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The impact of naturally-occurring, trans-placental bluetongue virus serotype-8 infection on reproductive performance in sheep

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ABSTRACT

Infection with bluetongue virus serotype (BTV)-8 occurred in ruminants in 2006 in Central-Western Europe. The trans-placental passage of this virus has been demonstrated in naturally- and experimentally-infected cattle and in experimentally-infected sheep. Trans-placental transmission is potentially important in the 'over-wintering' of this virus and its subsequent impact on reproductive performance. This epidemiological study was carried out on a sheep flock in Belgium that had experienced a severe outbreak of BTV-8 infection, and where the seroprevalence had increased from 1.3% to 88% between January and November 2007. In total, 476 lambs and 26 aborted fetuses from 300 ewes, lambing at four distinct time periods, were investigated between November 2007 and May 2008.

The following evidence suggested that BTV-8 infection occurred in utero: (1) positive PCR results from splenic tissue from aborted fetuses ($n = 4$); (2) fetal malformations suggestive of BTV infection ($n = 10$); (3) positive PCR results from red blood cells in-lambs ($n = 7$), and (4) the presence of antibody at birth in viable lambs prior to the intake of colostrum ($n = 9$). The evidence provided by this investigation strongly suggests that trans-placental BTV-8 infection occurs in naturally-infected sheep and the impact of infection on the reproductive performance of such a naïve flock was considerable, with up to 25% of ewes aborting and with flock fertility reduced by 50%. The contribution of in utero-infected lambs to the over-wintering of BTV appears limited.

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Introduction

Bluetongue (BT) is an infectious, non-contagious disease of ruminants caused by an arthropod-borne virus of the family *Reoviridae*, genus *Orbivirus*. The BT virus (BTV) includes 24 serotypes which are transmitted by haematophagus insects of the family *Ceratopogonidae*, genus *Culicoides* (Mellor and Wittmann, 2002). In cattle, goats and most wild ruminants, BT is mostly sub-clinical, but infection in sheep causes severe clinical signs such as nasal discharge, salivation, subcutaneous oedema (particularly of the head), ulceration of the oral mucosa, and occasionally cyanosis of the tongue (MacLachlan, 1994; Saegerman et al., 2007; Lefèvre et al., 2008).

Until recently, the distribution of BTV serotypes 1, 2, 4, 9, 15 and 16 was restricted to the Mediterranean basin, the Balkans and Central Europe, and between 1998 and 2006, outbreaks of BT, involving different serotypes occurred in Southern and Central Europe (Mellor and Wittmann, 2002; Saegerman et al., 2008a). In the summer of 2006, infection with BTV serotype-8 (BTV-8) occurred in numerous ruminants over a wide area of Central-Western Europe including The Netherlands, Belgium, Germany, and to a lesser extent, Luxemburg and Northern France (Saegerman et al., 2008a). The mean morbidity and mortality rates observed in sheep at the beginning of this epizootic were 20% and 5%, respectively (Elbers et al., 2007). In 2007, the morbidity and mortality rates increased in sheep older than 1 year to 49.4% and 9.2%, respectively (Saegerman et al., 2008b). More than 20,000 sheep died of BT in Belgium in 2007, which represents approximately 5–10% of the country's sheep population (FASFC, 2007).

Among the economic losses resulting from BTV infection are abortion and those due to congenital deformities such as hydranencephaly and cerebellar aplasia in calves and lambs (Oberst, 1993). The trans-placental passage of infection to ruminant fetuses

