although BT is considered a disease with an acute course, late muscle injuries and opportunistic infections can prolong the period of animals recovery and may even lead to late death, increasing the economic losses (Verwoerd and Erasmus 2004, MacLachlan *et al.* 2009). For this reason, lines and sloughing in the hoof wall and wool break (Figure 1) were observed up to 4 months after the clinical cases. These clinical signs are important as they may raise the suspicion of disease occurrence in the farm.

Partial or total loss of ram fertility, up to 90 days, due to testicular degeneration is a reproductive disorder associated with BT (Bürstel *et al.* 2009, Kirschvink *et al.* 2009). Scrotum hyperaemia and sagging, accompanied by testicle dislocation were observed in 1 of the rams. However, fertility was not compromised in the following breeding season, which started in October.

Several idiopathic oral lesions found in the sheep grazing on pasture could be attributed to trauma by thorny plants (Watson 2004). As for the mouth lesions observed in the stabled sheep, the elephant grass seed (*Pennisetum purpureum*) was considered partially responsible for these lesions (unpublished data). Only after the passage of the grass seed period, the oral mucosal lesions healed without remissions.

Serological BTV studies in Brazil demonstrate that the virus has a widespread distribution, although the serological prevalence varies among regions. In the Southern region, which is situated in the South Temperate Zone, BTV serological prevalence is lower than 1%, while in the South-Eastern region, where Rio de Janeiro is located, it varies between 20% or even above 40% (Cunha *et al.* 1988, Costa *et al.* 2006). The serological data revealed a seroprevalence of 80% (87/108). Nonetheless, it is important to highlight the differences in the rate of seropositivity according to the sheep age categories (Figure 2A).

Almost all the oldest ewes, between 2 and 5 years, were seropositive to BTV (Figure 2B) since the first serological analysis. Bluetongue outbreaks typically occur either when susceptible animals are introduced into endemic areas or when the virus spreads into immunologically naïve populations (MacLachlan 2004). Interestingly, in this monitored farm, ewes from the South region of Brazil, which is an epidemic area (Costa *et al.* 2006), were introduced into the South-Eastern region, Rio de Janeiro State, an endemic area. The high seropositive rate found since the monitoring started may be due to serological conversion or to previous contact of the flock with different BTV serotype(s).

The decrease of the seropositive rate found in the lambs 4 months after the beginning of the lambing period (May/2013) probably concurs with the decrease of passive immunity (Figure 2A). These animals would be seronegative during the rainy season, when the *Culicoides* population is more abundant (Carvalho and Silva 2014). The susceptibility of the lambs and some young ewes during this season, associated with the persistence of BTV in the farm, may be a risk factor for the occurrence of clinical disease. Associated with the decrease in passive immunity, 13 out of 19 lambs (68.4%) were positive by RT-PCR, at least once, demonstrating that they were exposed to BTV. Lower weight gain of the lambs, when compared with the database of the previous years in the farm, was observed. These results could be associated with BTV infection after the decrease of the maternal passive immunity and infections by other opportunist agents, causing the pulmonary and gastrointestinal disorders detected during the study.

Severe clinical signs and even deaths were observed at the first stage of the outbreak (Balaro *et al.* 2014). As the monitoring continued, clinical signs became less evident in some animals. However, the detection of viral RNA associated with the presence of *Culicoides* vector species throughout the study period suggests the continuous viral circulation, even during the dry and cool season in the region (Tables I and II). It is known that in BTV endemic areas, infected ruminants develop mild or no obvious disease, which may encroach the diagnosis (Gibbs and Greiner 1994, MacLachlan and Osburn 2006). The presence of BTV RNA was reported for more than 60 days in the blood of one of the sampled animals. This finding supports the information about extended viraemia usually associated with BTV infections, as it has been reported by MacLachlan (MacLachlan 2004). The prolonged detection of BTV RNA in the blood samples could also be due to recurrent virus infection by different serotypes, as it is still unknown which BTV serotypes circulate in the region.

These results show the BTV circulation in Brazil, causing clinical and subclinical disease and highlight the necessity for improving BT surveillance programme. The virus introduction in a farm, probably due to the characteristic of a free-range flock, associated with favourable climate conditions, caused economic losses, even in its subclinical form. The epidemiology of BT in Brazil is largely unknown. Therefore, surveillance programs based on monitoring of vector distribution, antibodies, and virus circulation, as well as animal trade, are urgently needed in this country.

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References

Antoniassi N.A.B., Pavarini S.P., Henzel A., Flores E.F. & Driemeier D. 2010. Aspiration pneumonia associated with oesophageal myonecrosis in sheep due to BTV infection in Brazil. *Vet Rec*, **166**, 52-53.

Balaro M.F.A., Lima M.S., Del Fava C., Oliveira G.R., Pituco E.M. & Brandão F.Z. 2014. Outbreak of Bluetongue virus serotype 4 in dairy sheep in Rio de Janeiro, Brazil. *J Vet Diag Invest*, **26**, 567-570.

Batten C., Darpel K., Henstock M., Fay P., Veronesi E., Gubbins S., Graves S., Frost L. & Oura C. 2014. Evidence for transmission of bluetongue virus serotype 26 through direct contact. *PLoS One*, **9**, e96049.

