Veterinary Microbiology 154 (2012) 257–265

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Experimental infection of South American camelids with bluetongue virus serotype 8

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ARTICLE INFO

Article history:
Received 23 December 2010
Received in revised form 9 April 2011
Accepted 21 July 2011

Keywords:
Bluetongue virus
BTV-8
South American camelids
Experimental infection
Blood cell binding

ABSTRACT

Bluetongue (BT) is an infectious, non-contagious disease of wild and domestic ruminants. It is caused by bluetongue virus (BTV) and transmitted by *Culicoides* biting midges. Since 1998, BT has been emerging throughout Europe, threatening not only the naïve ruminant population. Historically, South American camelids (SAC) were considered to be resistant to BT disease. However, recent fatalities related to BTV in captive SAC have raised questions about their role in BTV epidemiology.

Data on the susceptibility of SAC to experimental infection with BTV serotype 8 (BTV-8) were collected in an animal experiment. Three alpacas (*Vicugna pacos*) and three llamas (*Lama glama*) were experimentally infected with BTV-8. They displayed very mild clinical signs. Seroconversion was first measured 6–8 days after infection (dpi) by ELISA, and neutralising antibodies appeared 10–13 dpi. BTV-8 RNA levels in blood were very low, and quickly cleared after seroconversion. However, spleens collected post-mortem were still positive for BTV RNA, over 71 days after the last detection in blood samples. Virus isolation was only possible from blood samples of two alpacas by inoculation of highly sensitive interferon alpha/beta receptor-deficient ($IFNAR^{-/-}$) mice.

An *in vitro* experiment demonstrated that significantly lower amounts of BTV-8 adsorb to SAC blood cells than to bovine blood cells.

Although this experiment showed that SAC are generally susceptible to a BTV-8 infection, it indicates that these species play a negligible role in BTV epidemiology.

1. Introduction

Bluetongue (BT) is an infectious, non-contagious, multi-species disease (Afshar et al., 1995, reviewed by MacLachlan et al., 2009; Meyer et al., 2009) caused by bluetongue virus (BTV), an *Orbivirus* that belongs to the family *Reoviridae*. The main infection route for BTV is transmission by certain species of *Culicoides* biting midges (reviewed by Mellor and Wittmann, 2002). Currently, at least 24 serotypes of BTV have been described worldwide.

The severity of disease caused by BTV varies depending on the virus strain, host species, breed and individual resistance (MacLachlan, 1994; Darpel et al., 2007, reviewed by MacLachlan et al., 2009). In regions where BTV is endemic, it does not cause obvious disease in wild ruminants (Verwoerd and Erasmus, 2004), Old World camelids (Wernery et al., 2008) or in indigenous livestock breeds (MacLachlan, 1994).

Since 2006, BTV serotype 8 (BTV-8) has spread throughout Western and Central Europe causing a severe epizootic in sheep and, notably, in cattle (Saegerman et al.,

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doi:10.1016/j.vetmic.2011.07.025

2008; Dal Pozzo et al., 2009). In summer 2008, a serological survey was carried out in Switzerland. None of the 354 South American camelids (SAC) tested by a competitive ELISA were positive for BTV antibodies (Zanolari et al., 2010), even though Switzerland was affected by the BTV-8 epizootic at the time.

While field surveys and infection experiments in European wild ruminants (López-Olvera et al., 2010; Linden et al., 2010) and in Old World camels (Batten et al., 2010) suggest that those play a potential role as reservoirs for BTV, the relevance of SAC in BTV epidemiology is still unknown.

Serological evidence of subclinical infection in SAC (Rivera et al., 1987; Mattson, 1994) indicates a resistance to BT disease. This, however, is called into question by recent reports of BTV-related fatalities (Henrich et al., 2007; Meyer et al., 2009; Ortega et al., 2010).

Experimental BTV infections have only been conducted in two llamas (*Lama glama*) (BTV-10) (Afshar et al., 1995)—to evaluate a competitive ELISA (cELISA)—and in three dromedary camels (BTV-1) (Batten et al., 2010). BTV was detected in the dromedaries in a real-time quantitative reverse transcription-PCR (RT-qPCR) and was isolated in cell culture (Batten et al., 2010). The llamas and dromedaries seroconverted after BTV infection, but no clinical signs were observed in any animal (Afshar et al., 1995; Batten et al., 2010).

The unknown role of SAC in BTV epidemiology together with the reported fatalities prompted further investigations of BTV infection in SAC.

