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Short Paper

Akabane Virus Infection in a Calf in Aegean Region of Turkey

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Background/Aim: Akabane virus (AKAV) causes epizootic and sporadic, abortion in cattle, sheep and goats, premature or stillbirths and congenital anomalies characterized by arthrogryposis, hydranencephaly or microanencephaly. The presented study is to describe the immunohistochemical detection of Akabane Virus antigen in brain sections of a calf in Aydın province, Turkey.
Material and Method: The tissue samples were collected from a 3-day calf presented for laboratory diagnosis. For histopathologic examination, the tissues were stained with hematoxylin and eosin. Duplicate brain sections were also stained with streptavidin peroxidase method to detect AKAV antigens.
Results: Histopathologically, neuronal degenerative and necrotic changes characterized with central chromatolysis were detected in motor neurons of the brainstem and spinal cord. Slight inflammatory changes were seen in the brain sections, characterized with perivascular mononuclear infiltrations. Immunopositive labeling of AKAV antigens was detected in degenerated motor neurons and a few glial cells of the brainstem and spinal cord, in Purkinje cells of the cerebellum, and in neurons of the cerebral hemispheres.
Conclusion: This study showed that AKAV strains were circulated and caused clinical infection in cattle in Turkey. For future years, AKAV should be monitorized clinically, pathologically and virologically, and at the same time, its economic impact of animal husbandry on the country should be investigated.
Keywords: Akabane Virus, pathology, Immunohistochemistry.

Türkiye'de Ege Bölgesinde Bir Buzağıda Akabane Virus Enfeksiyonu

Özbilgi/Amaç: Akabane virus (AKAV) sığır, koyun ve keçilerde epizootic ya da sporadic seyirli, atık, konjenital anomaliler (artrogripozis, hidranensefali, mikroensefali), erken ya da yaşama gücü zayıf yavru doğumlarına neden olur. Sunulan **çaşışma** Türkiye'de Aydın ilinde bir buzağıda AKAV enfeksiyonunun histopatolojik bulguları ile viral antijenin immunohistokimyasal tanısını içermektedir.
Material and Method: Doku örnekleri nekropsi muayenesi için getirilen üç günlük buzağıdan alındı. Histopatolojik incelme için, dokular hematoksilen ve eosin ile boyandı. AKAV antijenini tesbit etmek için, seri kesitler streptavidin peroksidaz metot ile boyandı.
Results: Histopatolojik incelemede, beyin stemi ve medulla spinaliste sentral kromatolizis ile karakterize nöronal dejeneratif ve nekrotik değişiklikler saptandı. Perivasküler mononuclear hücre infiltrasyonu ile karakterize hafif yangısal değişiklikler belirlendi. AKAV antijenler beyin kökü ve medulla spinaliste dejenere motor nöronlar ile birkaç glial hücrede, serevbellumda Purkinje hücrelerinde, serbral hemisferde nöronarda saptandı.
Conclusion: Sunulan **çalışma** AKAV suşlarının Türkiye'de sirküle olduğunu sığırlarda klinik enfeksiyona neden olduğunu göstermektedir**.**
Anahtar Kelimeler: Akabane Virus, patoloji, Immunohistokimya.

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Introduction

The Akabane virus (AKAV) to be an arbovirus (arthropod-borne) was first isolated from the Aedes vexans and Culex tritaeniorhynchus mosquitoes in the Japanese village of Akabane (now a suburb of Tokyo) in 1959 (Matumoto and Inaba, 1980; Charles, 1994).

AKAV belong to the Simbu serogroup of the arthropod-borne *Orthobunyavirus* genus of the family *Bunyaviridae.* The virus is classified in the Simbu serogroup together with other important viruses (Aino, Douglas, Tinaroo and Peaton) (Charles, 1994; Kirkland, 2002; Kirkland 2015). The small flies of the genus Culicoides, also vectors of the blue tongue virus, are principal vectors. In countries with tropical and temperate climatic zones, especially in countries reported blue tongue viruses, these diseases should be discriminated with laboratory exmainations (Kirkland, 2002). Akabane virus is responsible for congenital artrogryposis-hydranencephaly epizooties in ruminants. It has been reported with clinical diseases in Australia, Israel, Japan, Korea, Taiwan and Turkey (Sellers and Pedgley, 1985; Charles, 1994; Kobayashi ve ark., 2007).

