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Published in:
The Veterinary Journal

DOI:
10.1016/j.tvjl.2008.06.008

Publication date:
2009

Available online at www.sciencedirect.com
ScienceDirect
The Veterinary Journal xxx (2008) xxx–xxx
The Veterinary Journal
www.elsevier.com/locate/tvjl

Impact of a natural bluetongue serotype 8 infection on semen quality of Belgian rams in 2007

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Accepted 11 June 2008

Abstract

In 2006, bluetongue (BT) virus serotype 8 emerged in northern Europe and numerous ruminants were affected in the following year. Infertility in males is one of the consequences of BT, although its severity and duration after natural infection has not been documented. In this report, the impact of BT-8 on clinical signs and semen quality of naturally infected rams is described through a longitudinal study of two Belgian ram populations ($n = 12$ and $n = 24$) and a cross sectional study in a further ram population ($n = 43$).

Macroscopic semen characteristics, semen concentration, motility, percentage of living and dead spermatozoa were assessed in 167 semen samples collected on 1–6 occasions from 79 BT-8 infected rams within 5–138 days after onset of clinical disease. These were compared with healthy control animals. Significant changes in all variables were observed after natural BT-8 infection. Total recovery occurred around 85 days after clinical disease in animals undergoing a close follow-up of semen quality. Good correspondence between the results of the longitudinal and cross sectional studies suggests that semen quality of BT-8 affected rams reached normal references values 63–138 days after clinical diagnosis of BT. In addition, semen concentration seems to be a sound epidemiological indicator of ram semen quality.

Keywords: Bluetongue serotype 8; Natural infection; Sheep; Semen; Infertility; Northern Europe

Introduction

Bluetongue (BT) is an infectious, non-contagious disease of ruminants caused by an arthropod-borne virus belonging to the family *Reoviridae*, genus *Orbivirus*. The BT virus (BTV) includes 24 serotypes, which are transmitted by haematophagus insects belonging to the family *Ceratopogonidae*, genus *Culicoides* (Mehlhorn et al., 2007; Mellor and Wittmann, 2002). Whilst cattle, goat and most wild ruminants are affected by subclinical BT infection, sheep are prone to develop severe clinical signs such as abundant nasal discharge, salivation, oedema (particularly in the region of the head), ulcerations of the oral mucosa, and sometimes by cyanosis of the tongue (MacLachlan, 1994; Saegerman et al., 2007; Lefèvre et al., 2008).

Until recently, BTV serotypes 1, 2, 4, 9, 15 and 16 have been contained within the Mediterranean basin, the Balkans and central Europe. However, outbreaks of BT occurred between 1998 and 2006 in southern and central Europe, involving several different serotypes (Mellor and Wittmann, 2002; Saegerman et al., 2008). In the summer of 2006, BTV serotype 8 emerged in northern Europe, infecting ruminants in a wide area in the Netherlands, Belgium, Germany, and to a lesser extent, in Luxemburg and northern France. The mean mortality and morbidity rates observed in sheep at the beginning of this epizootic were 20% and 5%, respectively (Elbers et al., 2007). More recent data indicate that morbidity and mortality rates increased in 2007 in sheep (FASFC, 2007). More than

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doi:10.1016/j.tvjl.2008.06.008

20,000 sheep died from BT in Belgium in 2007, or around 10% of the estimated national sheep population (FASFC, 2007).

The impact of BTV-8 infection on domestic ruminants in northern Europe is under evaluation in several countries. Direct economic losses due to mortality, morbidity, reproductive disorders such as abortions and infertility, reduced milk yields and reduced growth rate need to be estimated, as well as indirect losses due to exportation restrictions and treatment. Given the extent of BT and the increased morbidity in 2007, the economic losses due to BTV-8 infection are considerable for cattle and sheep farmers. Although vaccination campaigns will be undertaken in 2008, numerous questions about flock or herd management and the effects of BT on infected animals have still to be answered.

Infertility of male ruminants is one of the longer lasting effects of BT. In rams and bulls, BT infection is known to induce infertility, possibly in response to the deleterious effect of hyperthermia on spermatogenesis as well as to the effect of micro-vascular lesions in the reproductive tract (Osburn, 1994). There is little information about the duration of infertility and probability of recovery. To the best of the authors' knowledge, the effect of BTV-8 infection on rams' semen quality has not yet been documented. In the present study, the impact of BTV-8 on clinical signs and semen quality of 79 naturally infected rams in Belgium in 2007 is described using a longitudinal study of two ram populations and a cross sectional study.

Materials and methods

Animals

Healthy control rams

Reference values for normal ovine semen prior to the BT outbreak in northern Europe were obtained on several occasions from 11 healthy adult Dutch Texel or Belgian Texel rams that had belonged since 2004 to the University of Namur Centre for Ovine Insemination and Selection (CISO, Centre d'Insémination et de Sélection Ovines). At the time of testing (between 2005 and the beginning of 2007), all rams were clinically healthy and underwent regular 6-monthly testing for *Brucella ovis*, *B. melitensis*, Maedi-visna, border disease, ovine viral epididymitis and caprine arthritis–encephalitis virus.

