EXPERIMENTALLY INDUCED DISEASE

Vaccination against Louping Ill Virus Protects Goats from Experimental Challenge with Spanish Goat Encephalitis Virus

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Summary

Spanish goat encephalitis virus (SGEV) is a recently described member of the genus Flavivirus belonging to the tick-borne encephalitis group of viruses, and is closely related to louping ill virus (LIV). Naturally acquired disease in goats results in severe, acute encephalitis and 100% mortality. Eighteen goats were challenged subcutaneously with SGEV; nine were vaccinated previously against LIV and nine were not. None of the vaccinated goats showed any clinical signs of disease or histological lesions, but all of the non-vaccinated goats developed pyrexia and 5/9 developed neurological clinical signs, primarily tremors in the neck and ataxia. All non-vaccinated animals developed histological lesions restricted to the central nervous system and consistent with a lymphocytic meningomyeloencephalitis. Vaccinated goats had any clinical signs of disease or histological lesions, but all of the non-vaccinated goats developed pyrexia and 5/9 developed neurological clinical signs, primarily tremors in the neck and ataxia. All non-vaccinated animals developed histological lesions restricted to the central nervous system and consistent with a lymphocytic meningomyeloencephalitis. Vaccinated goats had significantly ($P < 0.003$) greater concentrations of serum IgG and lower levels of IgM ($P <0.0001$) compared with unvaccinated animals. SGEV RNA levels were below detectable limits in the vaccinated goats throughout the experiment, but increased rapidly and were significantly ($P <0.0001$) greater 2–10 days post challenge in the non-vaccinated group. In conclusion, vaccination of goats against LIV confers highly effective protection against SGEV; this is probably mediated by IgG and prevents an increase in viral RNA load in serum such that vaccinated animals would not be an effective reservoir of the virus.

Keywords: experimental infection; goat; Spanish goat encephalitis virus; vaccination

Introduction

Louping ill is a neurological disease characterized by a primarily lymphocytic non-suppurative meningoencephalitis; it is endemic in the upland and hill farming areas of the UK and Ireland (Jeffries et al., 2014). Louping ill is caused by louping ill virus (LIV), a member of the genus Flavivirus, which belongs to the tick-borne encephalitis (TBE) group of viruses. Current taxonomy states that the LIV group is comprised of LIV isolated in the UK and Norway (four genotypes), Spanish sheep encephalitis virus (SSEV), Greek goat encephalitis virus (GGEV) and Turkish sheep encephalitis virus (TSEV) (Marin et al., 1995; Gao et al., 1997; Gritsun et al., 2003; Grard et al., 2007). Tick-borne encephalitis virus (TBE) is recognized as a separate species to LIV (Pletnev et al., 2011).
In 2011, an outbreak of disease clinically and pathologically resembling louping ill occurred in a herd of Bermeya goats in Asturias (northern Spain) (Balseiro et al., 2012). Whole genome sequencing and phylogenetic analysis demonstrated that the virus isolated from the brain of an affected goat was significantly divergent from LIV genotypes and SSEV. Based on those observations, the virus was identified as a novel tick-borne Flavivirus and the name of Spanish goat encephalitis virus (SGEV) was proposed to distinguish it from SSEV (Mansfield et al., 2015). Naturally acquired infection with SGEV in the goat herd resulted in severe, acute encephalitis with a mortality rate of 100% (Balseiro et al., 2012). This high mortality rate had not been reported previously in infections in sheep caused by the related LIV or SSEV, suggesting a substantial difference in pathogenesis and pathology of this isolate or greater susceptibility of goats to this, possibly host-adapted, flavivirus. Two serological surveys in the region revealed that 5.1% of goats and sheep were antibody positive by FSME (TBE) IgM enzyme-linked immunosorbent assay (ELISA) detection kit, which recognizes any European TBE flavivirus, and 2.6% of chamois were positive by haemagglutination inhibition, which cross reacted with LIV antigen, demonstrating that exposure to flavivirus was neither common or rare (Balseiro et al., 2013; Ruiz-Fons et al., 2014).

The aim of this study was to determine the pathogenesis, pathology, onset and duration of SGEV RNA load in serum and the serological immune response of naïve goats challenged experimentally with SGEV, and to evaluate the efficacy in goats of the only commercially available vaccine to a flavivirus for use in animals (Louping ill BP vaccine, MSD Animal Health, Walton, UK).

