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Rasmussen, Lasse Dam; Savini, Giovanni; Palmarini, Massimo; Rasmussen, Thomas Bruun; Belsham, Graham; Bøtner, Anette

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ANALYSIS OF VIREMIA AND TRANSPLACENTAL TRANSMISSION OF FIELD AND RESCUED STRAINS OF BTV-2 AND BTV-8 FOLLOWING INOCULATION OF PREGNANT SHEEP

Lasse Dam Rasmussen[1], Giovanni Savini[2], Massimo Palmarini[3], Thomas Bruun Rasmussen[1], Graham J. Belsham[1] & Anette Bøtner[1].
[1]National Veterinary Institute, DTU, Lindholm, Denmark. [2]Istituto G. Caporale, Teramo, Italy
[3]Centre for Virus Research, University of Glasgow, Scotland.

Objectives

Live bluetongue virus (BTV) vaccine-strains and also, surprisingly, the European strain of BTV-8 can cross the placental-barrier and thus pass from one generation of animals to another without involvement of the insect vector.

A better understanding of the genetic basis for the transmission characteristics of the virus would help to identify the risks posed by further BTV incursions and facilitate the design of better control strategies. The development of reverse genetics for BTV enables investigation of the genetic traits conferred by individual genome segments within rescued viruses by making defined reassortants. To date, only a few experiments have investigated whether field and rescued virus strains behave similarly *in vivo.*

Methods

Twenty-four sheep (in 4 groups of 6) were inoculated (s.c.) with 4 strains of BTV in late pregnancy (approx. 1 month before lambing). The viruses used were: BTV-2 wt (Italian field strain), BTV-2 (rescued), BTV-8 wt (field strain from the Netherlands) and BTV-8 (rescued). Four sheep were non-inoculated controls. Blood samples from the sheep were tested frequently for viremia and anti-BTV antibodies (by ELISA) in the period until lambing. Pre-colostral blood samples were collected from all newborn lambs, except for one born dead, to determine if transplacental transmission had occurred. Milk from ewes was collected daily for 7 days after lambing and blood samples from the lambs were collected on days 0, 3 and 7 after birth. All samples have being tested for the presence of anti-BTV antibodies and for virus (RT-qPCR).

Results

All inoculated animals developed viremia. The viremia was significantly higher at all sampling points following inoculation ($p<0.01$ or $p< 0.05$, Mann-Whitney's U Test) in animals inoculated with BTV-2 wt compared to animals inoculated with BTV-2 rescued, whereas no significant difference was detected between BTV-8 wt and BTV-8 rescued. Wild type virus infected animals had a longer lag phase before antibodies were detected but the response increased at a faster rate. Some of the animals displayed clinical signs of infection, e.g. fever and panting. All the ewes delivered one lamb each, a few lambs born early did not thrive and were euthanized but most appeared healthy. Seven of the 28 lambs had been infected transplacentally; 2 from ewes inoculated with BTV-2 wt, 3 from ewes inoculated with BTV-2 rescued and 1 from a ewe inoculated with BTV-8 wt. The last infected lamb was from a non-inoculated control sheep, in the same stable but physically separated from, the BTV-2 wt inoculated ewes and became viremic with BTV-2 10 days after the others were inoculated.

Conclusion

Both wild-type and rescued BTVs induced viremia. Surprisingly, transplacental transmission occurred more frequently in ewes inoculated with BTV-2, both wt and rescued, than in ewes inoculated with BTV-8. The BTV-2 wt was passaged once in Kc and once in CPT-Tert cells. These very few passages may be enough to introduce changes enabling the virus to cross the placental barrier. This experiment indicates it will be difficult to identify a single BTV segment responsible for transplacental transmission in sheep using rescued BTV-2 and BTV-8 strains.