At time of the BT-8 outbreak in Belgium in August 2006, most of the rams were still housed at CISO. In August 2006 and in March 2007, they were tested for BT by an accredited laboratory (VAR, Veterinary and Agrochemical Research Centre, Uccle, Belgium) using real-time quantitative polymerase chain reaction (RTqPCR; Toussaint et al., 2007) and serology. All results were negative. Analysis of semen samples of these healthy rams provided data for reference values (noted in the text as control rams: CR). Macroscopically, the semen of these animals was always viscous and whitish.

Rams with natural BTV-8 infection

A total of 79 rams of different breeds (67% Belgian Texel or Dutch Texel, 10% French Texel, 7% Bleu du Maine, 4% Ile de France, 4% Suffolk, 8% other breeds), and aged between 1 and 6 years (mode: 1) underwent single or repeated semen collection and analysis within 5–138 days after the development of clinical signs of BT (called days post observation, DPO).

The animals came from two locations, namely the sheep centres of the University of Namur (CISO and the Centre for Ovine Research) or Belgian sheep breeders. Animals were divided into three groups:

(1) Twelve rams from the two sheep centres underwent repeated semen analysis (4–6 collections) between 5 to 138 DPO. This group is shown as SC in the text.
(2) Twenty-four rams belonging to sheep breeders underwent two or three semen analyses between 17 and 90 DPO. This group is recorded as LRF (longitudinal study on rams originating from the field).
(3) Forty-three rams belonging to sheep breeders underwent a single semen analysis between 23 and 86 DPO. This group is called CRF (cross sectional study on rams originating from the field). Table 1 shows the distribution of rams and the number of semen analyses performed within the groups.

All animal investigations were performed by the research staff of the Sheep Centres of the University of Namur and were approved by the local Ethical Committee for Animal Welfare.

Animals' history record and clinical signs

The date of onset of clinical BT of each ram was reported by the breeder/owner, as well as the nature of clinical signs present at onset of BT. A standardised questionnaire was used to assess the most prominent clinical signs detected by the breeder/owner at time of clinical disease (weakness, lameness, head oedema, submandibular oedema, peri-orbital oedema, salivation, oral lesions, respiratory distress, regurgitation). According to these observations, a clinical score ranging from 0 to 9 (0, absence of clinical signs; 9, presence of all the above clinical signs) was given once to each animal.

Blood collection, BTV serology and virology

In each ram tested in the summer and/or autumn of 2007, venous blood was collected by jugular venepuncture into dry tubes at the time of the first semen collection. Serum was divided into aliquots of 0.5 mL and

Table 1
Distribution of BTV-8 affected rams within groups and distribution of semen analyses within subgroups ($n = 167$).

Sheep centres (SC)		Longitudinal study of rams from the field (LRF)		Cross sectional study of rams from the field (CRF)	
Collection number	Number of rams	Collection number	Number of rams	Collection number	Number of rams
1	12	7	27	10	43
2	12	8	27	–	–
3	12	9	7	–	–
4	12		–	–	–
5	11	–	–	–	–
6	4	–	–	–	–
Total	63	–	61	–	43

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stored at −20 °C. BT serology was performed by an accredited laboratory (ARSIA, Association Régionale de Santé et d'Identification Animales, Ciney, Belgium) using a competition immuno-linked enzyme assay (ELISA) based on VP7 antibody detection. According to the manufacturer's instructions (Id-Vet), results were expressed as percentage of inhibition. All values <35% (the cut-off percentage) were considered positive (Vandenbussche et al., 2008).

All rams from the sheep centres underwent further viral diagnosis using RTqPCR by an accredited laboratory (VAR, Veterinary and Agrochemical Research Centre, Uccle, Belgium) using the technique described by Toussaint et al. (2007).

Semen collection

Semen collection was performed in a standardised procedure by presenting female sheep in oestrus to the rams. Semen was collected by use of a heated vagina and a heated collection vial. The semen was analysed immediately after collection.

Semen analysis

Macroscopic evaluation

Based on its macroscopic characteristics, the semen was classified as: viscous and white, viscous and yellow, slightly viscous and white, aqueous and white, or aqueous and translucent.

Semen motility

Mass motility was scored by a qualified and experienced investigator (MR) from 0 to 5. Semen was placed on a heated plate and scoring was performed at microscopic magnification of 200×. Each sample was evaluated twice; the mean value was used for data analysis.