To this end, a small-scale animal experiment has been conducted to collect data on the susceptibility of SAC to BTV-8 infection, as well as reference material for the validation of serological and virological assays.

An *in vitro* experiment with blood from cattle, pigs, alpacas and llamas was conducted to investigate the adsorption behaviour of BTV to camelid erythrocytes.

2. Material and methods

2.1. Animal experiment

2.1.1. Animals and experimental design

Three alpaca males (*Vicugna pacos*; age: 8, 15 and 18 months) and three llama males (*Lama glama* age: 7 months) were obtained from German breeders in January 2010. To exclude a previous exposure to BTV, serum and whole blood samples were examined with an ELISA (PrioCHECK® BTV DR, Prionics Deutschland, Planegg-Martinsried, Germany) and RT-qPCR (see below). Blood collection and animal transport were conducted outside the main vector season, when a BTV infection is very unlikely to occur (Wilson et al., 2007). Seven days before the experiment, all animals were housed in species groups in the containment facility of the Friedrich-Loeffler-Institut, Isle of Riems, Germany, with a 12 h light regimen. Feed and water were provided *ad libitum*. Blood samples were taken one day before the experiment to evaluate haematological parameters and to prove them free of BTV antibodies and BTV RNA (see respective sections below).

For BTV infection, the SAC were subcutaneously injected at multiple sites in the freshly clipped shoulder and dorsal thorax regions. Each animal received 10^5 TCID50 (50% tissue culture infective doses) of a recent German BTV-8 isolate (Eschbaumer et al., 2009) in 4 ml of tissue culture supernatant diluted in phosphate-buffered saline (PBS). Briefly, a Holstein calf had been inoculated with pooled blood from a BT outbreak in sheep. Virus was isolated from the calf and passaged twice on Vero cells (RIE15, Collection of Cell Lines in Veterinary Medicine, FLI Insel Riems).

The inoculum used for the SAC contained 5×10^8 BTV-8 genome copies/ml (quantification cycle (C_q) value 18.8). It was proven to be free of any bacterial contamination. After inoculation, whole blood and serum samples were taken at regular intervals. EDTA blood was stored at 4 °C, and serum samples were harvested within 4 h of collection, inactivated at 56 °C for 30 min, and stored at 4 °C until analysis.

Only three animals per species were available. Because three replicates were considered the absolute minimum for a meaningful experiment, it was decided to inoculate all animals and not to use a control group.

2.1.2. Clinical and haematological parameters

During the entire study, rectal body temperatures and close clinical monitoring were performed daily from 1 day before to 15 days post infection (dpi) (Fowler, 1998a; Hengrave Burri et al., 2005). This included examination of the eyes, conjunctival membranes, oral and nasal mucosa, auscultation of the heart and lungs, palpation of mandibular, axillar, cervical, inguinal and popliteal lymph nodes, examination of the skin of the coronary band and interdigital spaces for lesions and increased temperature. Blood samples taken on days −1, 2, 6, 8, 10, 13, 16, 20, 24, 28 and 35 were tested in a Cell-dyn® 3700 Hematology Analyzer (Abbott Laboratories, Abbott Park, IL, USA) using the 'goat' analysis profile.

2.1.3. Post-mortem examination

Euthanasia and post-mortem examination of all SAC was performed on 106 dpi. All animals were sedated by intramuscular (i.m.) application of 0.5 ml (llamas) or 0.3 ml (alpacas) of 1% detomidine (Cepesedan® RP, CP-Pharma, Burgdorf, Germany) and led to the necropsy room. For anaesthesia, each animal received an i.m. injection of 250 mg xylazine powder (Rompun® TS, Bayer HealthCare, Leverkusen, Germany) dissolved in 2 ml of 10% ketamine (Medistar, Ascheberg, Germany) ("Hellabrunner Mischung"; Wiesner, 1998). Then they were euthanized by intravenous injection of 25 ml of T61® (Intervet, Unterschleissheim, Germany) and exsanguinated through the jugular veins.

Spleen material of all SAC was collected for RT-qPCR analysis and for virus isolation in IFNAR$^{-/-}$ mice and tissue culture (see respective sections).

2.1.4. Serology

Serological data were collected from samples taken on −1, 2, 6, 8, 10, 13, 16, 20, 24, 28, 35, 48, 62, 70, 77, 83, 90, 97 and 106 dpi using two commercial test kits: the PrioCHECK® BTV double recognition ELISA (DR ELISA) and the