**Materials and Methods**

**Experimental Animals**

Eighteen female Alpine goats were sourced from Castilla y León where no cases of SGEV have ever been reported. Goats were kept in isolation under tick-free conditions to avoid potential exposure to SGEV. Additionally, all animals were treated with Butox® (Intervet Laboratories, Carbajosa, Spain) to further prevent tick infestation. The animals arrived 2 weeks prior to vaccination, were 2 months old at the time of initial vaccination and 3 months old at the time of challenge. Goats were housed in individual boxes in level-2 biocontainment facilities and were allocated randomly into two groups of nine animals each. Sampling procedures and SGEV challenge 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**

SGEV was isolated originally from the brain of a goat (Asturias, Spain, 2011) using ISE6 *Ixodes scapularis* tick cells cultured in L15B300 medium as described previously (Munderloh et al., 1999). Subsequently, SGEV was adapted for growth, propagation and titration in baby hamster kidney (BHK-21) cells 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 (penicillin/streptomycin solution, 100 units/µg per ml). The resultant virus stock had a titre of $1.4 \times 10^8$ plaque forming units (PFU)/ml in BHK-21 cells and it was diluted in BHK-21 cell tissue culture to $1.0 \times 10^7$ PFU/ml for experimental challenge in goats.

**Vaccination and SGEV Challenge**

One group was vaccinated with the louping ill vaccine as per the manufacturer’s instructions (subcutaneous injection of 1 ml) with minor modifications. This vaccine is licensed for use in sheep and comprised of tissue culture-derived inactivated virus with a liquid paraffin/montanide adjuvant (MSD Animal Health). Although sheep require only a single dose of vaccine, it is recommended that any other species given two doses 2 weeks apart (personal communication, H. Reid). Therefore, vaccinated goats were given two doses of vaccine subcutaneously over the right shoulder, one on day −27 and one on day −14. On day zero, the nine vaccinated and the nine unvaccinated (positive control group) goats were all challenged subcutaneously over the right thorax behind the elbow with a 1 ml suspension containing $1.0 \times 10^7$ PFU/ml of SGEV.

**Sampling, Serology and Evaluation of Clinical Signs**

Prior to vaccination, sera from all animals were subjected to the FSME (TBE) IgM and IgG ELISAs (PROGEN®, Heidelberg, Germany), which recognize antibodies to any European TBE flavivirus. Only animals with results below the manufacturer’s designated cut-off value were used in the experiment. Blood samples were taken weekly after vaccination, then daily after challenge with SGEV from 1 to 8 days post challenge (dpc) and then every 2 days until sacrifice. Rectal temperature was taken on the day of...
challenge and then daily thereafter 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, staring coat; and 2, in addition to the signs listed in 1, neurological clinical signs (e.g. tremors, ataxia or incoordination). Animals were killed at 12, 13, 21 or 28 dpc by intravenous overdose of pentobarbital (0.3 ml/kg) except for one vaccinated goat that was found dead at 4 dpc due to ruminal acidosis. Three vaccinated goats were killed at 13 dpc, two at 21 dpc and three at 28 dpc. Three goats from the positive control group were sacrificed at 12, 21 and 28 dpc.

One-step TaqMan Real-Time qRT-PCR Assay

Viral RNA was extracted from serum samples using the Speedtools RNA virus extraction kit (Biotools, Madrid, Spain), according to manufacturer’s instructions, and stored at −80°C until required. A SGEV specific quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed. Briefly, a 78 base pair (bp) fragment of the non-structural NS4b protein-coding region specific for SGEV was amplified using the primers LI-2 forward (9,199–9,221 bp) and LI-2 reverse (9,276–9,253 bp) and ‘visualized’ with the TaqMan probe Ast-LI (9,224–9,241 bp) labelled with FAM at 5’ and MGB quencher at 3’. The SGEV RNA standard control plasmid. The RNA was also present.

Lesions were ascribed to one of three levels of increasing severity (Table 1): grade I, only perivascular cuffing; grade II, perivascular cuffing and small foci of glial cells; and grade III lesions were classified as moderate non-suppurative encephalomyelitis. The latter consisted of (1) perivascular cuffing formed by an infiltrate of lymphocytes and histiocytes, (2) diffuse or focal proliferation of glial cells, (3) neuronal degeneration, neuron necrosis and neuronophagia with features including satellitism, nuclear pyknosis, chromatolysis, axonal swelling, atrophy and cell lysis, (4) demyelination and vacuolation of the neuropil and (5) meningitis. Microvascular changes consisting of reactive endothelium and perivascular oedema were also present.