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has, until recently, only been associated with the use of BTV modified live vaccines (MacLachlan et al., 2000). BTV serotypes -10, -11, -13 and -17 are able to cross the placenta and cause fetal infection (MacLachlan et al., 1985, 2000). Trans-placental infection with BTV-8 has occurred in cattle infected since 2006 in Central-Western Europe, resulting in hydranencephaly and abortion (Vercauteren et al., 2008; Wouda et al., 2008). Surviving, viraemic, 'dummy' calves have also been reported (Vercauteren et al., 2008). Of 102 pre-colostral serum samples from calves born from naturally-infected dams, 38 tested positive using a BTV group-specific ELISA (Desmecht et al., 2008). As epitheliochorial placentation in ruminants does not allow the transfer of immunoglobulins (Campbell et al., 1977), the presence of BTV antibodies in the serum of neonatal ruminants, born during periods of insect vector inactivity, strongly suggests in utero infection by BTV. Furthermore, BTV has been isolated from the blood of two neonatal calves prior to ingesting colostrum and BTV-RNA was detected in 11% of calves with clinical signs suspicious of BT, born during the vector-free period (De Clercq et al., 2008).

As in utero infection by BTV leads to viraemic animals, this process could contribute to the 'over-wintering' of the virus (Wilson and Mellor, 2008). An investigation into the rate of such trans-placental infection in naturally-infected sheep and cattle is thus vital. Although experimental trans-placental infection by BTV-8 in sheep has been described (Worwa et al., 2009), to the authors' knowledge, there are no published data relating to the situation in naturally-infected animals. The aims of the present study were to investigate the possibility of the trans-placental passage of BTV-8 in naturally-infected sheep, to quantify the viral transmission rate in a naïve flock by taking into account vector activity, and to determine whether BTV-8 over-winters in lambs following trans-placental infection.

Materials and methods

Animal selection

The sheep flock under investigation was held at the University of Namur, Belgium and consisted of 355 ewes. Of these, 29.3% were of the Ile de France breed, 27% were French Texels, 9% were Dutch Texels, 26.5% were Texel-Ile de France crossbreeds and 8.2% were Laitier Belge crossbreeds. The animals were studied between July 2007 and June 2008 and were pastured until approximately day 125 of gestation when they were housed prior to lambing. Both ewes and lambs were housed after lambing. The 23 rams under study were of the French or Dutch Texel, Ile de France or Laitier Belge breeds. The study was approved by the Ethical Committee for Animal Welfare of the University of Namur (Study 06/075).

Clinical history of BTV-8 infection in flock

Three clinical cases in November 2006 were confirmed by serology and PCR. The BTV seroprevalence in the flock, assessed in January and November 2007 by ELISA (ID-VET), increased from 1.3% to 88% between these dates. Approximately 60% of the ewes had been clinically affected between July and the end of September 2007. Details of each of the 219 clinical cases were recorded including the predominant clinical signs and the duration of treatment (N. Kirschvink et al., unpublished data). Infection was confirmed by the detection of antibody in serum and by PCR detection and virus isolation from red blood cells (Toussaint et al., 2007a; Vandenbussche et al., 2008).

Schedule of breeding and lambing periods

Four pregnancy (P)-lambing (L) periods were scheduled. In the period P1–L1, 100 ewes were oestrus synchronised in early July 2007 using vaginal progesterone sponges (60 mg medroxyprogesterone acetate, Veramix, Pfizer) and IM injection of 750 IU of pregnant mare serum gonadotrophin (PMSG) (Folligon, Intervet). The ewes underwent exocervical insemination with fresh semen 48–52 h after vaginal device removal and PMSG injection or were left with a ram to breed naturally. In the P2–L2 period, 114 ewes were left for 17 days with three 'teaser' rams at the end of August 2007 to induce oestrus and were then left for a further 17 days to breed naturally with several rams. For the P3–L3 period, 186 ewes were left with rams for 34 days in October 2007. In the P4–L4 period, following exposure to teaser rams for 17 days, 92 ewes were left with rams for 17 days at the beginning of January 2008.

Rams were equipped with coloured raddles to assess mounting of ewes and pregnancy diagnosis was performed approximately 45 days after the breeding period using ultrasonography. Non-pregnant ewes were re-used for the following period whereas pregnant ewes were pastured separately and housed for lambing around day 125 of pregnancy. Following this schedule, lambing occurred in November 2007 (L1), January 2008 (L2), March 2008 (L3) and May 2008 (L4).

