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EXPERIMENTALLY INDUCED DISEASE

Experimental Infection of Goats with a Newly Isolated Strain of Akabane Virus that Causes Encephalomyelitis

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Summary

In 2010, there was a large-scale outbreak of bovine encephalomyelitis in Korea, and 15 new strains of Akabane virus (AKAV) were isolated. To identify the pathogenicity of one of these strains, we infected adult goats with AKAV-7 via different routes. Twenty-five female goats were used in this study and were divided into five groups: intracerebral (IC) and intrasubarachnoid (IS) viral inoculation ($n = 8$ each), intravenous (IV) inoculation ($n = 4$), and vaccinated before IV inoculation ($n = 4$), in addition to a negative control animal. All animals inoculated with AKAV-7 had AKAV-neutralizing antibodies at 6–8 days post infection (dpi). During the experimental period, infected animals showed no clinical signs. In the IC group, 5/8 goats had non-suppurative encephalomyelitis affecting the cerebrum. Virus S RNA segments were detected in nearly all areas of the brain. In the IS group, 3/8 goats had encephalomyelitis affecting the cerebrum, cerebellum and spinal cord. At 7 and 21 dpi, virus S RNA segments were found mostly in the spinal cord, especially around the area of injection (L5–L6). Antibody titres in the serum of the vaccinated group had an early onset and slightly increased titre compared with the IV group. Histopathologically, there were no obvious lesions in the central nervous tissues in the vaccinated group, while one of four goats in the IV group showed encephalomyelitis in the parietal lobe of the cerebrum. The newly isolated AKAV-7 can cause encephalomyelitis in goats after experimental injection. The attenuated AKAV vaccine currently used in Korea may provide partial protective immunity against AKAV-7 infection, but the real effect of the vaccine requires further investigation in goats.

Keywords: Akabane virus; encephalomyelitis; experimental infection; goat

Introduction

Akabane virus (AKAV), a mosquito-transmitted Bunyavirus, is the major cause of congenital abnormalities and encephalomyelitis in ruminants. AKAV is usually transmitted by the bites of midges in the family Culicoides; this may include the species *Culicoides brevitarisis*, *Culicoides oxystoma* and *Cnebeculosus nebeculosus* (Kurogi *et al.*, 1987). AKAV infection occurs mainly during the summer rainy season when transmission vectors are active (Cho *et al.*, 2000). It is widely distributed throughout Australia, Southeast Asia, East Asia, the Middle East and Africa (Ogawa *et al.*, 2007). AKAV disease in ruminants can lead to abortion, stillbirth and congenital abnormalities in newborns. The prototype strain of AKAV, JaGAr39, was first isolated in Japan from mosquitoes in 1959 (Oya *et al.*, 1961) and in Korea, the first case of AKAV infection was reported in 1980 (Bak *et al.*, 1980).

Although the primary sign of AKAV infection is congenital deformity, the AKAV variant strain

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'Iriki', isolated from 10 calves ranging in age from 3 days to 16 months, caused non-suppurative encephalitis and neurological signs in calves in southern Japan from October to November, 1984 (Miyazato *et al.*, 1989). Since then, some AKAV strains have caused outbreaks of encephalomyelitis in calves and adult cattle (Uchida *et al.*, 2000; Kamata *et al.*, 2009). In 2000, Akabane viral encephalomyelitis in calves was reported in South Korea (Lee *et al.*, 2002).