Akabane virus causes infection in a wide variety of domestic ruminants and wild animals. In endemic areas, cattle, buffalo, sheep, goats and horses show a high prevalence of antibodies (Kirkland, 2002). It has been shown that AKAV causes epizootic and sporadic, abortion in cattle, sheep and goats, premature or stillbirths and congenital anomalies characterized by arthrogryposis, hydranencephaly or microcephaly (…….). In particular, the virus passes through the placenta in the early stages of pregnancy and causes many congenital anomalies, including artrogriposis-hydranencephaly in the offspring (Taylor and Mellor, 1994). It causes encephalitis in the infected young animals during or after birth (Kirkland, 2002).

In a recent Turkish study, the disease had a regional distribution and Akabane virus was isolated from the sheep in Aydin province. Moreover, sequence analysis on the basis of the the partial S segment of the isolates revealed that the virus was near genogroup II on the phylogenetic tree (Oğuzoğlu et al, 2015). This report represents the pathological and immunohistochemical findings of Akabane disease in a calf.

Materials and methods

A 3 day old death calf, from a herd in Çine of Aydin province represented for the diagnosis to pathology laboratory of Faculty of Veterinary Medicine, Adnan Menderes Unıversity. To anamnesis, only a few cases of abortion with no congenital anomaly were noted in this herd. Tissue samples were collected from the brain, intestines, lymph nodes, spleen, liver, lungs and kidneys and were fixed in a 10 % buffered formalin solution and then embedded in paraffin. For histopathologic examination, the tissues were cut in thickness of 5 µm and stained with hematoxylin and eosin. Duplicate unstained sections were used for immunohistochemistry (IHC).

To detect AKAV antigens in brain tissues, avidin-biotin peroxidase complex (ABC, Invitrogen, Histostain Plus kit, USA) was used. Rabbit anti-AKAV antiserum (AKAV Antibody ELISA kit, IDVET, France) was diluted to 1:10 and used as the primary an-