All rams used in natural service or for artificial insemination underwent semen quality assessment (Kirschvink et al., 2009). At the time of breeding they were either free of (period P1) or had completely recovered from (periods P2, P3 and P4) BTV-8 infection. Only rams reaching the semen quality equivalent of that required by the Ovine Insemination and Selection Centre of Namur University (semen motility score $\geqslant 4$, concentration $\geqslant 2.5$ billion spermatozoa/mL, $\geqslant 70\%$ living spermatozoa and <5% abnormal spermatozoa) were used (Kirschvink et al., 2009). At lambing, care was taken to insure that each lamb received colostrum from its own ewe and animals were excluded from the study if other colostrum was supplied.

Reproductive performance

The number of ewes used per breeding period, the number of pregnant ewes after day 45, the number of lambing ewes, the number of abortions occurring after day 125 of pregnancy and the numbers of live and aborted lambs were carefully recorded for each lambing period. Abortion occurring at pasture between pregnancy diagnosis (day 45) and day 125 was considered 'undetected' because fetuses could not be recovered.

Sample collection during lambing

Blood was obtained from ewes by jugular puncture from 48 h before to 48 h after lambing. Serum aliquots were prepared and kept at −20 °C until analysis. Several pieces of placenta were collected 2–4 h after lambing and were kept at −20 °C until analysed. Lambs were blood sampled by jugular puncture immediately after birth and before they ingested colostrum (T0). Serum aliquots were prepared and held at −20 °C. The lambs were re-sampled 36 h later when transfer of maternal antibodies had occurred (T36) (N. Kirschvink et al., unpublished data). Twenty randomly selected lambs, in addition to all lambs with positive BTV serology at T0, were blood sampled into EDTA tubes at 14 days (D14). The red blood cells (RBC) were immediately separated and stored at −80 °C. Where abortions occurred, the ewe was identified and the fetus was subjected to post-mortem examination when tissue autolysis was not too advanced. Wherever possible, the spleen was removed and stored at −20 °C.

Follow-up sampling of BTV-8 PCR-positive lambs

Seven clinically normal lambs with positive T0 serology and PCR, born in November 2007, were re-sampled until week 18. Serum samples were collected bi-monthly in order to compare with those from T0 BTV-8-negative lambs that became positive after ingesting colostrum ($n = 7$). Seven lambs born to BTV-8-negative ewes were also included in the follow-up sampling and served as negative controls. All serum samples were stored at −20 °C.

One 'dummy' lamb with neurological deficits, born in November 2007, had a positive T0 serology but was PCR-negative at D14. This lamb was euthanased at 5 months old, at which time photographs were taken of its cerebrum and samples of cerebrum and spleen were removed and stored at −80 °C.

Sample analyses

Serological examination

A competitive ELISA to detect the VP7 antibody was used (Id-Vet) and results were expressed as a percentage of negativity. All values with a percentage of negativity <66% (the cut-off point) were considered positive (Vandenbussche et al., 2008).

RNA detection

The detection of viral RNA in RBCs, placenta, spleen and brain using a two-step RT-qPCR targeting segment 5 of BTV (RT-qPCR_S5), was performed by the Belgian National Reference Centre for Bluetongue (Veterinary and Agrochemical Research Centre, UCCLE, Belgium). The technique used was as described by Vandenbussche et al. (2008) and the group-specific primers and Taqman probe targeting BTV-segment 5 were as detailed by Toussaint et al. (2007a) but with slight modifications to the extraction method. Briefly, total RNA was purified from 200 μL of RBCs by adding 800 μL Trizol. After adding 160 μL of chloroform and a 15 min centrifugation step at 12,000 *g*, the aqueous phase was mixed with an equal volume of 100% ethanol. The extraction was completed using the NucleoSpin RNA Virus Kit (Macherey–Nagel). When a positive result was achieved (cycle threshold [Ct] value <40), virus isolation was carried out in embryonated chicken eggs (Toussaint et al., 2007b).

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