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Transplacental transmission of BTV-8 in sheep: BTV viraemia, antibody responses and vaccine efficacy in lambs infected in utero

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ABSTRACT

Bluetongue virus (BTV) is an insect vector transmitted virus which causes an economically important disease in ruminants. BTV infection during pregnancy can result in infection of the foetus, which may lead to the birth of persistently infected or immunotolerant offspring. Since persistently infected animals continuously produce large amounts of virus they could be a source of infection for the insect vector. This could significantly influence the epidemiology of the virus and hence might require additional measures to control a BTV outbreak.

Therefore, we investigated the potential of BTV-8 to induce persistent infection or immunotolerance in lambs in an experimental setting.

Infection of eighteen 70–75 days pregnant ewes with wild type BTV-8 led to the birth of 25 out of 44 BTV RNA positive lambs (foetal infected, FI). All 23 FI lambs born alive also had anti BTV antibodies at birth; infectious virus could be recovered from 5 out of 25 FI lambs.

Viral RNA loads decreased rapidly after birth; 19 out of 20 FI lambs that remained in the experiment until week 14 after birth, were RNA negative at that time. Since persistence of BTV-8 infection could not be demonstrated, we investigated whether foetal infection had an effect on protection against a field virus infection and on efficacy of vaccination. To this end, 5 FI lambs and 5 foetal non-infected (FNI) lambs were vaccinated with the inactivated Bovilis® BTV-8 vaccine, five months after birth. Three weeks after the vaccination, all lambs were infected with wild type BTV-8. The foetal infection did not interfere with vaccination efficacy. In contrast, foetal BTV-8 infection induced an immune response which afforded protection against BTV challenge comparable to the level of protection induced by vaccination.

1. Introduction

Bluetongue virus (BTV), an Orbivirus (Reoviridae), causes disease in ruminants characterized by fever, oedema, and ulceration of the mucosae [1]. The virus is mainly transmitted by certain *Culicoides* species.

Various means of vaccination have been used to prevent BTV infection already since 1908 [2]. The use of a live, chicken embryo passaged BTV vaccine in pregnant ewes in the USA led to transplacental transmission of the virus, resulting in severe teratogenic effects [3], which later have been reproduced experimentally [4–10]. There has been some controversy on whether BTV could induce viral persistence or merely a prolonged infection. Luedke et al. [11,12] reported the induction of persistent infection and immunotolerance after foetal BTV infection of cattle. However, more recent experiments in pregnant sheep and cattle were unsuccessful in inducing persistent infection or immunotolerance [4,5,9].

BTV-8\net 2006, the strain which caused the European outbreak, has shown the ability to transmit to the foetuses in the field [13,14], with similar teratogenic effects [15–18]. Although the birth of immunotolerant (i.e. RT-PCR positive, antibody negative) calves has been described, the follow-up of these calves is limited [15,19,20].

This paper describes, for the first time, the effects of a foetal BTV-8 infection on lambs after birth and on BTV vaccine efficacy later in life.

In our experimental setting, persistent BTV-8 infection or immunotolerance could not be induced. In contrast, foetal infection induced protection against BTV-8 infection similar to vaccination with an inactivated vaccine.

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2. Materials and methods

2.1. Animals

Twenty-one Swifter ewes were as described earlier [21] and mated with three rams.

Nine weeks after mating, the pregnant ewes were relocated from the conventional stables to the isolation facilities. The animal room had been treated with cyfluthrin (Solfac®, Bayer CropSciences); the ewes were treated with deltramethrin (Butox® MSDAH) every 8 weeks until the end of the experiment.

Blood samples taken just before transport of the ewes to the isolation facilities were free from BTV, BTV antibodies and pestiviruses.

The lambs were separated from the ewes immediately after birth, marked with individual ear marks in the order of birth and transferred to a different isolation room. Stillborn lambs were given the earmark number of the ewe with a suffix number. All ewes were euthanized immediately after parturition.

The lambs were fed maximum 400 ml BTV antibody negative bovine colostrum. Thereafter, the lambs were fed commercial lamb milk replacer (Bellam Uniek, Hendrix UTD, Boxmeer, The Netherlands), hay, and concentrates.

2.2. Design of the experiment

The design of the experiment was approved by an independent ethical committee. The experiment was conducted in compliance with national and European regulations on animal experiments. Ten weeks after mating, 18 randomly chosen pregnant ewes were inoculated subcutaneously with 10 ml BTV-8. Three other pregnant ewes served as untreated controls. The ewes were observed daily for clinical signs and for signs of abortion. Blood samples for detection of antibodies and viraemia were taken at regular intervals until parturition. From birth onwards, the general health of the lambs was monitored daily. Blood samples were taken immediately after the birth of the lambs (before the ingestion of colostrum) and every 1–4 weeks until 14 weeks after birth. Post mortem examinations were performed on lambs which were stillborn or died in the course of the experiment; tissue samples from spleen, liver, thymus, cerebrum and cerebellum were taken for virus detection.

