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Detection of Akabane Virus Antigen from Aborted Fetal Calf Brain Tissue by Immunohistochemistry

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유산 송아지의 뇌조직으로부터 Immunohistochemistry를 이용한 아까바네 바이러스 항원 검출

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요 약 : 아까바네 바이러스로 인하여 유산된 태아의 뇌조직으로부터 아까바네 바이러스 항원을 면역학적으로 검출하는 기법을 확립하였다. 아까바네 바이러스로 유산된 태아의 뇌는 조직이 거의 손실되거나 유약하여 부검 즉시 포르말린 등에 보존하여야 하므로, 포르말린에 보존된 뇌조직을 절편 하여 파라핀으로 포매된 조직표본으로부터 immunohistochemistry 방법으로 아까바네 바이러스 특이 항원을 검출하였다. 또한 이들 조직으로부터 직접 마우스 뇌내 접종과 조직배양내 바이러스 분리를 통하여 immunohistochemistry 법의 항원 검출 효율이 높음을 확인하였다. 유산된 태아의 뇌조직에서 단크론 항체를 이용한 항원 검출 실험에서 세포의 세포질내에서 아까바네 특이 항원이 검출되었고 hematoxylin-eosin 대조 염색으로 항원을 특이적으로 구분하여 진단할 수 있었다. 바이러스에 감염된 세포는 조직학적으로 변성이 심한 부위에서 다수 관찰되었고 맥관계에 가까운 세포에서도 독립적으로 감염된 세포가 관찰되었다.

Key word : Akabane virus, immunohistochemistry, aborted fetal calf brain

Introduction

Akabane virus belongs to *Bunyaviridae* simbu group containing three segmented single stranded RNAs with negative polarity[8]. Akabane virus cause no clinical signs in pregnant cow but outcome reside epizootic abortion, still birth, premature birth and arithrogryposis-hydranencephaly (AH) syndrome to the fetus if dams are infected with the virus[1-5,7]. The virus transmitted infected animal to naive animal via mosquitos and infected animals showed short duration of the viremia which make difficult in diagnosis with the virus isolation[10]. Histopathology and serology are the mainly used diagnostic methods for the Akabane infection but there is controversy remained for the specificity to differentiate with other viral pathogens such as Chuzan virus and bovine viral diarrhea virus (BVDV)[9,11,12]. Virus isolation is considered to be a specific diagnostic method for the Akabane infection in claves but tedious cell culture and is time consuming process. Immunohistochemistry was sensitive and specific test for the detection of the Akabane viral antigen from formalin fixed fetal calf brain tissue.

Materials and Methods

Aborted fetal brain samples

Aborted fetal brain samples were collected during late autumn abortion cases throughout nation wide

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in 1997. Brain tissues were collected aseptically and transported to the laboratory on ice without freezing. Brain samples from Akabane-like cases were divided for virus isolation and immunological examination. Brain tissue was fixed in 10% neutral formalin for the immunohistochemistry and further processed with appropriate steps.

Virus isolation

Brain homogenate from aborted fetus has been prepared apetically and inoculated into suckling mouse brain and passaged 3 times in the same manner. Third passaged mouse brain was used for the virus isolation in cell culture. Akabane virus cultured in the cell culture was detected by indirect immunofluorescent test using monoclonal antibody specific to the Akabane virus.

Immunohistochemistry

A paraffin embaded brain tissue blocks from formalin fixed brain tissue section were prepared as same manner for the conventional tissue section for the hematoxylin-eosin staining. A thin section of the brain tissue was de-paraffinized with solvent and incubated with monoclonal antibody specific to Akabane virus. Immunochemical procedure was adopted from Lyoo et al6 and minor modification was made. Briefly, deparaffinized tissue sections were incubated with monoclonal antibody specific to Akabane virus for three hours and washed three times with PBS. Tissue sections were reacted with biotinylated anti-mouse IgG. ABC kit (Vector, USA) was used for the chromogenic reaction to visualize Akabane antigens.

