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Genetic and pathogenic characterization of Akabane viruses isolated from cattle with encephalomyelitis in Korea

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ABSTRACT

A large-scale outbreak of Akabane viral encephalomyelitis in cattle was reported in the southern part of Korea in 2010. Fifteen Akabane virus (AKAV) strains were isolated from the brain and spinal cord samples by using BHK-21 and/or HmLu-1 cells. To examine the genetic relationships and characteristics of the isolates, nucleotide sequences of the S, M, and L segments of the 15 isolates were determined and analyzed. Complete sequence analysis of the 15 AKAV isolates showed 99.9–100% amino acid identities, indicating that the 15 isolates originated from a single strain. The S and M RNA segments of a representative isolate (AKAV-7/SKR/2010) were also compared with the segments of representative reference sequences. This AKAV-7/SKR/2010 strain showed the highest identity with the Iriki and KM-1/Br/06 strains. Neighbor-joining phylogenetic trees of S and M RNA segments were constructed. Four representative AKAV isolates were classified into subgroup Ia, which contains the Iriki and KM-1/Br/06 strains recognized to cause encephalomyelitis in calves and adult cattle in Japan. Moreover, experimental intraperitoneal infection was performed using the AKAV-7/SKR/2010 and AKAV-17/SKR/2010 strains to assess pathogenesis in suckling mice. The 2 isolates, genetically related to the Iriki strain, were neurovirulent and caused neurological signs in suckling mice. In contrast, the 93FMX strain and the K0505 strain, related to the OBE-1 strain, were avirulent in mice. The present results indicate that these isolates most likely had originated from the Iriki strain and are closely related to the Iriki strain both genetically and pathogenically.

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1. Introduction

Akabane virus (AKAV), a member of the *Orthobunyavirus* genus in the family *Bunyaviridae*, is an important bovine pathogen. To date, AKAV infections have been reported in Australia, Southeast Asia, East Asia, and the Middle East (Bak et al., 1980; Konno et al., 1982; Taylor and Mellor, 1994; Jagoe et al., 1993; Liao et al., 1996). The prototype strain of AKAV, JaGAr39, was first isolated in Japan from mosquitoes in 1959 (Oya et al., 1961). However, recent entomological studies have revealed that *Culicoides* insects serve as the principal vector of AKAVs (Kurogi et al., 1987; Yanase et al., 2005). In pregnant cattle, AKAV causes epizootic and sporadic outbreaks of abortions, premature births, stillbirths, and congenital abnormalities such as arthrogryposis–hydranencephaly (AH) syndrome. Bovine encephalitis was first reported in 10 calves in Kagoshima Prefecture, Japan (Miyazato et al., 1989), from which the Iriki strain (a variant of AKAV) was isolated and used to cause encephalitis in experimentally inoculated calves. Since then, other strains of AKAV have caused small-scale sporadic outbreaks of encephalomyelitis in calves and

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adult cattle in Taiwan and Korea (Liao et al., 1996; Lee et al., 2002). In Japan in 2006, one large-scale outbreak affected nearly 200 cattle (Kono et al., 2008).

AKAV has a lipid envelop and its genome consists of three segments of single-stranded negative-sense RNA, designated as S (small), M (medium), and L (large), according to their size (Nichol et al., 2005). The L RNA segment encodes the L protein, which has RNA polymerase activity involved in viral replication and transcription. The M RNA segment is 4300 bp long and encodes a precursor polyprotein, which is cleaved into two envelope glycoproteins (Gn and Gc) and a nonstructural protein (NSm). Gc induces neutralizing antibodies and plays a role in attachment to mammalian cells, while Gn functions in viral attachment to insect cells (Ludwing et al., 1991). NSm likely plays a role in viral assembly and morphogenesis (Shi et al., 2006). The S RNA segment is 858 bp long and encodes a nucleoprotein (N) and a small nonstructural protein (NSs) in overlapping open reading frames (ORFs). The N protein shares antigenic determinants with other species in the *Orthobunyavirus* genus, and NSs is believed to act as an alpha/beta-interferon antagonist to help regulate host–protein synthesis (Bridgen et al., 2001; Weber et al., 2002).