Fourteen weeks after birth, 20 lambs were selected for the vaccination experiment: 10 lambs that were BTV RT-qPCR positive at birth (designated foetal infected FI) and 10 lambs that were BTV RT-qPCR negative at birth, 9 of which were born to the control ewes (foetal non-infected FNI). The remaining lambs were euthanized.

At the age of 25 weeks, 5 of the FI and 5 of the FNI lambs were vaccinated with 1 ml Bovilis® BTV-8 vaccine in accordance with the manufacturer's instructions. The vaccines were administered subcutaneously in the axillary fold using 21 G × 40 mm disposable needles. The other 10 lambs were used as unvaccinated controls. Three weeks after the vaccination, all lambs were inoculated subcutaneously with 10 ml BTV-8. Body temperature measurements and clinical observations were done daily. Blood samples were taken every 2–4 days to monitor viraemia and antibody response. Three weeks after the challenge inoculation, all lambs were euthanized.

2.3. Vaccines and inocula

The BTV-8 inoculum OvBTV-8Ver1291007 07K07 (titre 7.3 $\log_{10}$ $TCID_{50}$/ml) was produced as described earlier [21] and diluted in culture medium to 3.3 $\log_{10}$ $TCID_{50}$/ml for the inoculation of the ewes and 6.3 $\log_{10}$ $TCID_{50}$/ml for the inoculation of the lambs.

Bovilis® BTV-8 (lot B651A, MSDAH) contains inactivated BTV-8 as antigen and aluminium hydroxide and saponine as adjuvant.

2.4. Virus isolation (VI)

EDTA blood samples were treated as described earlier [22].

Approximately 0.5 cm^3 of tissue sample was homogenized in 2 ml culture medium containing antibiotics and antimycotics. A volume of 1 ml of blood or tissue homogenate was incubated for 1–1.5 h on a 1 day old, 25 cm^2 Vero cell monolayer. After 7 days of incubation at 37 °C and 5% CO_2, the monolayers were examined microscopically for the presence of cytopathogenic effect (cpe). If no cpe was present, the monolayers were propagated and incubated for another 7 days. This step was repeated twice. If the cpe was ambiguous, the presence of BTV was confirmed by an immunofluorescence test (IF) or by RT-qPCR on the supernatant as described before [21].

2.5. RT-qPCR

Spleen samples of stillborn lambs were homogenized using the MagNa Pure LC Total Nucleic Acid Isolation Kit in the MagNa Lyser (Roche Diagnostics). The samples were shaken 5 times for 20 s at 7000 rounds per minute (rpm), and cooled for 30 s in between. After centrifugation, the tissue samples were lysed at 70 °C for 10 min.

Viral RNA was extracted from duplicates of 200 μl of tissue or blood sample using the MagNa Pure 96 System extraction robot (Roche Diagnostics) and the MagNA Pure 96 DNA and Viral NA Small Volume Kit. RNA was eluted in 50 μl and 6 μl was tested in a validated real-time reverse transcriptase polymerase chain reaction (RT-qPCR) designed to detect all serotypes of BTV [23], with minor modifications. The two Ct values of the duplicates were averaged; a value of 46.00 was used if no CT value was obtained. Based on the likelihood of isolating infectious virus from a sample, Ct values ≥35 were interpreted as negative, values <30 as positive and values between 34.99 and 30.00 as doubtfully positive. For the estimation of the duration of the viraemia, doubtfully positive samples were regarded as positive.

2.6. BTV specific antibody tests

BTV antibodies were detected using the VMRD® competitive inhibition antibody ELISA in accordance with the manufacturer's instructions.

Neutralizing antibodies were detected using a virus neutralization test (VN) in accordance with the description in Moulin et al. [24].

3. Results

3.1. Transmission experiment

3.1.1. Ewes

All ewes were negative for BTV RNA at the start of the experiment. The three non-infected control ewes remained negative throughout the experiment.

All BTV infected ewes except from ewe 3153 were BTV RNA positive during at least 2 weeks after the infection; ewe 3153 remained doubtfully positive during the whole post inoculation period.

At 8 weeks after the inoculation, all BTV infected ewes were still BTV RNA positive (15/18 ewes) or doubtfully positive (3/18 ewes).

Ewe 3147 died from the complications of a prolapsed uterus 1 week before the expected parturition date; unfortunately, her lambs also died.

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