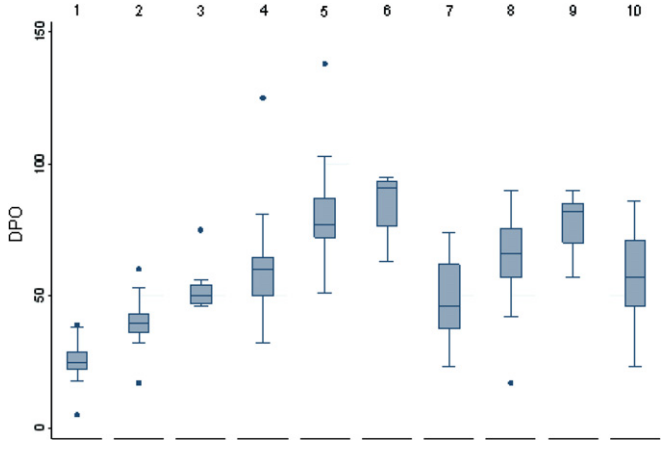

Fig. 1. Semen collections by group of BT-affected rams and the number of days after the first clinical observation of BT (days post-observation, DPO). *X* axis: 1–6 = collections 1–6 performed in rams from the university sheep centres (SC, $n = 12$); 7–9 = collections 1–3 performed in the group of rams included in the longitudinal study of rams originating from the field (LRF, $n = 24$); 10 = collection performed in the group of rams included in the cross sectional study of rams originating from the field (CRF, $n = 43$). *Y* axis: days post-observation (DPO) of clinical signs. Data are presented as box plots: •, outside values; distal and proximal horizontal adjacent lines, upper and lower adjacent values; distal, central, and proximal horizontal lines of the box, 25th percentile, median, and 75th percentile of values, respectively.

Semen concentration

Semen was diluted in saline (0.09%) and absorbance was measured at 500 nm by use of a calibrated photo-spectrometer (Vital Scientific). Semen concentration was calculated using the following formula:

$$\text{Concentration} = \text{absorbance} \times 4.98 + 0.11 \text{ billion spermatozoa/mL of semen}$$

Semen viability and morphology

Semen was coloured with eosin-nigrosin and at least 100 spermatozoa were counted on a video screen after microscopic magnification (1000×). The percentage of normal and alive (uncoloured) spermatozoa, normal and dead spermatozoa (red coloured with normal morphology) and abnormal dead spermatozoa (red coloured with abnormalities such as separate heads, curled tails etc.) were established for each sample.

Data analysis

As the onset of clinical signs of BT had been detected by the animal breeders/owners and not by the same investigator, data were analysed according to their time of collection, which was known precisely. Fig. 1 shows when single or repeated semen collections were taken against the presumed time point of clinical disease (DPO).

Comparisons of semen characteristics between subgroups of rams and control rams were performed using Wilcoxon rank tests assuming an unequal variance and that the data were not normally distributed (Dagnelie, 1998). The limit of statistical significance of the tests performed was defined as $P \leqslant 0.05$.

Results

Clinical pattern

The first clinical signs of BT were observed by sheep breeders/owners from mid-July through to late September 2007 with the highest frequency (mode) in late July and the beginning of August. The number of clinical signs observed in the rams ranged from 1 to 9 with a mode of 2–3. The most frequently observed clinical signs were the following: weakness (83%), salivation (70%), oedema of head (45%), regurgitation (45%), lameness (44%), oral lesions (21%), submandibular oedema (16%), peri-orbital oedema (16%) and respiratory disorders (11%).

Serological and virological testing

All animals included in the SC, LRF or CRF group tested positive for BT by the competitive ELISA test. Some healthy rams without a history of clinical BTV and normal semen quality tested negative (data not shown). The rams of the SC were also tested by RTq-PCR and had all positive results.

Qualitative semen analysis

The appearance and the colour of the semen in each of the groups of rams and the collection time are presented in Fig. 2. In CR rams, semen was viscous and white (i.e. normal). In SC rams, semen appearance was highly altered

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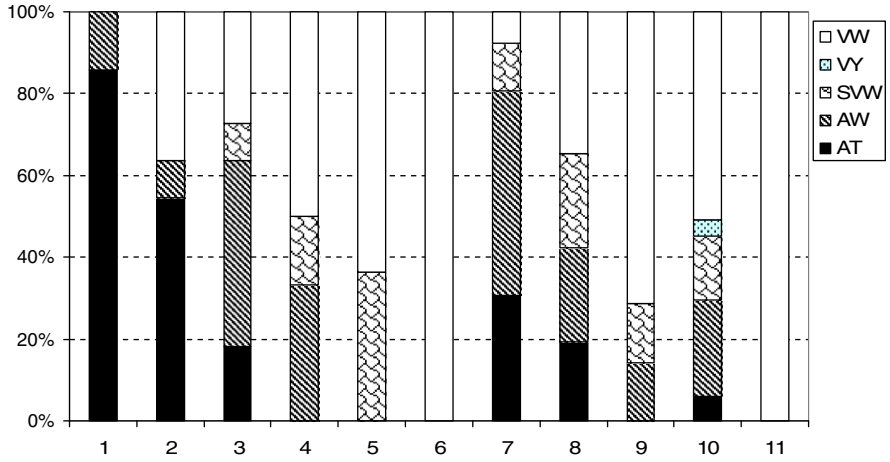

