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Chuzan Disease as congenital Hydranencephaly-Cerebellar Hypoplasia Syndrome in Calves

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Abstract

Chuzan disease, which is characterized by a hydranencephaly-cerebellar hypoplasia (HCH) syndrome in calves, took place in the Kyushu district of Japan during the period November 1985 to April 1986. The etiological agent of Chuzan virus was isolated from blood of sentinel cattle and Culicoides oxystoma in the period September to October 1985, and classified as a new member of the Palyam subgroup of the genus Orbivirus. The main clinical abnormalities of the disease are impairment of mobility and nervous signs. Almost all the affected calves could not suckle by themselves and some showed central nervous symptoms. Gross lesions of the affected calves were confined to the brain. The HCH syndrome was observed at a high rate. On the basis of virological and sero-epidemiological investigations and experimental infections with pregnant cows, it was confirmed that Chuzan virus was the etiologic agent of HCH syndrome of calves. The sero-epidemiological survey indicated that antibody to Chuzan virus was present at a high rate in the Kyushu district, while it was at a low rate in the Chugoku and Shikoku districts. The antibody was not found in the east and northeastern region of Japan. The sero-epidemiological investigations strongly suggested that Chuzan virus come to the main islands of Japan from the Southeast Asian countries through Okinawa. To control this disease in Japan, an inactivated vaccine is scheduled for practical use in the field in the near future.

Discipline: Animal health
Additional key words: congenital anomalies of calves

During the period November 1985 to April 1986, an epizootic incidence of congenital anomalies of calves characterized by hydranencephaly-cerebellar hypoplasia (HCH) syndrome took place in the Kyushu district of Japan. Approximately 2,400 calves were born with an HCH syndrome[1,2]. The histopathological findings, the seasonal outbreak and the geographical distribution of that disease suggested an infectious etiology.

A serological survey indicated that there was a high correlation between the HCH syndrome of calves and the antibody to Chuzan virus in precolostral sera, and also between the distribution of the antibody and the prevalence of Chuzan virus[1,2]. These findings strongly suggested that Chuzan virus be the etiologic agent of the above disease [2]. This conclusion was further confirmed in the course of the study on etiological role of Chuzan virus for congenital abnormalities with an HCH syndrome of calves by inoculating pregnant cows with that virus[10].

Miura et al. designated the HCH syndrome caused by Chuzan virus infection as Chuzan disease[10].

Etiology

1) Taxonomy: Chuzan virus was primarily isolated from blood of sentinel cattle and *Culicoides oxystoma* in the cell culture of HmLu-1 cell line derived from hamster lung cells, in the period September to October in 1985. The term of Chuzan virus came from the name of a town in Kagoshima city of Japan, where it was first isolated from cattle and

midges. Chuzan virus was classified in a new member of the Palyam subgroup of the genus Orbivirus on the basis of its physicochemical, morphological and antigenic properties[8].

2) Propagation in cell culture: Chuzan virus propagated with a cytopathic effect (CPE) in primary cell cultures as well as in cultures of cell lines. The virus grew well with an obvious CPE in cell lines originating from the tissues of bovine, swine, monkey and hamster, and in the primary swine, guinea pig and hamster cells. Propagation was most clear in BHK_{21} from baby hamster kidney and it was inapparent in the rabbit kidney cell line RK-13. Furthermore, the virus propagated well in suckling mice and hamsters by intracerebral inoculation and induced their death[3].

3) Properties and morphology: Treatment with ether or chloroform reduced the infectivity of Chuzan virus by one-tenth to one-hundredth as compared with that of the untreated virus, but the infectivity was not completely inactivated with the treatment of organic solvents. However, the virus was almost abolished at pH 3.0. The virus passed through membrane filters with pore size ranging from 100 to 450 nm, but failed to pass through a 50 nm pore filter. The replication of the virus was not inhibited by 5'-iodo-2'-deoxyuridine, indicating the virus was an RNA virus[1].

Haemagglutinating (HA) activity of Chuzan virus was demonstrated by using bovine erythrocytes. The virus presented enhanced HA with an increase of the salt concentration in the diluents[4].

