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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 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).

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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 1.4×10^8 plaque forming units (PFU)/ml in BHK-21 cells and it was diluted in BHK-21 cell tissue culture to 1.0×10^7 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 is 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 1.0×10^7 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

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