Fig. 2. Macroscopic aspects of semen samples by group of rams and the collection time. *X* axis: lanes 1–6 = collections 1–6 of the SC group ($n = 12$); lanes 7–9 = collections 1–3 of the LRF group ($n = 24$); lane 10 = collection of the CRF group ($n = 43$ rams); lane 11 = collection of the control rams ($n = 11$). *Y* axis: percentage of samples; VW, viscous appearance and white colour of the semen; VY, viscous appearance and yellow colour of the semen; SVW, slightly viscous appearance and white colour of the semen; AW, aqueous appearance and white colour of the semen; AT, aqueous appearance and translucent colour of the semen; *Y* axis: Percentage of animals in each category.

after around 25 DPO. Semen appearance progressively improved over time until a complete recovery occurred at 85 DPO (the mean value for recovery of normal macroscopic characteristics). In LRF rams, semen appearance at collection times 7 and 8 was altered. The characteristics at these times were similar to the SC group semen at time 2. In CRF rams, half of the semen samples were abnormal (approximately 30% had an aqueous appearance).

Quantitative semen analysis

Motility of spermatozoa

The motility of spermatozoa was strongly affected by BT (Fig. 3a). In SC rams, the motility measured at collection times 1–4 was significantly lower than in CT rams. At collection times 5 and 6, i.e. around 80–85 DPO, motility was similar to that of healthy control animals. In LRF rams, the motility measured at collection times 7 and 8 was significantly lower than in controls. At collection time 9, i.e. around 77 DPO, motility did not differ from the semen motility of controls. In CRF rams, motility was more variable but significantly decreased when compared to control rams.

Concentration of spermatozoa

The concentration of spermatozoa was strongly affected by BT (Fig. 3b). In SC rams, concentration measured at collection times 1–5 was significantly decreased in comparison to healthy controls. At collection time 6, i.e. around 85 DPO, concentration was similar to the values recorded in controls. In LRF rams, concentration measured at collection times 7 and 8 remained significantly lower than in con-

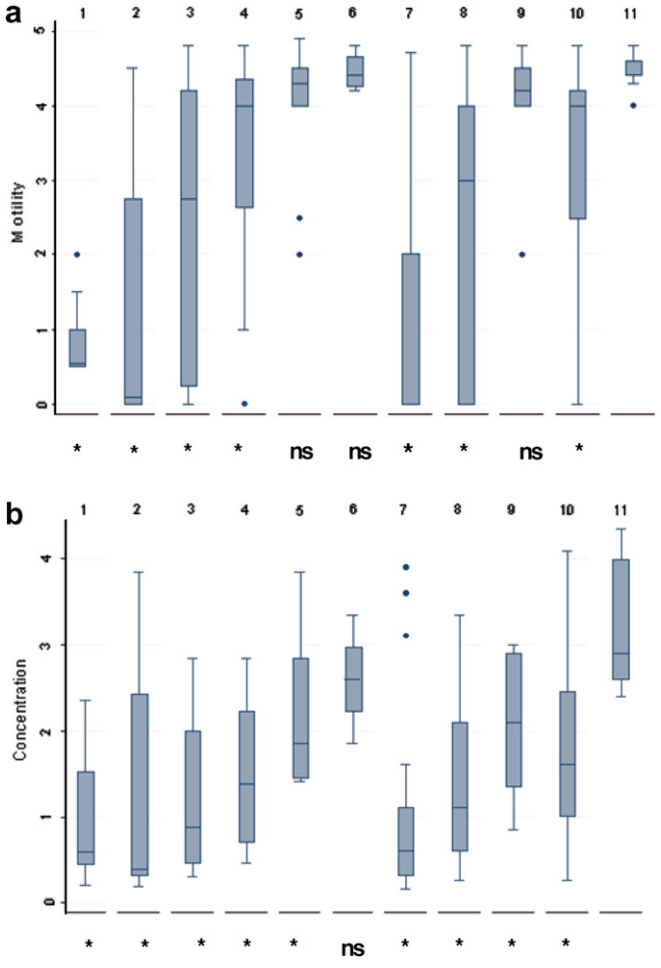

Fig. 3. Motility (a) and concentration (b) of the semen by ram group and the collection time. Refer to Fig. 2 for explanation of *X* axis and for the codification of box plots. *Y* axis: mobility expressed by a score between 0 and 5. *Significantly different from CR-value, $P < 0.02$; ns: not significant.

trols. In CRF rams, semen concentration varied strongly but the mean value was significantly different from healthy control rams.