Post-mortem Examination

Goats were subjected to full necropsy examination and gross lesions were recorded. Samples for histopathology were taken from the central nervous system (CNS), extracted whole and fixed; these consisted of sections of the brain (cerebral cortical regions including the frontal, parietal and occipital lobes, optic chiasma, corpus callosum, hypothalamus, thalamus, midbrain, cerebellum, pons and medulla oblongata) and four sections of the spinal cord (cervical, thoracic, lumbar and sacral), as well as representative samples of lung, kidney, liver, spleen, gut (distal jejunum, ileocaecal valve and associated lymph nodes), skeletal muscle (biceps brachii) and brachial and sciatic plexuses. Samples for histology were fixed in 10% neutral buffered formalin prior to trimming and selected samples were processed routinely and embedded in paraffin wax. Sections (4 μm) from each block were stained with haematoxylin and eosin (HE).

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antibody binding was blocked with 25% normal goat serum (NGS, Vector Laboratories, Peterborough, UK) in phosphate buffered saline (PBS) for 30 min prior to addition of the primary mouse monoclonal anti-LIV IgG antibodies (clone 3.3, Moredun Research Institute, Edinburgh, UK raised to an envelope protein of LIV [Venugopal et al., 1992] and shown to cross react with SGEV [Balseiro et al., 2013; Salinas et al., 2017]), diluted 1 in 3,000 in 25% NGS in PBS and incubated overnight at 4°C. Slides were washed with PBS (3 × 5 min) and ‘visualization’ of the bound primary antibodies was by an EnVision Kit (goat anti-mouse horseradish peroxidase conjugate, DakoCytomation, Ely, UK) as per the manufacturer’s instructions followed by addition of 3,3′-diaminobenzidine (DAB, Vector Laboratories) chromogen for 10 min. Slides were then washed with tap water, counterstained with haematoxylin for 1 min, washed in tap water again, dehydrated through graded alcohols, rinsed in xylene and mounted under DPX (Sigma–Aldrich). Negative control sections were comprised of semiserial sections of all tissues subjected to IHC with the primary antibodies substituted by isotype matched, mouse IgG antibodies and sections of known LIV-positive sheep brain were included in each run as positive controls.

**Statistical Analyses**

Quantitative data such as rectal temperature, SGEV RNA load and anti-European flavivirus IgM and IgG absorbance readings were tested for differences between groups and time of sampling by an analysis of variance using GLM procedure (SAS statistical package, SAS Inc., Cary, North Carolina, USA). Differences between pairs of least square means were tested with the Tukey–Kramer t-test for multiple comparisons in the same statistical package and procedure. Statistical significance was taken at \( P \leq 0.05 \).

### Results

#### Clinical Findings

All of the vaccinated goats, except for the one that died suddenly of ruminal acidosis at 4 dpc, remained clinically normal throughout the study. Clinical signs were observed in unvaccinated positive control goats only. Rectal temperature began to rise at 3 dpc, peaking at 6–7 dpc. The rectal temperatures of the goats in the positive control group were significantly higher (\( P < 0.0001 \)) than those of goats in the vaccinated group from 3 to 8 dpc (Fig. 1). Typically, clinical signs classified as severity 1 (primarily dullness and staring coat) affected all animals in this group and appeared at 4 dpc, which coincided with the increase in rectal temperature. Mild neurological clinical signs (severity 2), consisting of muscular tremors (primarily in the neck), ataxia and/or incoordination, affected five animals and began variably at 4 dpc (goats 81 and 86), 8 dpc (goat 85) or 10 dpc (goats 77 and 78) and persisted until sacrifice (Table 1).

#### Table 1

<table>
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*Maximum clinical signs severity score: 0, no clinical signs; 1, dullness, loss of condition and staring coat; 2, in addition to the previous clinical signs (in 1), also neurological clinical signs including tremors, ataxia and incoordination.

†Histological lesion severity score: 0, no lesions; I, perivascular cuffing; II, perivascular cuffing and small foci of gliosis; III, prominent perivascular cuffing, numerous foci of gliosis, neuronophagia, necrosis of Purkinje cells and non-suppurative meningitis.