Bürstel D., Adams W. & Ganter M. 2009. Evaluation of the reproductive performance in rams following recovery of Bluetongue disease. *Tierärztl Prax*, **37**, 289-295.

Carvalho L.P.C. & Silva F.S. 2014. Seasonal abundance of livestock-associated *Culicoides* species in northeastern Brazil. *Med Vet Entomol*, **28**, 228-231.

Clavijo A., Sepulveda L., Riva J., Pessoa-Silva M., Tailor-Ruthes A. & Lopez J.W. 2002. Isolation of bluetongue virus serotype 12 from an outbreak of the disease in South America. *Vet Rec*, **151**, 301-302.

Coetzee P., Stokstad M., Venter E.H., Myrmel M. & Van Vuuren M. 2012. Bluetongue: a historical and epidemiological perspective with the emphasis on South Africa. *Virology Journal*, **9**, 198.

Costa J.R.R., Lobato Z.I.P., Herrmann G.P., Leite R.C. & Haddad J.P.A. 2006. Prevalência de anticorpos contra o vírus da língua azul em bovinos e ovinos do sudoeste e sudeste do Rio Grande do Sul. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, **58**, 273-275.

Cunha R.G., Souza D.M. & Teixeira A.C. 1988. Incidência de anticorpos para o vírus da língua azul em soros de caprinos e ovinos do estado do Rio de Janeiro. *Arquivo Fluminense de Medicina Veterinaria*, **3**, 53-56.

Favero C.M. 2009. Padronização e aplicação de técnicas de diagnóstico para o vírus da língua azul e da doença hemorrágica epizootica dos cervídeos. Dissertation, Universidade Federal de Minas Gerais, Escola de Veterinária.

Gibbs P.J. & Greiner E.C. 1994. The epidemiology of Bluetongue. *Comp Immunol Microbiol Infect Dis,* **17**, 207-220.

Kirschvink N., Raes M. & Saegerman C. 2009. Impact of a natural bluetongue serotype 8 infection on semen quality of Belgian rams in 2007. *Vet J*, **182**, 244-251.

Legisa D., Gonzalez F., De Stefano G., Pereda A. & Dus Santos M.J. 2013. Phylogenetic analysis of bluetongue virus serotype 4 field isolates from Argentina. *J Gen Virol*, **94**, 652-662.

Maan N.S., Maan S., Belaganahalli M.N., Ostlund E.N., Johnson D.J., Nomikou K. & Mertens P.P.C. 2012. Identification and differentiation of the twenty six Bluetongue virus serotypes by RT–PCR amplification of the serotype-specific genome segment 2. *PLoS One*, **7**, e32601.

Maclachlan N.J., Drew C.P., Darpel K.E. & Worwa G. 2009. The pathology and pathogenesis of Bluetongue. *J Comp Path*, **141**, 1-16

MacLachlan N.J. & Osburn B.I. 2006. Impact of bluetongue virus infection on the international movement and trade of ruminants. *J Am Vet Med Ass*, **228**, 1346-1349.

MacLachlan N.J. 2004. Bluetongue: pathogenesis and duration of viraemia. *Vet Ital*, **40**, 462-467.

Mellor P.S., Boorman J. & Baylis M. 2000. *Culicoides* biting midges: their role as arbovirus vectors. *Ann Rev Entomol*, **45**, 307-340.

Mo C.L., Thompson L.H., Homan E.J., Oviedo M.T., Greiner E.C., González J. & Sáenz M.R. 1994. Bluetongue virus isolations from vectors and ruminants in Central America and the Caribbean. *Am J Vet Res*, **55**, 211-215.

Sambrook J. & Russell D.W. 2001. Molecular cloning: a laboratory manual, 3rd Ed. (Cold Spring Harbor Laboratory) Cold Spring Harbor, New York, 1124-1125, A1.27.

Van der Sluijs M.T., Schroer-Jooster D.P., Fid-Fourkour A., Vrijenhoek M.P., Debyser I., Moulin V., Moormann R.J. & de Smit A.J. 2013. Transplacental transmission of Bluetongue virus serotype 1 and 8 in sheep: virological and pathological findings. *PLoS One*, **8**, e81429.

Verwoerd D.W. & Erasmus B.J. 2004. Bluetongue. *In* Infectious disease of livestock, 2nd Ed. (J.A.W. Coetzer & R.C. Tustin, eds). Oxford Univertsity Press, Cape Town, 1201-1220.

Viarouge C., Lancelot R., Rives G., Bréard E., Miller M., Baudrimont X., Doceul V., Vitour D., Zientara S. & Sailleau C. 2014. Identification of bluetongue virus and epizootic hemorrhagic disease virus serotypes in French Guiana in 2011 and 2012. *Vet Microbiol*, **174**, 78-85.

Watson P. 2004. Differential diagnosis of oral lesions and FMD in sheep. *In practice*, **26**, 182-191.

World Organisation for Animal Health (OIE). 2014. Bluetongue. *In* Manual of Diagnostic Tests and Vaccines for Terrestrial animals.

Zientara S., Sailleau C., Viarouge C., Höper D., Beer M., Jenckel M., Hoffmann B., Romey A., Bakkali-Kassimi L., Fablet A., Vitour D. & Bréard E. 2014. Novel bluetongue virus in goats, Corsica, France, 2014. *Emerg Infect Dis*, **20**, 2123-2132.