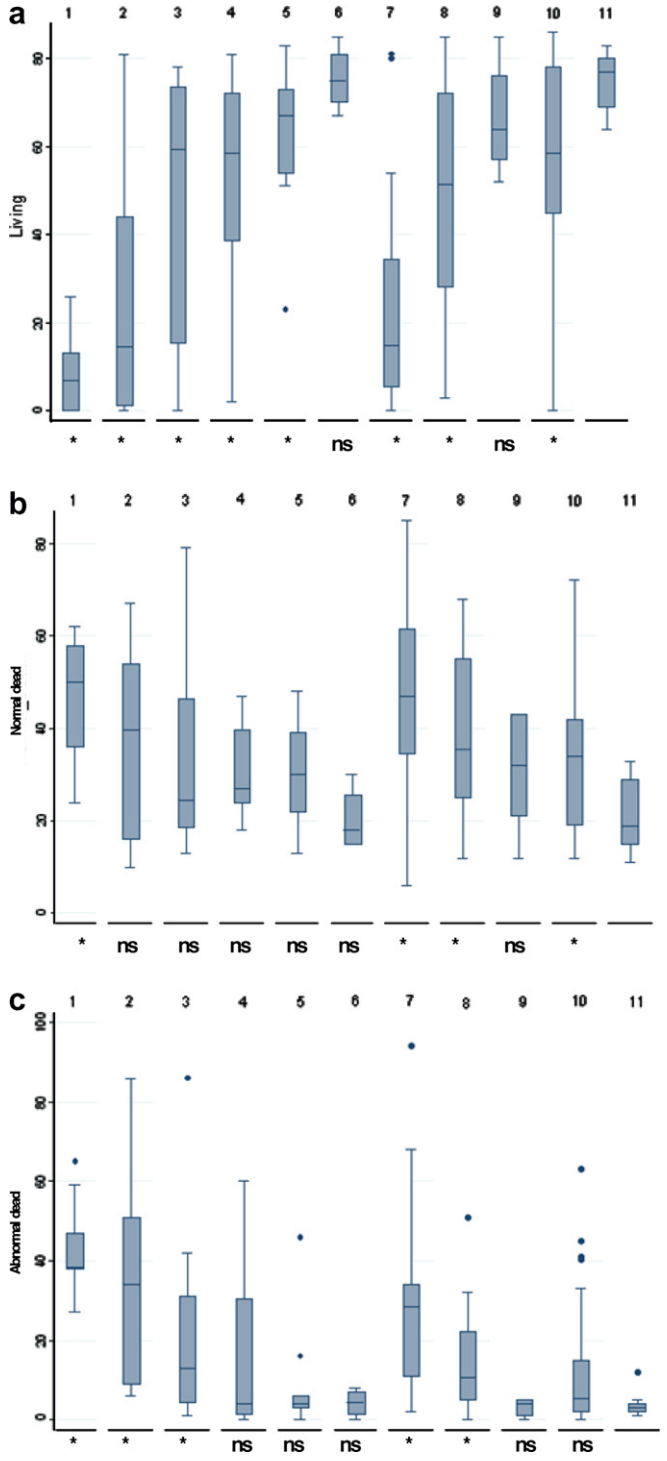

Fig. 4. Percentage of living spermatozoa (a), normal dead spermatozoa (b) and abnormal dead spermatozoa (c) by ram group and collection time. Refer to Fig. 2 for explanation of *X* axis and for the codification of box plots. *Y* axis: Percentage of living spermatozoa. *Significantly different from CR-value, $P < 0.05$; ns: not significant.

Percentage of living spermatozoa

The percentage of living of spermatozoa was reduced after BTV infection (Fig. 4a). In SC rams, the percentage of living spermatozoa measured at collection times 1–5 progressively increased but was significantly lower than in control rams. At collection time 6, i.e. around 85 DPO, normal values of living spermatozoa were measured. In LRF rams, the percentage of living spermatozoa measured at collection times 7 and 8 was significantly decreased, whilst values measured at collection time 9, i.e. around 77 DPO, were similar to those recorded in control rams. In CRF rams, the percentage of living spermatozoa measured was significantly lower than in healthy rams.

Percentage of normal dead spermatozoa

The percentage of normal dead spermatozoa was strongly increased after BT infection (Fig. 4b). In SC rams, only the percentage of normal dead spermatozoa recorded at collection time 1 was significantly higher than in healthy controls, whereas percentages recorded at collection times 2, 3, 4 and 5 remained higher, but not significantly different from controls. At collection time 6, i.e. around 85 DPO, values were similar to those recorded in healthy control rams. In LRF rams, the percentage of normal dead spermatozoa recorded at collection times 7 and 8 was also significantly increased, whilst values were similar to those of controls at collection time 9, i.e. around 77 DPO. In CRF rams, the percentage of normal dead spermatozoa was significantly higher than in controls.

Percentage of abnormal dead spermatozoa

The percentage of abnormal dead spermatozoa strongly increased in BT-affected sheep (Fig. 4c). The most frequent abnormalities were separated head and tails and abnormally curled tails. In SC rams, the percentage of abnormal dead spermatozoa measured at collection times 1–3 was significantly higher than in controls. A progressive decrease occurred and values recorded at collection times 4, 5 and 6 (with collection time 6 corresponding to around 85 DPO) did not significantly differ from those of control rams. In LRF rams, the percentage of abnormal dead spermatozoa measured at collection times 7 and 8 was significantly increased, whereas percentage recorded at collection time 9, i.e. around 77 DPO, was similar to that recorded in healthy controls. In CRF rams, the percentage of abnormal dead spermatozoa measured was not significantly higher than in healthy rams.

Discussion

Numerous domestic ruminants have been infected by BTV-8 in Northern Europe since the outbreak in the summer of 2006. The disease had more severe effects in 2007 in sheep and cattle than in 2006 (FASFC, 2007). The present

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study aimed to assess the impact of natural BTV-8 infection on semen quality in rams that underwent either repeated or single semen analyses between 5 and 138 DPO. The work also provides some information about the primary clinical signs of BTV-8.