In 2010, there was a large-scale outbreak (involving more than 500 cattle) of bovine encephalomyelitis in the southern part of Korea and 15 strains of AKAV were isolated from these cattle. The affected cattle generally showed clinical signs of locomotor ataxia, astasia, tremors, hypersensitivity and paralysis of the hind- and/or fore-limbs. Microscopical examination revealed the presence of non-suppurative encephalomyelitis, composed mainly of lymphohistiocytic perivascular cuffing, gliosis and neuronal degeneration and necrosis in the brain and spinal cord (Oem et al., 2012a). Recent studies have demonstrated substantial variations in virulence and antigenic properties among field isolates of AKAV (Miyazato *et al.*, 1989; Akashi and Inaba, 1997; Akashi et al., 1997; Yoshida and Tsuda, 1998). Although attenuated and inactivated vaccines have been used to prevent the disease (Kurogi *et al.*, 1978, 1979), evaluation of vaccine efficacy for this newly isolated virus is essential. To identify the pathogenicity of one of the new virus strains causing encephalomyelitis, we infected adult goats experimentally with AKAV-7 via different routes.

Materials and Methods

Animals

Twenty-five female Korean native goats (*Capra hircus*), aged 6 months, were used for the study. All animals were seronegative for AKAV by virus neutralization test (VNT) prior to the experimental procedures. All goats were clinically healthy and maintained in the animal facility at the College of Veterinary Medicine, Chonbuk National University, Republic of Korea, under standard conditions prescribed by the Institutional Guidelines. The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Chonbuk National University (CBU 2013-0031).

Virus

Virus isolated from brain of cattle with neurological signs and lesions of encephalomyelitis was passaged twice in BHK-21 cells and was provided by the Viral Disease Diagnostic Laboratory of the Animal Disease Diagnostic Division, Animal and Plant Quarantine Agency, Republic of Korea. The virus was grown in BHK-21 cells. After elimination of cell debris by ultracentrifugation for 15 min at 896 g, a 250 ml aliquot of cleared culture medium containing virus was concentrated to a final volume of 10 ml by Vivacell 250 (Sartorius Stedim Biotech, Gottingen, Germany). Virus titre was determined as tissue culture infective dose ($TCID_{50}$) by endpoint titration. The final virus titre was $5 \times 10^{6.167}$ $TCID_{50}$/ml. All procedures were performed at 4°C.

Animal Inoculation and Sample Collection

Goats were divided into five groups: intracerebral (IC) and intrasubarachnoid (IS) inoculation, each with eight animals, intravenous (IV) inoculation and vaccinated before intravenous inoculation, each with four animals, and a negative control animal. Goats were anaesthetized with an intramuscular injection mixture of tiletamine/zolazepam (80 mg/kg body weight, Zoletil; Virbac SA, Carros, France) and xylazine (50 mg/kg body weight, Rompun; Bayer Healthcare, Seoul, Korea), and IC and IS inoculations were undertaken as described below, after which the goats were allowed to recover. For the IC inoculation, the right-hand side of the skull was trepanated and the inoculum (1 ml virus solution containing $5 \times 10^{6.167}$ $TCID_{50}$ AKAV-7) was delivered to the frontal lobe to a depth of approximately 5 mm. For the IS inoculation, goats were inoculated with 1 ml inoculum into the subarachnoid space at the level of L4–L5. For the IV group, a 5 ml inoculum was injected slowly through the jugular vein. Goats in the vaccine-treated group were vaccinated with live vaccine (Akabane cattle Vac, Daesung, Korea) subcutaneously and inoculated intravenously with 5 ml inoculum 3 weeks after vaccination. The control animal received sterile phosphate buffered saline intravenously. After inoculation, goats were observed daily for clinical changes and for neurological signs in particular. Blood samples (5 ml) were collected every 2 days after inoculation until 14 days post infection (dpi) to evaluate viraemia and seroconversion. Peripheral blood mononuclear cells (PBMCs) were isolated by differential centrifugation over Histopaque-1077 (Sigma, Poole, UK) to detect viraemia. Goats were killed under deep anaesthesia at 7 dpi (four animals each from the IC and IS groups) and on 21 dpi (four animals each from the IC, IS, IV and vaccine-treated groups). Necropsy examination was performed and samples of brain, spinal cord, lymph node and spleen were collected for histopathological and virological analyses.

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