![Fig. 1. Rectal temperature (mean ± standard error of mean [SEM]) over 28 days post challenge (dpc) in positive control (blue diamonds, \( n = 9 \)) and vaccinated (pink squares, \( n = 8/9 \)) goats. Goats in the positive control group had significantly higher (\( P < 0.0001 \)) rectal temperatures compared with those in the vaccinated group between 3 and 8 dpc.](image-url)
Serology

Sera from all 18 goats were below the manufacturer’s cut-off level in the IgM and IgG ELISAs prior to the commencement of the experiment. Following vaccination at days −27 and −14, and challenge at day zero, IgM antibody concentrations remained below the manufacturer’s designated cut-off value until 8−14 dpc (peaking at 10 dpc) in the vaccinated group and from 6 to 21 dpc (also peaking at 10 dpc) in the positive control group (Fig. 2A). The IgM concentrations in the positive control group were significantly higher ($P < 0.0001$) than in the vaccinated group from 6 to 21 dpc. The rise in IgM antibody concentrations coincided with the second increase in rectal temperature and was associated also with the increasing severity of clinical signs and the presence of neuropathological lesions in goats in the positive control group (Fig. 2 and Table 1).

Serum IgG concentrations in animals of the vaccinated group began to rise after the second vaccination at day −14, but did not exceed the manufacturer’s cut-off value until 7 dpc and remained elevated until the animals were killed at 28 dpc (peaking at 12 dpc, Fig. 2B). In the positive control group, the IgG concentrations did not begin to rise until 8 dpc and only exceeded the manufacturer’s cut-off value at 12−21 dpc (peaking at 16 dpc) before falling below the cut-off value on the day of sacrifice at 28 dpc (Fig. 2B). The serum IgG concentrations were significantly lower ($P < 0.003$) in the positive control group compared with the vaccinated group between 4 and 16 dpc.

Pathology and Immunohistochemistry

Other than congestion of meningeal vessels, which was present in animals from both groups, no gross lesions were observed in any animal. No histological lesions were found in any of the vaccinated animals (Fig. 3). Histological lesions were morphologically similar irrespective of the day of sacrifice. Goats in the positive control group showed a primarily lymphocytic non-suppurative encephalomyelitis of varying severities.

Two positive control goats had grade I lesions, three had grade II and four had grade III lesions. The right side of the brain showed more severe lesions than the left in 4/9 goats and all of these animals had grade III lesions. In goats with grade I and II lesions (5/9), there was no obvious difference in severity between different sides of the brain.

Histological lesions were consistently more severe (grade III) and multifocal in the midbrain, cerebellum, medulla oblongata and cervical spinal cord compared with other anatomical levels of the CNS (Fig. 3). The cerebral cortex typically showed grade I lesions and the thalamus, hypothalamus and hippocampus were devoid of lesions. The midbrain had perivascular cuffing, proliferation of glial cells and neuron degeneration and necrosis, mainly in the ventral aspects (Figs. 3A, B). Foci of gliosis were present mainly in the pons and the molecular layer of the cerebellum (Figs. 3D, E). Changes in the Purkinje cells varied from mild degeneration of a small number to widespread necrosis and depletion variably affecting individual cerebellar folia. In such cases there was often proliferation of Bergmann glial cells and in some instances the loss of Purkinje cells was marked by empty spaces (Fig. 3E). The medulla oblongata also showed perivascular cuffing, proliferation of glial cells and neuron degeneration and necrosis. The cervical spinal cord was consistently more severely affected than the thoracic, lumbar
and sacral regions, with lesions present in both the white and grey matter. In the white matter, perivascular cuffing was usually present and in the grey matter perivascular cuffing, gliosis and neuron necrosis were present, with the ventral horns consistently more severely affected than the dorsal horns (Figs. 3G, H). Neuronal loss was present in several nuclei of the brain including the red, olivary, medial accessory olivary and trigeminal nuclei, plus the reticular formation and nuclei of the vagal and hypoglossal nerves.

No microscopical lesions were found in the samples of lungs, kidney, liver, spleen, gut or brachial and sciatic plexuses. No positive labelling for SGEV was found by IHC in any brain section or the brachial and sciatic plexuses from any goat in the positive control group. All negative control preparations were devoid of labelling and all positive control sections of sheep brain showed intense cytoplasmic labelling in large numbers of neurons.