Onset of disease, clinical signs and serology within the tested ram population

The first rams with clinical signs of BTV-8 infection were detected in mid-June 2007, whilst the last animal tested developed disease at the end of September 2007. The greatest number of detected cases occurred at end of July 2007. This information indicates a slightly earlier onset of BTV-8 in Belgium than reported in a survey performed among Belgian sheep holders (C. Saegerman and N. Kirschvink, unpublished data) as well as with official data originating from the Belgian Federal Agency for the Safety of the Food Chain (FASFC, 2007), where the peak of clinical cases was recorded at the end of August 2007.

The clinical signs displayed by BTV-8 infected rams were most frequently (in descending order) weakness, salivation, oedema of the head, regurgitation, lameness, lesions of the oral cavity, submandibular oedema, peri-orbital oedema and respiratory disorders. Most rams presented with two or three of these clinical signs. As the animals in this study could not be examined systematically by the same investigator when they developed disease, a clinical sign such as pyrexia was not included in the questionnaire because most sheep farmers had not measured rectal temperature of their animals. The presence of lesions within the oral cavity might also have been underestimated as well as early and sometimes less easily detectable signs such as peri-orbital oedema. Nevertheless, the clinical signs we detected agreed with those reported in experimentally BTV-8 infected sheep (Backx et al., 2007; Darpel et al., 2007) and in naturally infected animals (Elbers et al., 2007; C. Saegerman and N. Kirschvink, unpublished data).

The age of the rams included in our study ranged from 1 to 6 years, and most of the rams were between 1–2 years old. This particular age distribution was essentially due to the fact that the majority of the rams tested were Texel rams (as the Texel breed is predominant in Belgium, very young and promising individuals are mainly used for reproduction). Apart from the rams from the sheep centres of Namur University, the animals we used were not selected by the investigators: each sheep farmer interested in an evaluation of the semen quality of his ram(s) could request semen collection and analysis by qualified personnel from the sheep centres. If the serological analysis confirmed BTV infection, the animal was included in the study. This selection procedure certainly induced a bias with regard to breed and age distribution in animals included in the database, which should not therefore be considered to be representative of the Belgian ram population affected by BTV-8.

Clinical suspicion or clinical diagnosis of BTV-8 was confirmed in all rams from the sheep centres by positive RTqPCR and ELISA. Each animal that had shown clinical signs of BT was seropositive; 13 rams reported without clinical signs were positive and showed semen alterations, whilst no ram with normal semen quality and without clinical signs was positive for BT.

Virus detection on semen samples was not carried out in the present study. It would have been interesting to assess whether viral shedding occurred and over what length of time, although the probability of viral isolation in semen seems very low. Indeed, viral isolation of BT in frozen semen of 18,000 bulls revealed only two positive results (Osburn, 1994) and no isolation of virus was possible in semen from rams after vaccination with an attenuated BTV-2 vaccine (Darpel et al., 2007). As BTV-infected sheep have a relatively short viraemia in comparison to bulls (8–15 vs. 63 days) (Singer et al., 2001), it is unlikely that viral shedding was still occurring when semen sampling was feasible. Although semen collection had been performed in one ram within five days of supposed infection, sampling <25 days after BTV-8 infection was rarely possible because of low libido. Some rams had to be exposed to ewes in oestrus on several occasions (2–3 weeks apart) before semen collection was successful.

Longitudinal and cross sectional assessment of semen quality in BTV-8 infected rams

The results from repeated semen analyses performed on 11 healthy rams from the insemination centre before the BTV-8 outbreak were used as reference values for normal semen. Differences between separate semen collections (up to five) within a ram and between rams did not significantly differ (data not shown) and one semen analysis from each ram was used to generate reference values. The same investigator performed these tests and the semen evaluation after the BTV-8 outbreak (MR).

Up to six semen collections were taken from the rams from the sheep centres, whereas up to three collections came from rams in the LRF group and a single collection was taken from rams in the CRF group. In Fig. 1, collection times are expressed as DPO. It appears that collection times 3, 4 and 5 of the SC group corresponded relatively well to collection times 7, 8 and 9 of the LRF group. Collection times 3–4 of the SC group and collection times 7–8 of the LRF group coincided with collection time 10 of the CRF group. Although data obtained within a BTV-8 affected group should not be compared statistically with that of another BTV-8 affected group, a comparison of semen quality within different ram populations over time is possible, allowing to some extent an extrapolation of data of the SC group.

An assessment of age- and breed-related differences on the impact of BTV-8 infection on semen quality would have been interesting to ascertain, but due to the high percentage of young animals and Texel sheep, the two effects

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could not be satisfactorily tested in this study. However, some complementary results (data not shown) have indicated that for semen concentration (the most important epidemiological indicator for assessing a ram's semen quality) no differences appear between Texels and other breeds whichever collection time was tested.

