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INFECTIOUS DISEASE

Bovine Epizootic Encephalomyelitis caused by Akabane Virus Infection in Korea

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Summary

A large-scale epidemic of Akabane virus (AKAV) encephalomyelitis in cattle aged 4–72 months occurred in the southern part of Korea from late summer to late autumn in 2010. Affected cattle exhibited neurological signs including locomotor ataxia, astasia, tremor and hypersensitivity. Samples of brain ($n = 116$), spinal cord ($n = 116$) and whole blood ($n = 205$) were submitted to the National Veterinary Research and Quarantine Service for diagnosis. Microscopical analysis of the brains and spinal cords revealed the presence of non-suppurative encephalomyelitis in 99 of 116 brains and/or spinal cords (85%). The brains and spinal cords were evaluated by reverse transcriptase polymerase chain reaction and AKAV antigens were detected by immunohistochemistry using rabbit antiserum against AKAV strain OBE-1. Fifteen AKAVs were isolated from the brain and spinal cord samples. Antibodies against AKAV in a virus neutralization test were detected in 188 of 205 serum samples (91.7%). This is the first report of a large-scale outbreak of bovine epidemic encephalomyelitis caused by AKAV infection in Korea.

Keywords: Akabane virus; cattle; encephalomyelitis

Akabane virus (AKAV) was first isolated in Japan and is transmitted primarily by *Culicoides* biting midges and mosquitoes (Oya *et al*., 1961; Kurogi *et al*., 1987; Yanase *et al*., 2005). AKAV infection has been reported in Australia, Southeast Asia, East Asia and the Middle East (Bak *et al*., 1980; Jagoe *et al*., 1993; Taylor and Mellor, 1994; Liao *et al*., 1997). Infection of adult cattle causes a transient viraemia without obvious clinical signs, while infection of pregnant cattle often causes fetal damage resulting in abortion, stillbirth or various congenital abnormalities (Inaba *et al*., 1975).

AKAV is a member of the Simbu serological subgroup of the genus *Orthobunyavirus* in the family Bunyaviridae (Nichol *et al*., 2005). AKAV infection does not normally cause clinical signs in mature cattle. However, the Iriki variant of AKAV, isolated from a calf with non-suppurative encephalitis and neurological signs in southern Japan in 1989, caused encephalitis in experimentally inoculated calves (Miyazato *et al*., 1989). Certain AKAV strains also caused outbreaks of encephalomyelitis in calves and adult cattle in Taiwan, Japan and Korea (Miyazato *et al*., 1989; Liao *et al*., 1997; Lee *et al*., 2002; Kono *et al*., 2008).

In addition, a large-scale outbreak that affected nearly 200 cattle in Japan was reported in 2006 (Kono *et al*., 2008). Non-suppurative encephalomyelitis was observed in cattle showing neurological signs. AKAV antigen and genetic material were detected in the brains and spinal cords from affected cattle. The isolates were characterized as belonging to genogroup Ia within the Iriki strain.

In 2010, a large-scale outbreak of bovine encephalomyelitis occurred in the southern part of Korea. In this study, we determined whether bovine encephalomyelitis was associated with AKAV infection and examined the clinical, epidemiological and histopathological features of the disease.

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From mid-August to the end of October 2010, more than 500 cattle with neurological disorders were reported in five provinces (Jeonbuk, Jeonnam, Gyeongbuk, Gyeongnam and Chungbuk) in the southern part of Korea. Most of the affected cattle were from two provinces (Jeonbuk and Jeonnam). The affected cattle displayed clinical signs including locomotor ataxia, astasia, tremor and hypersensitivity. Paralysis of the hind and/or forelimbs occurred in most affected cattle. The ages of affected cattle ranged from 4 months to 6 years. No significant relationship was observed between clinical signs and the age or sex of the cattle.

Necropsy examinations were performed by pathologists in the regional Veterinary Service Laboratory. Samples of brain ($n = 116$), spinal cord ($n = 116$) and whole blood ($n = 205$) from cattle on 178 farms in five provinces were submitted to the National Veterinary Research and Quarantine Service. Samples from other organs including the heart, kidney and liver were also submitted. Portions of the brain (cerebral hemisphere, midbrain, cerebellum, pons and medulla oblongata) and spinal cord were fixed in 10% neutral buffered formalin, and stored at -70°C or immediately minced and homogenized for virus isolation. For histopathology, haematoxylin and eosin (HE) staining was performed.