SGEV RNA Load

In the positive control group, two peaks of SGEV RNA load in serum were detected, at 3 and 7 dpc, which subsequently declined at 4 and 8 dpc to reach the lowest levels at 5 and 12 dpc (Fig. 4). In the vaccinated goats, viral RNA copy numbers never varied noticeably from the limit of detection of the assay and never reached the lowest level found in the positive control group throughout the experiment. Viral RNA copy numbers in the positive control group

![Fig. 3. Histological lesions observed in positive control (severity III: A, B, D, E, G and H) and vaccinated goats (no lesions: C, F and J). All sections are stained by HE. (A) Midbrain: note the large number of foci of gliosis (asterisks) and perivascular cuffs (arrows). Bar, 200 μm. (B) Midbrain: note detail of gliosis (asterisk) and perivascular cuffing (blue arrow), neuron degeneration in the red nucleus (black arrows) and vacuolation of the neuropil (arrowhead). Bar, 100 μm. (C) Midbrain of a vaccinated goat: no histopathological changes were present. Bar, 200 μm. (D) Cerebellum: note perivascular cuffing (black arrow) and numerous foci of gliosis (blue arrows) in the molecular layer and meningitis (yellow arrow). Bar, 200 μm. (E) Cerebellum: note degeneration and necrosis of Purkinje cells (black arrows) and proliferation of Bergmann cells (asterisk) in the molecular layer. Perivascular cuffing was also present (blue arrow). Bar, 50 μm. (F) Cerebellum of a vaccinated goat: no lesions were present in the molecular or granular layers or the Purkinje cells. Bar, 200 μm. (G) Cervical spinal cord: note foci of gliosis (black arrows) and perivascular cuffing (blue arrow) in the ventral horns. Bar, 200 μm. Inset: note perivascular cuffing and neuron degeneration. Bar, 20 μm. (H) Cervical spinal cord: note gliosis (asterisk) and vacuolation (black arrows) in the grey matter. Bar, 100 μm. (I) Cervical spinal cord from a vaccinated goat: note no lesions present. Bar, 200 μm.]
Vaccination for Spanish Goat Encephalitis Virus

Fig. 4. SGEV RNA load in serum samples (mean ± standard error of mean) from positive control (blue) and vaccinated (pink) goats for initial 12 days post challenge with SGEV. Note that there is a biphasic rise in RNA load in positive control goats and no such rise in vaccinated animals. Viral RNA copy numbers in the positive control group were significantly higher ($P < 0.0001$) than in the vaccinated group at 2–10 dpc, except at 5 dpc. Viral RNA loads were determined by a qRT-PCR assay. A standard curve created using an RNA standard control plasmid was used for quantification. Data represent the average of eight vaccinated and nine positive control independent goats per time point. The dashed line represents the limit of detection of the assay.

were significantly higher ($P < 0.0001$) than in the vaccinated group at 2–10 dpc, except for at 5 dpc (Fig. 4).

Discussion

This is the first report of experimental challenge of goats with SGEV. The challenge successfully induced clinical disease and histological lesions in the CNS of the unvaccinated animals (positive control group, 100% attack rate). The commercial vaccine against LIV, which is licensed for use in sheep only, was highly effective in protecting goats challenged experimentally by the subcutaneous route with SGEV, as no clinical disease or histological lesions were present in any of the vaccinated animals (100% efficacy). Furthermore, vaccination reduced any SGEV challenge-induced RNA load in serum to such low levels (i.e. below that of the detection limit of qRT-PCR) that transmission of the virus to feeding ticks would be prevented. This suggests that vaccination would be a highly effective method of controlling the disease in individual animals and may also reduce the prevalence of SGEV-infected Ixodes spp. ticks, the presumed vector of natural transmission.