From a qualitative point of view, the semen of BTV-8 affected rams was strongly altered in all groups with the modifications being most striking soon after infection (Fig. 2). As macroscopic semen evaluation relies on the subjectivity of the investigator, such observations need a strict codification and should be interpreted with care. Only rams of the SC group had a normal appearance of semen at collection time 6, i.e. when all other variables were similar to those of healthy control rams. Although semen volume per collection had been recorded (data not shown), this variable has not been considered for qualitative assessment because of high parameter variability independent of the presence of BTV infection (Bréard et al., 2007).

Quantitatively, BTV-8 infection had significant repercussions on semen motility, concentration and the percentage of living and normal dead spermatozoa of rams in all groups (Figs. 3 and 4a and b). The percentage of abnormal dead spermatozoa was significantly different from controls in the SC and LRF group, but due to wide parameter variability within the CRF group, differences from controls were not seen in these animals (Fig. 4c). Data generated from the SC group were from a limited number of individuals ($n = 12$), but a prolonged follow-up was possible that showed that variables became similar to control values at collection time 5 (motility, percentage of normal dead spermatozoa and percentage of abnormal dead spermatozoa) or at collection time 6 (concentration, percentage of living spermatozoa), i.e. at 77 and 91 DPO, respectively. Despite the reduced number of collections (2/3), data from the LRF group provide interesting results that corroborate results from the SC group. Indeed, results of all variables at collection times 7, 8 and 9 are similar to those of the SC group at collection times 3, 4 and 5, respectively. A similar relationship can be established for results of the CRF group and those of the SC and LRF groups at collection times 3–4 and 7–8, respectively.

The data from all BTV-8 affected groups show similar trends and suggest that a total recovery of fertility may occur over time, i.e. within 63–138 DPO. The variables most rapidly returning to normal values were the percentage of normal and abnormal dead spermatozoa, followed by semen motility. Semen concentration and percentage of living spermatozoa only reached control values in the SC group at collection time 6, suggesting that these variables needed the longest time to recover. These observations are in agreement with the study of Bréard et al. (2007) where an attenuated BTV-2 vaccine was reported to induce a transient decrease of semen quality in rams. Although the effect of serotype 8 has been investigated in the present study, it seems that recovery of normal semen concentration appears to be the longest-lasting process of spermatogenesis; semen concentration could therefore play an interesting role as epidemiological indicator.

As the data presented here have been generated through a field study where natural BTV infection occurred, a certain lack of precision with regard to time of infection and onset of clinical signs in relation to times of semen collection exists. Pyrexia has been shown to be a constant and early indicator of BTV-8 infection (Darpel et al., 2007) but could not be systematically assessed in this study. However, semen quality has been assessed in rams before and after vaccination against BTV-2 using an attenuated virus strain (Bréard et al., 2007). In that study, sheep were either vaccinated or sham-vaccinated twice at 0 and 47 days and the impact on semen quality was monitored on a weekly basis until day 90. In vaccinated rams, a significant decrease of semen motility (lowest mean score 2.3), semen concentration (lowest mean concentration 3.1 billion spermatozoa/mL) and spermatozoa with an intact membrane (i.e. normal living spermatozoa; lowest mean percentage 50%) was recorded and reached its minimum around days 30–35, whereas the percentage of abnormal spermatozoa significantly increased and reached its maximum at the same time point (highest mean percentage 50%). When a second injection was given to these rams on day 49, a further less intense effect was observed on all variables and complete recovery was achieved by day 70.

This study did not allow a comparison with our data with regard to recovery, but some considerations regarding the interval between virus inoculation and measurable effects on semen quality are possible. A significant alteration in semen in the absence of clinical signs occurred within 30 days of vaccination and semen alterations were moderate compared to those observed in rams with natural BTV-8 infection. The earliest semen analyses of rams were performed in our study around 25 DPO, which (taking into account an incubation period of 5–10 days) would correspond to 30–35 days after infection by the vector *Culicoides* (Backx et al., 2007; Darpel et al., 2007).

Semen quality was lowest at the first collection time and then progressively improved, suggesting that the period of semen quality decrease had not been monitored. Given that natural BT infection induced clinical disease, collection at earlier time points was often not possible. It could be that semen changes occurred more rapidly during natural infection than after inoculation of an attenuated BTV-2 vaccine. Potential serotype-related differences could also account for the differences. It can be concluded that natural BTV-8 infection induces much more severe and longer lasting semen alterations than an attenuated BTV-2 vaccine strain. Recovery of normal semen quality seems however to occur in BTV-8 infected rams, although a prolonged follow-up including reproductive performances of the rams was not possible in the present study.