Results

Formalin fixed brain tissues from aborted fetus showed Akabane specific positive reaction (Fig 1A, B) and broad range of the positive cells also observed with degeneration of the brain tissues (Fig 1B). Arrows indicate dark brown immunohistochemistry positive cells which is result of chromogenic reaction labeled on secondary antibodies.

An isolated cell nearby vascular system showed positive reaction with monoclonal antibody (Fig 2A). A positive cells with dark brown cytoplasmic chromogenic reaction was detected without severe degeneration of brain tissue which may indicate initial infection of the tissue through vessels (Fig 2B).

Brain homogenate was intracerebrally inoculated into suckling mice for the virus isolation. From third passaged mouse brain tissue infected cell culture Akabane viral antigen was detected by indirect immunofluorescent test. An animal inoculation, cell culture isolation and immunohistochemistry showed all positive reaction but animal inoculation and cell culture inoculation was time and labour consuming process. Immunohistochemistry for the Akabane antigen detection from formalin fixed fetal brain tissue was sensitive and had good correlation with animal inoculation and cell culture virus isolation (Table 1).

Discussion

Sensitive and specific detection for the calf abortion in cattle industry is very important to set an appropriate vaccination program against pathogenic agent involved in reproductive failures in the farm. Akabane disease can be transmitted by mosquitos and showing similar signs in aborted fetus with other viral agent such as Chuzan and BVDV. Several diagnostic methods for the Akabane disease has been introduced include virus isolation and serodiagnosis. Virus isolation is considered to be a specific diagnostic method for the Akabane infection in claves but tedious cell culture and is time consuming process. Serodiagnosis positive is an only indication of the viral infection in cow and calves. Direct evidence of the Akabane virus infection in the aborted fetus should made from viral antigen or nucleic acid detection from the fetus. To satisfy this condition immunohistochemistry using monoclonal antibody specific to Akabane virus was used. This developed immunohistochemistry was sensitive and specific test for the detection of the Akabane viral antigen from formalin fixed fetal calf brain tissue. An animal inoculation, cell culture isolation and immunohistochemistry showed all positive reaction but animal inoculation and cell culture inoculation was time and la-