Naturally acquired SGEV infection caused a 100% mortality rate in clinically affected goats (Balseiro et al., 2012) and although the challenge isolate used for this study was derived from that original natural outbreak, no fatalities occurred in this study. This was despite the induction of histological lesions in the CNS in all animals in the positive control group and 5/9 of these animals developing neurological clinical signs, all of which is similar to experimental infection of sheep with LIV (Doherty and Reid, 1971a; Reid et al., 1984; Sheahan et al., 2002). This difference in mortality rate between the naturally acquired and experimentally induced disease may be due to a number of factors. The initial outbreak of SGEV occurred in Bermeya goats (Balseiro et al., 2012), a rare breed in danger of extinction that are consequently highly inbred and probably have a restricted genetic diversity. The herd had been translocated to an area previously devoid of this breed and it is unlikely they had been exposed previously to the virus prior to this. Furthermore, if the Bermeya goats had not been exposed to ticks previously, they may have had a combined infection of SGEV and anaplasmosis (Anaplasma phagocytophilum), which has been shown to increase dramatically the mortality rate of flavivirus infection (Reid et al., 1986). Additionally, the differences in mortality rate may be due to the challenge dose, the route of inoculation, the individual immunocompetence and general condition of the animals and/or the loss of virulence of the original viral strain after culture in tick and mammalian cell lines, plus laboratory manipulation (Reid et al., 1984; Sheahan et al., 2002). However, considering the severity of the lesions in the brain, which were similar to those in naturally infected goats (Balseiro et al., 2012), it is unlikely that the virus had become significantly less pathogenic. As SGEV is thought to be transmitted by ticks, the route of challenge is also unlikely to have affected the mortality rate. The most likely reasons for the lack of mortality in the experimentally challenged animals is the different breed of goat used and the low-stress environment of the experimental facilities, which remove all nutritional, climatic, production and predator-associated stress. This is analogous to subcutaneous experimental challenge with LIV in sheep and goats at a similar dose ($2.5 \times 10^6–10^6.6$ PFU/animal), where only 2/8 sheep and 1/7 goats developed neurological clinical signs (Reid and Doherty, 1971; Reid et al., 1984). The much higher proportion (5/9) of goats developing neurological clinical signs in this study suggests that SGEV may be more pathogenic than LIV. The pathogenicity of SGEV in sheep has been studied and is described elsewhere (Salinas et al., 2017). Furthermore, as SGEV was described relatively recently, it is unknown if goats are truly the natural host or if they are a spill-over from another species, such as the sympatric chamois (Rupicapra spp.)
(Ruiz-Fons et al., 2014). Sheep and goats have different susceptibilities to various diseases (e.g. blue-tongue; Maclachlan et al., 2009) and differences in the individual immune response of each species should be taken into account. Experimental infection of lambs with LIV results in neurological clinical signs 8–13 dpc (Reid and Doherty, 1971; Sheahan et al., 2002), but in goats, pyrexia, which correlates with viraemia, occurs at 3–10 dpc, followed by the appearance of neurological clinical signs, such as tremors, at 12 and 13 dpc (Reid et al., 1984). Our findings showed initial clinical signs, such as pyrexia and depression, began at 3 dpc, coinciding with the initial peak of RNA load (also at 3 dpc), but with onset of neurological clinical signs later (i.e. at 10 dpc), which suggests that goats have a shorter incubation period when challenged with SGEV compared with LIV (Reid et al., 1984).

In the present study, the three goats with the most severe pyrexia (between 41.5 and 42°C) developed the most severe histological lesions (grade III). Animals with moderate or severe histological lesions (grades II and III) also developed neurological clinical signs and had higher SGEV RNA loads compared with those with mild histological lesions (grade I). This is similar to other encephalitic viruses, as the initial viral load in serum after infection and the control of early viraemia are two important factors in the development of encephalitis (Prow et al., 2014; de Wispelaere et al., 2015). The severity of clinical signs in louping ill has been directly related to the extent of damage to neurons (Doherty and Reid, 1971a; Sheahan et al., 2002), with neuropathological changes in moribund sheep most marked in the Purkinje cells, the neurons of the motor and vestibular nuclei and ventral horns of the spinal cord (Doherty and Reid, 1971a). This is similar to the locations of the lesions caused by SGEV in this study, which suggests that the virus has the same neurotropic characteristics as LIV.

The distribution of lesions in the spinal cord has not been studied previously in detail (Doherty and Reid, 1971b). In the present study, lesions were found throughout the spinal cord, but the most severe and extensive lesions were consistently in the cervical spinal cord, which may explain why tremors in the neck were the most common clinical sign. The lack of any immunolabelling in the CNS and brachial plexuses, despite high levels of virus, suggests either that the virus had disappeared previous to the day of sacrifice after the initial rise of RNA load in serum, and/or that any virus present was bound by challenge-induced endogenous antibody, which blocked the specific epitope recognized by the anti-LIV monoclonal antibody.

The mechanism of translocation in natural LIV infections, and other tick-borne encephalitides, from the site of the infected tick-bite to the CNS, is thought to be haematogenous during the phase of viraemia after initial viral replication in the lymphoid tissues (Reid and Chianini, 2007). The ipsilateral nature of the CNS lesions in relation to the site of challenge with SGEV, although suggestive of a neurotropic rather than a haematogenous spread from the point of inoculation, is too short a time course for this to have occurred. The goats in this study were devoid of lesions in the thalamus and hippocampus, which is in contrast to lesions found in lambs challenged experimentally with LIV by the subcutaneous route (Doherty and Reid, 1971b; Sheahan et al., 2002), but we cannot explain this observation.