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Conclusions

This field study demonstrates that natural BTV-8 infection has a significant impact on semen quality in rams, leading to infertility. Longitudinal investigations suggest that recovery of semen quality can be expected within 63–138 DPO. As clinical signs are sometimes inapparent and as libido reappears earlier than fertility, semen quality testing should be performed in rams before mating.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

The authors thank Benoît Bolkaerts, Christine Baricalla, Nicolas Mauguit, Laetitia Wiggers and François Claine (University of Namur) for helpful technical assistance with animals and semen sample analyses and are grateful to Delphine Cassart (University of Namur) for setting the manuscript. The collaboration with ARSIA (Association Régionale de Santé et d'Identification Animales, Ciney, Belgium) for BTV antibody determination is gratefully acknowledged, as well as the collaboration with VAR (Veterinary and Agrochemical Research Centre, Uccle, Belgium) for RTqPCR. This study was financed by the University of Namur and the University of Liège.

References

Backx, A., Heutink, C.G., van Rooij, E.M., van Rijn, P.A., 2007. Clinical signs of bluetongue virus serotype 8 infection in sheep and goats. Veterinary Record 161, 591–592.

Bréard, E., Pozzi, N., Sailleau, C., Durand, B., Catinot, V., Sellem, E., Dumont, P., Guerin, B., Zientara, S., 2007. Transient adverse effects of an attenuated bluetongue virus vaccine on the quality of ram semen. Veterinary Record 160, 431–435.

Dagnelie, P., 1998. Statistique théorique et appliquée. Inférence statistique à une et à deux dimensions. (Tome 2), De Boeck and Larcier, Bruxelles and Paris. p. 659.

Darpel, K.E., Batten, C.A., Veronesi, E.A.E., Anthony, S., Bachanek-Bankowska, K., Kgosana, L., bin-Tarif, A., Carpenter, S., Muller-Doblies, Takamatsu, H.H., Mellor, P.S., Mertens, P.P., Oura, C.A., 2007. Clinical signs and pathology shown by British sheep and cattle infected with bluetongue virus serotype 8 derived from the 2006 outbreak in northern Europe. Veterinary Record 161, 253–261.

Elbers, A.R.W., Mintiens, K., Staubach, C., Gerbier, G., Meroc, E., Ekker, H.M., Conraths, F.L., van der Spek, A.N., Backx A., 2007. Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe. Annexe 2. Nature and severity of disease in sheep and cattle. European Food Safety Authority, Parma, Italy, p. 14.

Federal Agency for the Safety of the Food Chain (FASFC) of Belgium, 2007. <http://www.afsca.be/crisis/sa-blueT/catarrhale_fr.asp>.

Lefèvre, P.-C., Mellor, P.S., Saegerman, C., 2008. Chapter 74: Bluetongue. In: Lavoisier (Ed.), Infectious and Parasitic Disease of Livestock. Provigny, France, pp. 959–989.

MacLachlan, N.L., 1994. The pathogenesis and immunology of bluetongue virus infection of ruminants. Comparative Immunology Microbiology and Infectious Diseases 17, 197–206.

Mehlhorn, H., Walldorf, V., Klimpel, S., Jahn, B., Jaeger, F., Eschweiler, J., Hoffmann, B., Beer, M., 2007. First occurrence of *Culicoides obsoletus*-transmitted Bluetongue virus epidemic in Central Europe. Parasitological Research 1001, 219–228.

Mellor, P.S., Wittmann, E.J., 2002. Bluetongue virus in the Mediterranean Basin 1998–2001. The Veterinary Journal 164, 20–37.

Osburn, B.I., 1994. The impact of bluetongue virus on reproduction. Comparative Immunology Microbiology and Infectious Diseases 17, 189–196.

Saegerman, C., Hubaux, M., Urbain, B., Lengelé, L., Berkvens, D., 2007. Regulatory aspects concerning temporary authorisation of animal vaccination in case of an emergency situation: example of bluetongue in Europe. In: Pastoret, P.-P., Schudel, A., Lombard, M. (Eds.), Animal Vaccination. Part 2: Scientific, Economic, Regulatory and Socio-Ethical Aspects. Chapter 4: Regulatory Aspects. Revue Scientifique et Technique, Office International des Epizootics (Special Issue) 26, pp. 395–414.

Saegerman, C., Berkvens, D., Mellor, P.S., 2008. Bluetongue Epidemiology in the European Union: current status and perspectives. Emerging Infectious Diseases 14, 539–544.

Singer, R.S., MacLachlan, N.J., Carpenter, T.E., 2001. Maximal predicted duration of viremia in bluetongue virus-infected cattle. Journal of Veterinary Diagnostics and Investigations 13, 43–49.

Toussaint, J.F., Sailleau, C., Breard, E., Zientara, S., De Clercq, K., 2007. Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments. Journal of Virological Methods 140, 115–123.

Vandenbussche, F., Vanbinst, T., Verheyden, B., Van Dessel, W., Demeestere, L., Houdart, P., Bertels, G., Praet, N., Berkvens, D., Mintiens, K., Goris, N., De Clercq, K., 2008. Evaluation of antibody-ELISA and real-time RT-PCR for the diagnosis and profiling of bluetongue virus serotype 8 during the epidemic in Belgium in 2006. Veterinary Microbiology 129, 15–27.

Please cite this article in press as: Kirschvink, N. et al., Impact of a natural bluetongue serotype 8 infection on semen ..., The Veterinary Journal (2008), doi:10.1016/j.tvjl.2008.06.008