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Schmallenberg virus experimental infection of sheep

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ABSTRACT

Since late 2011, a novel orthobunyavirus, named Schmallenberg virus (SBV), has been implicated in many cases of severely malformed bovine and ovine offspring in Europe. In adult cattle, SBV is known to cause a mild transient disease; clinical signs include short febrile episodes, decreased milk production and diarrhoea for a few days. However, the knowledge about clinical signs and pathogenesis in adult sheep is limited.

In the present study, adult sheep of European domestic breeds were inoculated with SBV either as cell culture grown virus or as virus with no history of passage in cell cultures. Various experimental set-ups were used. Sampling included blood collection at different time points during the experimental period and selected organ material at autopsy.

Data from this study showed, that the RNAemic period in sheep was as short as reported for cattle; viral genome was detectable for about 3–5 days by real-time RT-PCR. In total, 13 out of 30 inoculated sheep became RNAemic, with the highest viral load in animals inoculated with virus from low cell culture passaged or the animal passaged material. Contact animals remained negative throughout the study. One RNAemic sheep showed diarrhoea for several days, but fever was not recorded in any of the animals. Antibodies were first detectable 10–14 days post inoculation. Viral RNA was detectable in spleen and lymph nodes up to day 44 post inoculation.

In conclusion, as described for cattle, SBV-infection in adult sheep predominantly results in subclinical infection, transient RNAemia and a specific antibody response. Maintenance of viral RNA in the lymphoreticular system is observed for an extended period.

1. Introduction

In late 2011 a novel orthobunyavirus of the Simbu serogroup, referred to as "Schmallenberg virus" (SBV), was discovered near the German-Dutch border and thereafter spread rapidly to other European countries (European Food Safety Authority, 2012; Hoffmann et al., 2012). Recently, it has been shown that SBV is most related to Douglas and Sathuperi virus (Goller et al., 2012). Affected adult cattle

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show none or only mild clinical signs, however, an infection of SBV-naïve cows and ewes during a critical period of pregnancy may lead to severe foetal malformations (Garigliany et al., 2012; Herder et al., 2012); reviewed in (Beer et al., 2013; Wernike et al., 2013b). Insect vectors play an essential role in the spread of SBV; viral genome has been detected in different field collected *Culicoides* spp. (De Regge et al., 2012; Rasmussen et al., 2012).

Based on an experimental infection study with calves, the viraemic period in cattle seems to be short (Hoffmann et al., 2012). However, very little is known about the duration of viraemia as well as the progress of the disease and development of antibodies to SBV in adult sheep. In the present study, two different types of inoculum were compared regarding the ability to induce RNaemia and humoral immunity in sheep: culture-grown virus and an SBV field strain that was passaged in cattle only (delivered as infectious serum). In addition, to investigate the tissue tropism and potential persistence in any of the organ systems, the animals were euthanized at different time points after infection and analyzed for the presence of SBV.

2. Materials and methods

All experimental protocols were reviewed by a state ethics commission and have been approved by the competent authority (Denmark: Danish Animal Experimentation Inspectorate, licence no. 2008/561-1541; France: "Secrétariat Permanent du Comité d'Ethique en Expérimentation Animale Val de Loire" C 37-175-3, 21 of June 2012; Germany: State Office for Agriculture, Food Safety and Fisheries of Mecklenburg-Vorpommern, Rostock, Germany, ref. LALLF M-V TSD/7221.3-1.1-004/12).

2.1. Inocula

Two different types of inoculum were used in the different experiments: (1) cell culture-grown SBV, and (2) cattle serum containing SBV.

Cell culture grown SBV was produced as follows: For group A and B, virus was isolated from cow blood on KC cells (cell line L1062, Collection of Cell Lines in Veterinary Medicine, Friedrich-Loeffler-Institut, Insel Riems, Germany; derived from Culicoides variipennis midges (Wechsler et al., 1991)). After initial isolation on KC cells as previously described (Hoffmann et al., 2012) the virus was passaged five times in baby hamster kidney (BHK) cells (cell line L0164) (inoculum I: KC/BHK_5), alternatively once in BHK cells, again in KC cells and again in BHK cells (inoculum II: KC/BHK/KC/BHK). For group C, virus was isolated from lamb brain and passaged 3 times on Vero cells (inoculum III: Vero). Inoculum I contained $10^{6.2}$ tissue culture infectious doses per ml ($TCID_{50}$/ml), inoculum II 10^7 $TCID_{50}$/ml and inoculum III contained 10^8 $TCID_{50}$/ml, as determined by end-point titration on the respective cells.

Cattle serum containing SBV was collected for group E and F. Briefly, a whole blood sample of a SBV-positive cow was injected into another heifer, two and three days after inoculation serum was taken, tested in further cattle regarding infectivity and stored at −70 °C until use (Wernike et al., 2012).

2.2. Sheep and experimental design

The animal experiments were conducted at different time points in the BSL-3 facilities of the National Veterinary Institute, Lindholm, Denmark, of the Plate-Forme d'Infectiologie Expérimentale, Institut National de la Recherche Agronomique, Tours, France and of the Friedrich-Loeffler-Institut, Insel Riems, Germany.

An overview of the experimental setup is shown in Fig. 1. In total 37 SBV-naïve adult sheep of European domestic breeds were assigned to 7 groups. Male and female animals were distributed equally. Twenty-three sheep in 3 groups were inoculated with culture-grown virus: 4 sheep received 1 ml of inoculum I subcutaneously (s.c.) (group A), 3 animals received 1 ml of inoculum II s.c.

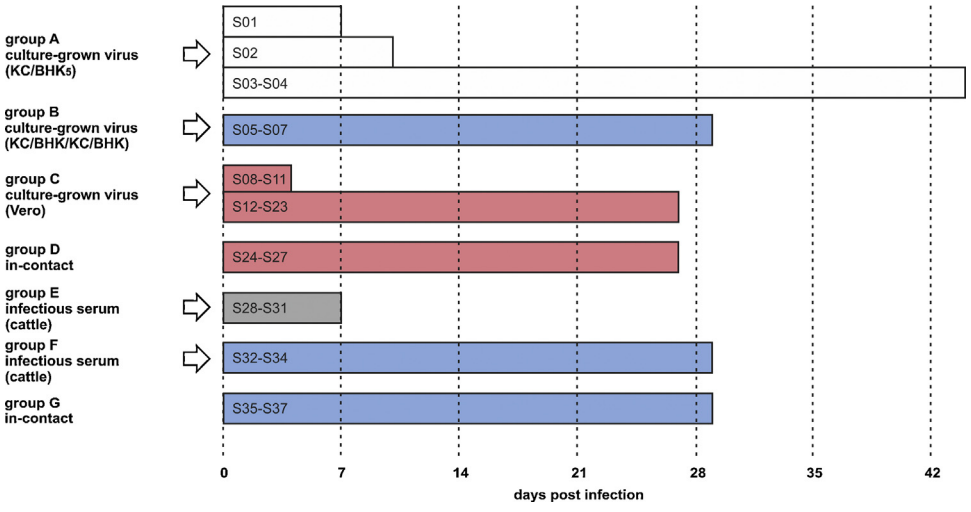

Fig. 1. Experimental design. Animal groups highlighted in the same colour were housed together. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)