Vaccination of goats with the LIV vaccine failed to induce a detectable IgM response despite the application of two doses of vaccine given 2 weeks apart. Furthermore, vaccination appears to have both delayed and reduced the IgM response to challenge with live SGEV, as the unvaccinated positive control animals reacted more quickly, with a significantly greater concentration of IgM, which was also of longer duration. The initial lack of an observed IgM response to vaccination may be due to the difficulty in measuring low concentrations of this antibody due to its pentameric nature, which reduces the accessibility of surface epitopes for binding by other antibodies. Alternatively, the sensitivity of the ELISA may be low as it was not developed specifically to detect goat antibodies to SGEV and there may be low cross reactivity. However previous studies have demonstrated cross reactivity with LIV (Klaus et al., 2014), so it is not unreasonable to assume cross reactivity with SGEV. In the vaccinated group, the lower concentration of IgM found in response to challenge with SGEV was probably due to the presence of vaccine-induced IgG binding rapidly to the virus, thereby negating the induction of a large or rapid IgM response.

IgG concentrations increased in the LIV-vaccinated group after the initial dose and continued to increase steadily after the second vaccine dose and the challenge with live SGEV, such that IgG antibodies were notably and significantly higher in concentration throughout the whole experiment. This would suggest that the LIV vaccine induced an IgG response and that the resultant 100% efficacy of the vaccine shows that this antibody cross reacts with SGEV and that IgG levels present prior to challenge were probably critical for the protection conferred. Conversely, the rising IgM concentrations in the non-vaccinated animals did not protect them from disease, nor did the lower and later developing IgG
antibodies, despite the rapid decrease in viral load at 10 dpc, which coincided with peak IgM concentrations. This suggests that IgM would be not be as efficient at clearing the RNA load or that the concentrations induced over this timescale were not high enough to contribute to protection.

The reduction in the viral RNA load observed in the positive control group following the appearance of the humoral immune response was probably due to the major role that neutralizing antibodies play in clearing viraemia in infected animals (Diamond et al., 2003). Serological tests are very useful for evaluating the epidemiological status of herds. In the UK, where louping ill is endemic, the presence of IgM antibodies to LIV is used as an indicator of a recent infection (Reid and Chianini, 2007). Our findings indicate that, when using the IgM ELISA in goats, it would be possible to detect recently infected animals between 6 and 15 days post infection, a very short time interval and then only if natural infection mimics our experimental challenge (Fig. 1). However, under field conditions and in a large population, natural infections would not occur simultaneously and this would broaden the window of usefulness of the IgM ELISA.

The initial increase in SGEV RNA load seen in the positive control group between 2 and 5 dpc, probably represents the primary viral replication in lymphoid tissues resulting in infectious virus spreading to the CNS via the haematogenous route. This initial rise in RNA load would be the one that infects co-feeding larvae, nymphs and ticks for persistence of the disease in the environment. The second rise in blood-borne virus seen 5–10 dpc and peaking at 7 dpc probably denotes secondary replication within the CNS. The virus released from this secondary replication in the CNS into the cerebrospinal fluid and thence to the blood was probably less infectious, as it would have been highly antibody associated, especially with IgM. Therefore, for diagnostic purposes in endemic areas, a combination of selective blood sampling of pyrexic animals to evaluate IgM and/or viral load should be used to indicate recent infection with SGEV. Conversely, evaluation of serum IgG concentrations would be useful for evaluating the level of exposure of a population, as well as assessing the response to vaccination.

At present there is no treatment, other than symptomatic, for louping ill and control of the disease relies on vaccination or control of the tick vector (Jeffries et al., 2014). The present study showed that vaccination of goats against LIV conferred highly effective protection against SGEV-induced histological lesions and clinical disease, which was probably IgG dependent, and that vaccination is a viable option for control of the disease. However, this study did not determine the duration of immunity to SGEV conferred by the LIV vaccine and future work should address this and also establish the optimal age and vaccination schedule, including whether one dose of vaccine would be effective.

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Conflict of Interest Statement

The authors declare no conflict of interest with respect to publication of this manuscript.

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