Microscopically, moderate-to-severe non-suppurative meningoencephalomyelitis was observed in the brain and spinal cord. Characteristic lesions were perivascular infiltration of lymphocytes and histiocytes with fewer plasma cells, multifocal gliosis and neuronal necrosis and neuronophagia (Fig. 1A). These lesions were more severe in the midbrain, medulla oblongata and spinal cord than in the cerebrum and cerebellum. In the spinal cord, lymphohistiocytic perivascular cuffing, gliosis and neuronal generation and/or necrosis were mainly observed in the ventral horn of the grey matter (Fig. 1B). Although the severity and distribution of lesions were different, non-suppurative meningoencephalomyelitis was observed in 99 of 116 brains and/or spinal cords (85%).

Immunohistochemistry (IHC) for detection of AKAV within lesions was performed with an automated immunostainer (Ventana, Bethesda, Maryland, USA). All reagents (enzyme, DABMap kit and haematoxylin) were from Ventana. Rabbit antiserum against the OBE-1 strain of AKAV was used as the primary antibody (kindly provided by Dr. S. Tanaka; Kono *et al.*, 2008). The antiserum was diluted 1 in 16,000 and was incubated with the sections for 3 h at room temperature. Biotinylated mouse anti-rabbit immunoglobulin (Ig) G (Dako, Glostrup, Denmark) was used as the secondary antibody.

Strong labelling of AKAV antigens was observed within the cytoplasm of neurons and axons in the midbrain (Fig. 1C) and in the spinal cord (Fig. 1D), as well as occasionally in endothelial cells and macrophages aggregated within perivascular cuffs (data not shown).

Viral nucleic acids were extracted from the brains and spinal cords of affected cattle using an RNeasy Mini Kit (Qiagen, Valencia, California, USA). Primer sets for reverse transcriptase polymerase chain reaction (RT-PCR) amplification of the AKAV S gene were selected based on the sequence of the OBE-1 strain (GenBank accession No: AB000851). The primers used were AKA424F (5′-CAGAAGAAGGCCAAGA TGGT-3′) and AKA425R (5′-AATGCAGCCTTG ACTGCGTCC-3′). A One-Step RT-PCR Kit (INtRON, Seongnam, Korea) was used to detect the S gene of AKAV. A reverse-transcription reaction was conducted at 50°C for 30 min with subsequent heating to 95°C for 15 min. PCR reactions were carried out in a final volume of 20 μl for 35 cycles at 94°C for 20 sec, 55°C for 20 sec and 72°C for 30 sec, followed by incubation at 72°C for 5 min. PCR products were resolved by electrophoresis in a 1.2% agarose gel and visualized by ethidium bromide staining. For differential diagnosis, PCR was also performed for other arboviruses (Aino virus, Chuzan virus, Ibaraki virus and bovine ephemeral fever virus [BEFV]), bovine viral diarrhoea virus (BVDV), bovine herpesvirus-1 (BHV-1), bovine herpesvirus-5 (BHV-5) and *Chlamydia* spp. (Vilcek *et al.*, 1994; Ros *et al.*, 1999; Madico *et al.*, 2000; Ohashi *et al.*, 2004; Hsieh *et al.*, 2005).

In RT-PCR analyses of the brain and spinal cord samples using primers specific for the AKAV S gene, specific PCR products of 476 base pairs were detected in 101 of 116 cattle (87%). However, no PCR products were produced for any of the other infectious agents investigated.

The brains and spinal cords of affected cattle were rinsed, minced and homogenized in serum-free Eagle's minimum essential medium (MEM; Cellgro®; Mediatech, Manassas, Virginia, USA). The supernatant of a 10% homogenate was used for virus isolation. The supernatant was inoculated into monolayer cultures of hamster lung (HmLu-1) and baby hamster kidney (BHK-21) cells. After incubation at 37°C for 7 days, the cell culture fluids were subinoculated into freshly prepared cell cultures at least three times until the infected cells exhibited a cytopathic effect.

Viruses were isolated from 15 PCR-positive samples by HmLu-1 and/or BHK-21 cell cultures. All 15 isolates were identified as AKAV by RT-PCR and sequencing. The isolates were classified into the same subgenogroup, together with the Iriki strain, which caused encephalitis in calves in 1984 (data not shown).