The L RNA segment of the OBE-1 strain was recently sequenced (Ogawa et al., 2007b), while the L RNA segment of AKAV remains uncharacterized. The S and M RNA segments of the AKAV strains have been completely sequenced and characterized (Akashi et al., 1997; Yanase et al., 2003; Kobayashi et al., 2007; An et al., 2010). Phylogenetic analysis of the S and M RNA segments indicated that AKAVs can be grouped into four distinct genogroups (I–IV) and two subgroups (Ia and Ib) (Yamakawa et al., 2006; Kobayashi et al., 2007; Kono et al., 2008). Genogroup Ia primarily includes isolates found in Japan and Taiwan (PT-17, CY-77, and NT-14). An Israeli strain (ISR-01) and several Japanese strains are placed in Genogroup Ib (Yamakawa et al., 2006). No Korean AKAV strains belonging to genogroup I have been previously reported. Genogroup II mainly contains isolates from Japan and Korea (An et al., 2010). The MP496 strain isolated in Kenya in 1972 and two strains, B8935 and R7479, isolated in Queensland, Australia, in 1968 are included in prototype group IV and group III, respectively.

A large-scale epidemic of Akabane viral encephalomyelitis in cattle aged 4–72 months occurred in the southern part of Korea from late summer to late autumn in 2010. More than 500 cattle with neurological disorders were reported in five provinces (Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam, Chungbuk) in the southern part of Korea. Most of the affected cattle were found in two provinces (Jeonbuk and Jeonnam). The affected cattle mainly showed clinical signs such as locomotor ataxia, astasia, tremors, and hypersensitivity.

In this study, we investigated the genetic and pathogenic characterization of the AKAV strains isolated from cattle with encephalomyelitis in the southern part of Korea in 2010.

2. Materials and methods

2.1. Viral isolates

The 93FMX strain and the K0505 strain were isolated from bovine whole blood in 1993 and 2005, respectively. The two strains used in this study were registered in the Biological Research Center (BRC) of Korea. Fifteen AKAVs were isolated from the brain and spinal cord samples of infected cattle during a large-scale epidemic of Akabane viral encephalomyelitis in Korea in 2010 (Table 1). Isolated viruses were propagated on a monolayer culture of BHK-21 cells using α-MEM containing 5% fetal bovine serum (FBS, Cellgro®; Mediatech Inc., Manassas, VA, USA) at 37 °C. When the infected cells exhibited cytopathic effects (CPEs), virus-containing cell culture supernatants were separated from the cellular debris by centrifugation.

2.2. PCR amplification, sequencing, and phylogenetic analysis

Viral nucleic acids were extracted from virus-containing cell culture supernatant using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) and stored at −80 °C until analysis. RT-PCR procedures were performed in a one-tube system for sequencing. A 794-bp fragment covering the

Table 1
Summary of the AKAV isolates used in this study.

Strain	Cell lines	Clinical sample	Profile of cattle	Collected date	Geographic location
AKAV-7/SKR/2010	BHK-21, HmLu-1	Brain	Korean native, female, 8-month-old	25-September-2010	Jeonbuk
AKAV-17/SKR/2010	BHK-21	Brain, spinal cord	Korean native, male, 7-month-old	28-September-2010	Jeonbuk
AKAV-32/SKR/2010	HmLu-1	Brain	Korean native, castrated male, 30-month-old	1-October-2010	Jeonbuk
AKAV-35/SKR/2010	HmLu-1	Brain	Korean native, male, 22-month-old	1-October-2010	Jeonbuk
AKAV-37/SKR/2010	BHK-21, HmLu-1	Brain, spinal cord	Holstein, female, 12-month-old	3-October-2010	Jeonbuk
AKAV-40/SKR/2010	HmLu-1	Brain	Korean native, castrated male, 15-month-old	7-Oct-2010	Jeonbuk
AKAV-44/SKR/2010	BHK-21, HmLu-1	Brain	Korean native, female, 18-month- old	7-October-2010	Jeonbuk
AKAV-67/SKR/2010	BHK-21	Brain, spinal cord	Korean native, female, 12–month-old	15-October-2010	Jeonbuk
AKAV-80/SKR/2010	HmLu-1	Brain	Korean native, female, 12-month-old	20-October-2010	Jeonbuk
AKAV-83/SKR/2010	HmLu-1	Brain	Korean native, female, 5-year-old	22-October-2010	Jeonnam
AKAV-90/SKR/2010	BHK-21	Brain	Korean native, female, 6-year-old	25-October-2010	Jeonbuk
AKAV-92/SKR/2010	BHK-21, HmLu-1	Brain, spinal cord	Korean native, female, 6-year-old	25-October-2010	Jeonbuk
AKAV-94/SKR/2010	BHK-21	Brain	Korean native, female, 6-year-old	26-October-2010	Jeonbuk
AKAV-97/SKR/2010	HmLu-1	Brain	Korean native, female, 3-year-old	29-October-2010	Jeonbuk
AKAV-99/SKR/2010	BHK-21	Brain	Korean native, female, 25-month-old	3-November-2010	Jeonnam

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