Veterinary Microbiology 166 (2013) 394–404

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

# Evidence of transplacental transmission of bluetongue virus serotype 8 in goats

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ARTICLE INFO

*Article history:*
Received 5 February 2013
Received in revised form 16 June 2013
Accepted 24 June 2013

*Keywords:*
Bluetongue
BTV-8
Transplacental transmission
Goat
Mid and end pregnancy

ABSTRACT

During the incursion of bluetongue virus (BTV) serotype 8 in Europe, an increase in the number of abortions in ruminants was observed. Transplacental transmission of BTV-8 in cattle and sheep, with subsequent foetal infection, is a feature of this specific bluetongue serotype.

In this study, BTV-8 ability to cross the placental barrier at the beginning of the second third of pregnancy and at the end of pregnancy was investigated in goats in two separate experiments. In the first experiment, nine goats were experimentally infected with BTV-8 at 61 days of pregnancy. Foetuses were collected 21 dpi. BTV-8 was evidenced by real time RT-PCR and by viral isolation using blood from the umbilical cord and the spleens of 3 out of the 13 foetuses. All dams were viraemic (viral isolation) at the moment of sampling of the foetuses. Significant macroscopic or histological lesions could not be observed in foetuses or in their infected dams (notably at the placenta level).

In the second experiment, 10 goats were infected with BTV-8 at 135 days of pregnancy. Kids were born by caesarean section at the programmed day of birth (15 dpi). BTV-8 could not be detected by rt-RT-PCR in blood or spleen samples from the kids.

This study showed for the first time that BTV-8 transplacental transmission can occur in goats that have been infected at 61 days of pregnancy, with infectious virus recovered from the caprine foetuses. The observed transmission rate was quite high (33%) at this stage of pregnancy. However, it was not possible to demonstrate the existence of BTV-8 transplacental transmission when infection occurred at the end of the goat pregnancy.



## 1. Introduction

Bluetongue is an infectious non contagious disease caused by bluetongue virus (BTV). BTV belongs to the genus *Orbivirus* and the *Reoviridae* family (Mertens et al., 2005). Twenty six different serotypes have been described (Maan et al., 2011). This arbovirus affects naturally domestic and wild ruminants and is mainly transmitted through biting by midges belonging to the genus *Culicoides*. The most clinically affected species is sheep, while clinical signs were normally not seen in cattle (Saegerman et al., 2007) until the appearance of serotypes 1 and 8 in Northern Europe.

BT was considered exotic in Europe until the 90s, even if some serotypes (1, 2, 4, 9 and 16) were encountered since

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http://dx.doi.org/10.1016/j.vetmic.2013.06.020

1998 around the Mediterranean basin (Mellor and Wittman, 2002). In 2006, BTV-8 was introduced in Europe and rapidly spread all over northern Europe causing a major epizooty and huge economical losses (Toussaint et al., 2006). Clinical signs were observed both in sheep and in cattle.

Goats are less susceptible to BTV-8 infection than sheep and cattle, even if clinical manifestations have been reported both in natural conditions (Dercksen et al., 2007) and in experimental studies (Bréard et al., 2011; Backx et al., 2007). However, even if symptomatology is rougher than in cattle and sheep, caprine species remains susceptible and constitutes a reservoir for that disease (Chartier et al., 2009).

One of the main features observed for the BTV-8 strain that circulated in northern Europe is its ability to cross the placental barrier as reported in numerous experimental (Backx et al., 2009; Worwa et al., 2009; van der Sluijs et al., 2011) and field (De Clercq et al., 2008; Desmecht et al., 2008; Menzies et al., 2008; Zanella et al., 2012; Saegerman et al., 2011) observations in cows and ewes. Transplacental transmission rate varied according to the studies, from around 10% (De Clercq et al., 2008; Santman-Berends et al., 2010) to 41.7% (Batten et al., 2009) in cows, and can reach as much as 69% in mid-pregnancy infected ewes (van der Sluijs et al., 2011). One of the consequences of this transplacental transmission was the birth of RT-PCR positive calves after the transportation of BTV seropositive pregnant heifers in Northern Ireland, which led to the introduction of BTV-8 in a previously free region (Menzies et al., 2008).

