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EXPERIMENTALLY INDUCED DISEASE

Lambs are Susceptible to Experimental Challenge with Spanish Goat Encephalitis Virus

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Summary

Spanish goat encephalitis virus (SGEV) is a member of the genus *Flavivirus*, family Flaviviridae, and causes encephalomyelitis in goats. The aim of this study was to determine whether sheep are susceptible to experimental challenge with SGEV by two different routes. The results show that SGEV can infect sheep by both the subcutaneous and intravenous routes, resulting in neurological clinical disease with extensive and severe histological lesions in the central nervous system. Lambs challenged subcutaneously developed more severe lesions on the ipsilateral side of the brain, but the lesion morphology was similar irrespective of the route of challenge. The clinical presentation, pathogenesis, lesion morphology and distribution shows that SGEV is very similar to louping ill virus (LIV) and therefore any disease control plan must take into account any host species and SGEV vectors as potential reservoirs. Furthermore, discriminatory diagnostics need to be applied to any sheep or goat suspected of disease due to any flavivirus in areas where SGEV and LIV co-exist.

Keywords: experimental infection; immunology; lamb; Spanish goat encephalitis virus

Introduction

Spanish goat encephalitis virus (SGEV) is a member of the genus *Flavivirus*, family Flaviviridae (Mansfield *et al.*, 2015), and causes encephalomyelitis in goats (Balseiro *et al.*, 2012); however, it is still uncertain if goats are the primary host. Whole genome comparison showed that SGEV shares 89.4% homology with Spanish sheep encephalitis virus (SSEV) and between 88.7 and 89.3% homology with three louping ill virus (LIV) isolates (Mansfield *et al.*, 2015). All three viruses (SGEV, SSEV and LIV) are members of the tick-borne flavivirus lineage and SGEV is considered to be a subspecies within the louping ill species of tick-borne flaviviruses. LIV is endemic in upland areas of the UK where sheep and red grouse are considered to be the reservoir (Jeffries *et al.*, 2014). The commercially available vaccine to protect sheep against LIV (Louping ill BP vaccine, MSD Animal Health, Milton Keynes, UK) has been shown to protect goats against experimental challenge with SGEV (Salinas *et al.*, 2017), but it is unknown if sheep are susceptible to infection with SGEV.

The aims of this study were: (1) to determine whether sheep are susceptible to experimental challenge with SGEV, by two different routes, and describe any resultant viraemia, pathogenesis and immune response, and (2) to compare these parameters with a previously reported SGEV experimental challenge of goats (Salinas *et al.*, 2017).

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Materials and Methods

Experimental Animals

Twenty-two female lambs (Assaf breed) were sourced from Castilla y León, where no cases of SGEV have ever been reported. The lambs were raised in isolation under tick-free conditions in order to avoid potential exposure to SGEV or any other tick-borne virus, treated preventively with Butox® (Intervet Laboratories, Carbajosa, Spain) to further prevent the possibility of tick infestation and subsequently housed in biocontainment level 2 facilities. Once in the experimental facilities, the animals were allocated randomly to one of three separately housed groups: two groups of nine lambs and a further group of four. The latter were used as negative controls. Sampling and challenge procedures were approved by the Animal Research Ethics Committee of the Community of Junta de Castilla y León, Spain (reference number ULE_010_2015). Experiments were conducted in accordance with the current Spanish and European legal requirements and guidelines regarding experimentation and animal welfare.

Culture of SGEV and Experimental Challenge

SGEV was grown in cell culture and titrated as described elsewhere (Salinas *et al.*, 2017). Briefly, SGEV was adapted for growth in baby hamster kidney (BHK)-21 cells. BHK-21 cells were grown in Glasgow medium supplemented with 0.37% (weight/volume) sodium bicarbonate, 5% tryptose phosphate broth, 2 mM L-glutamine, 10% fetal calf serum and antibiotics, and used for the propagation and titration of SGEV.

The virus stock had a titre of 1.4×10^8 plaque forming units (PFU)/ml in BHK-21 cells and it was diluted in BHK-21 cell culture to 1.0×10^7 PFU/ml for experimental challenge in lambs.

One group ($n = 9$, group SC) was challenged subcutaneously on the right thorax caudal to the elbow with 1 ml of a suspension containing 1.0×10^7 PFU/ml in BHK-21 cells. A second group ($n = 9$, group IV) was challenged intravenously via the jugular vein with an identical dose. The remaining group ($n = 4$) were unchallenged negative controls.

Serum Sampling, Serology and Evaluation of Clinical Signs

Prior to challenge all animals were tested using the FSME (tick-borne encephalitis [TBE]) IgM and IgG enzyme-linked immunosorbent assays (ELISAs) (PROGEN®, Heidelberg, Germany), which recognize antibodies to any TBE due to flaviviruses, including LIV (Klaus *et al.*, 2014), to confirm the absence of previous exposure to any European TBE flavivirus. Blood samples, for harvesting serum, were taken from the jugular vein on the day of challenge, daily for 8 days post challenge (dpc) and at days 11, 15 and 16 dpc until sacrifice. Blood samples were centrifuged at 240 g for 5 min, the sera removed and stored at −80°C until examined for IgM and IgG levels by ELISA as described above. Rectal temperatures were taken prior to challenge and at intervals of 24 h post challenge (hpc) and clinical signs, including general condition and neurological signs, were monitored daily with the severity designated as: 0, no clinical signs; 1, dullness, loss of condition and staring coat; 2, in addition to changes for a score of (1), neurological clinical signs (e.g. tremors, ataxia or incoordination). The experiment was terminated at 12 or 17 dpc by intravenous overdose of pentobarbital (0.3 ml/kg) (Table 1).

Real-time Reverse Transcriptase Polymerase Chain Reaction

Serum samples were subjected to specific TaqMan quantitative reverse transcriptase polymerase chain

Table 1
Clinical signs and histopathological lesion scores

Lamb ID	*Day of necropsy post challenge*	**Severity of clinical signs (day of onset)*	*†Severity of histological lesions*
Subcutaneous route			
14	12	0	III
18	17	1 (4)	IV
19	12	2 (4)	I
21	12	0	III
25	17	0	III
26	12	0	III
28	17	2 (12)	IV
29	17	2 (9)	III
50	17	0	0
Intravenous route			
00	17	0	III
01	12	2 (4)	III
05	12	0	III
15	12	2 (2)	IV
16	17	2 (3)	III
22	17	2 (13)	I
24	12	0	I
30	17	2 (8)	IV
32	17	2 (3)	III

*Neurological clinical signs: 0, none; 1, dullness, loss of condition and staring coat; 2, in addition to the previous clinical signs (in 1), neurological clinical signs such as tremors, ataxia and incoordination.
†Histological lesion score: 0, none; I, very mild with only perivascular cuffing; II, mild with perivascular cuffing and a small number of glial foci; III, moderate with numerous perivascular cuffs, numerous glial foci, neuronophagia, necrosis of Purkinje cells and meningitis; IV, severe with frequent perivascular cuffing, diffuse gliosis and neuron necrosis.

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