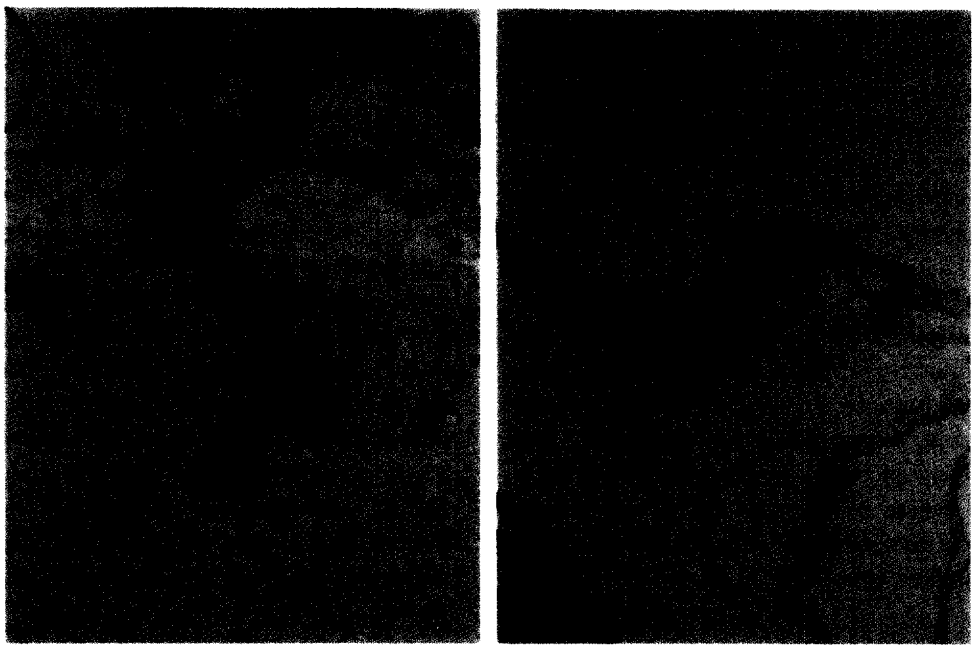

Fig 1. Immunohistochemistry for the detection of the Akabane virus viral antigen in fetal calf brain using monoclonal antibody to Akabane virus. Dark brown cells which indicate Akabane virus positive were accumulated in the area of mild degeneration (Fig 1A, closed arrow) and remarkable degeneration of the brain tissue from aborted fetus (Fig 1B, open arrow).

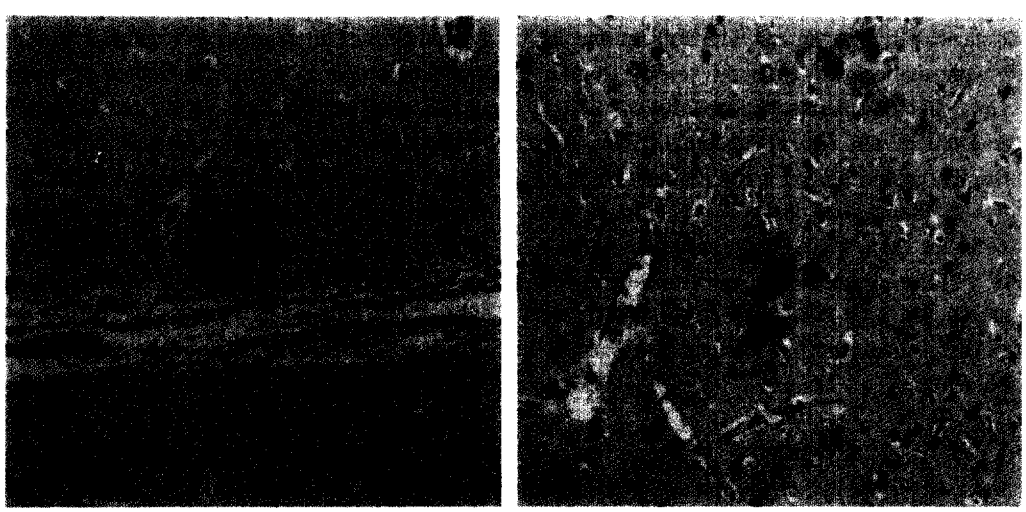

Fig 2. Isolated individual cell infected with Akabane virus was detected nearby vascular system in the brain tissue by immunohistochemistry.

Table 1. Detection of Akabane virus from brain tissue by mouse inoculation and tissue culture

Fetal brain sample	Mouse inoculation	Cell culture	Formalin fixed tissue
Test methods	Intracerebral inoculation	CPE[1]/IFA[2]	IHC[3]
Result	positive	positive	positive

1: Cytopathic effect, 2: Indirect immunofluorescent assay, 3: Immunohistochemistry

bour consuming process. Immunohistochemistry for the Akabane antigen detection from formalin fixed fetal brain tissue was sensitive and had good correlation with animal inoculation and cell culture virus isolation. This test could be effectively used for the detection of the Akabane virus antigen in aborted fetal brain tissue.

Conclusion

Detection of the Akabane virus antigen from aborted fetal calf brain tissue by immunohistochemistry was sensitive and applicable for the diagnosis of Akabane disease from field samples with unknown cause of abortion cases. The test was sensitive and practical for the fragile soft tissue such as brain tissue which is delicate to handle in the farm. Formaline fixed brain tissue was appropriate for the diagnosis of the Akabane disease by detecting virus antigen. The reliability of the test was confirmed by animal inoculation, virus isolation and indirect immunofluorescent test using monoclonal antibody specific to the Akabane virus.

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