Transplacental transmission has never been described earlier with a wild type BTV strain but has been reported with attenuated vaccine strains belonging to serotypes 10, 11, 13 and 17 (Maclachlan et al., 2000). This type of transmission appeared to be limited to cell culture adapted viruses, which led some authors to postulate that the European BTV-8 strain has acquired either cell culture adapted strains' properties or attenuated vaccine strains properties (Maclachlan et al., 2009).

Interestingly, transplacental transmission in ovine, bovine and caprine species could be involved in the overwintering and introduction of BTV in free regions as described in Northern Ireland (Menzies et al., 2008).

Congenital anomalies (*e.g.* hydranencephaly, cavitary encephalopathy, cerebral hyperaemia) have been observed in the field as well as in experimental studies following transplacental BTV-8 infection of foetuses (Vercauteren et al., 2008; Maclachlan and Osburn, 2008; Wouda et al., 2008; van der Sluijs et al., 2011; Saegerman et al., 2011). These congenital anomalies mainly affect the nervous tissue because of the neurological tropism of BTV-8 in the foetus, especially for non-differentiated cells of the brain during development. Such congenital anomaly or birth defect is determined by the date of infection during pregnancy and therefore by the stage of foetal development.

This report presents two experiments conducted in order to investigate the possible transmission of BTV-8 *via* the placenta at mid-pregnancy (infection at 61 days pregnancy) and end-pregnancy (infection at 135 days pregnancy), and possible birth defects associated with the transplacental transmission.

## 2. Materials and methods

### 2.1. Animals

In the first experiment, thirteen goats (10 Alpine and 3 Saanen) aged 10 months old on average were included. The animals came from a farm in the West of France (Deux-Sèvres) with no history of major disease (no abortion reported) and not implementing vaccination against bluetongue. Goats had been cycled, and 2 billy-goats were added for directed mating on November 9, 2010. A pregnancy diagnosis by ultrasound was performed in mid-December 2010 on the farm. Goats were 61 days pregnant at the beginning of the experiment.

For the second experiment, sixteen Saanen goats, aged about 1 year old on average and nearly at the same stage of pregnancy (about 4 months and a half) were selected. Directed mating without previous oestrus cycling was performed, and pregnancy was assessed by ultrasonography. The animals came from a conventional farm in the Center of France (Corrèze) with no bluetongue history and without vaccination against bluetongue. No history of abortion was reported by the farmer for the last five years.

In both experiments (conducted separately), animals were housed in two high containment units (ABSL3) (Centre de Recherche Biomédicale, Maisons-Alfort, France, agreement number 94-046-2) throughout the inoculation phase. The local ethical committee approved the experimental protocol (dossier n. 10-0005). One week before the beginning of the experiment, pregnancy was confirmed by ultrasonography. Goats were also confirmed negative for the presence of BTV (by RT-PCR for the detection of all serotypes of BTV) or anti-BTV antibodies (by VP7 ELISA). Finally, a comprehensive examination of all goats was carried out to ensure the absence of any intercurrent disease. Preventive antibiotic (tulathromycin, Draxxin®, Pfizer Animal Health) and anti-inflammatory (carprofen, Rimadyl®, Pfizer Animal Health) treatment was administered to all animals upon arrival, in the absence of clinical signs, to limit the risk of developing respiratory symptoms related to transport and housing in confined rooms, which could interfere with the detection of BTV-8 related clinical signs.

The animals were identified by their number on ear tags, and foetuses or kids (depending on the experiment) were subsequently identified by the number of the dam's tag followed by a letter (A, B, C or D) corresponding to the order of umbilical cord blood sampling and necropsies (first experiment) or kidding (second experiment).

### 2.2. Inocula

The inoculum used to infect goats consisted in the supernatant of cultured BHK cells infected with a BTV-8 wild strain. This virus was isolated in France in 2008 from a bovine blood sample and was amplified on embryonated eggs before a passage on BHK21 cells.