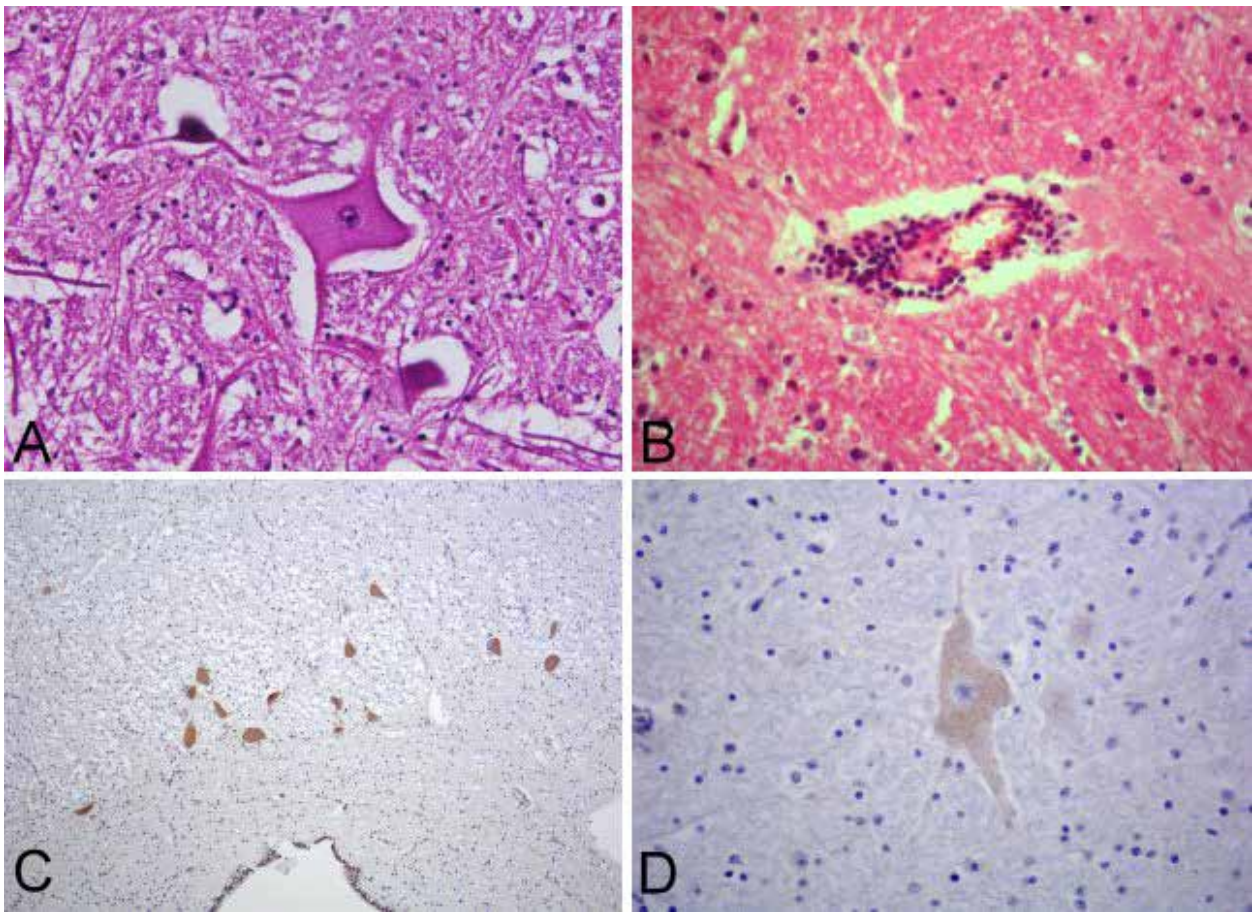

Figure 1. A. Central chromatolysis and neuronal shrinkage in motor neurons of brainstem. H&E. 40X. B. Perivascular lymphocyte infiltration in the brain tissue. C. AKAV labeling in neurons. Streptavidin peroxidase method. D. AKAV labeling in a neuron. Streptavidin peroxidase method.

Şekil 1.A.Beyin stem motor nöronlarında senral kromatolizis ve nöronal büzüşme. H&E.40X. B.Beyin dokusunda perivasküler lenfosit infiltrasyonu. C.Nöronlarda AKAV pozitif antijenler. Streptavidin peroksidaz metot. 20X D. Bir nöronlarda AKAV pozitif antijenler. Streptavidin peroksidaz metot.40X

tibody. For control purposes, replicate sections of selected infected tissues were processed, substituting normal rabbit serum for rabbit anti-AKAV serum.

Results and Discussion

On postmortal examination, no macroscopic findings were observed. Histopathologically, inflammatory changes in the brain sections were slight infiltrations of perivascular mononuclear cells and focal or scattered gliosis. The most conspicuous lesions were in motor neurons, characterized with central chromatolysis of the brainstem and spinal cord. There was also satellitosis and neuronophagia in the brain and brain stem. IHC revealed that AKAV antigens were complementary to histologic lesions in the brainstem and spinal cord. The viral antigen labeling of AKAV antigens was detected especially in degenerated motor neurons and a few glial cells of the brainstem and spinal cord, in Purkinje cells of the cerebellum and in neurons of the cerebral hemispheres (Figure 1).

Previous reports showed that AKAV circulated irregularly in ruminants in Turkey (Taylor and Mellor, 1994; Karaoglu et al., 2007; Urman et al., 1980; Haligur et al., 2014). Our previous study indicated that AKAV, near genogroup II on the phylogenetic tree, was circulated and causes death in newborn lambs in Çine district of Aydin province of Turkey (Oguzoglu et al., 2015). Similar, the presented calf case diagnosed in a cattle herd showed that AKAV also circulated in cattle herds in Aydin province, Turkey.

This case show that AKAV infection reappeared in Aydin province of Turkey. For future years, AKAV should be monitorized clinically, pathologically and virologically, and at the same time, its economic impact of animal husbandry on the country should be investigated. Moreover, AKAVs isolated from different ruminant species and/or hematophagous mosquitoes in Turkey should be carried out antigenic characteristics and phlogenetic analysis.

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