Chuzan virus was observed in a single particle, or occasionally in the form of two or more particles wrapped in a membrane called pseudoenvelope. The diameter of particles ranged from 50 to 60 nm[8].

4) Antigenic relationship: Chuzan virus was not antigenically related to viruses of the genus Orbivirus such as epizootic hemorrhagic disease (EHD) of deer, Bluetongue, Corriparta, Eubenangee, and Warrego subgroup in the agar-gel immunodiffusion (AGI) and neutralization (NT) test, but was closely related to viruses of Palyam subgroup in AGI. Chuzan virus, however, could be distinguished from the tested viruses of the subgroup in NT test[8].

Clinical and pathological findings

1) Clinical signs: No clinical abnormalities have been observed in the cows during pregnancy[1,2]. During the prevalence of Chuzan disease, there was no increase of abortion and stillbirth.

The affected calves in uterus were of normal size and weight. They showed no deformities in appearance, except for corneal nebula which was seen occasionally. These neonates had impairment of mobility such as ataxia, repeated stumbling and falling, circling and colliding against a barrier. Some affected calves showed nervous symptoms, including intermittent or persistent opisthotonus, flapping of legs, tremor, and nystagmus (Plate 1). The calves appeared to be completely or partially blind, and almost could not suckle, although more than half of them could suck weakly if given artificially[2]. The prognosis of anomalies was poor, but it was presumed that in mild cases the calves might survive for some period under artificial feeding.

2) Necropsy findings: Gross lesions of affected calves were confined to the brain, but not in other organs. HCH syndrome was observed at a high rate (Plates 2 & 3). Hydranencephaly was found in more than 80% of the affected calves, while microencephaly with hydrocephalus or hydrocephalus was seen at a low rate[2,6,7]. Defect or marked hypoplasia and apparent hypoplasia of the cerebellum were recognized at a high rate[2,6,7]. It appeared that structures such as basal ganglia, hippocampus and thalamus were generally normal.

Histological lesions were correlated with gross lesions. Hydranencephaly was a principal lesion. The walls of the thin cerebral hemispheres consisted

Plate 1. An affected calf with opisthotonus

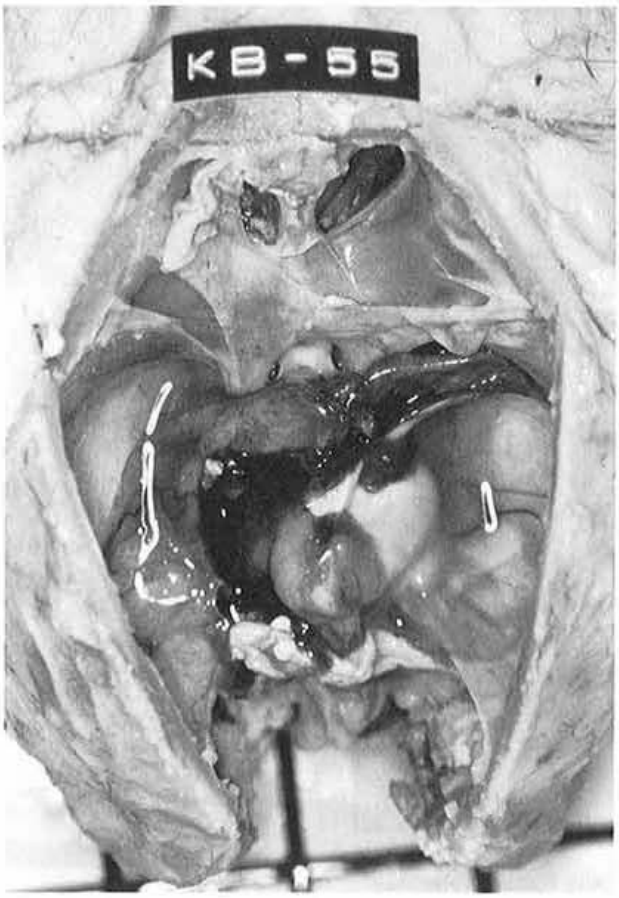

Plate 2. An affected calf with hydranencephaly and cerebellar hypoplasia

of remaining nervous tissues with adherent leptomeningings and ependymal cells. The degeneration of white matter was generally observed. Calcification was seen in the nervous tissue. Inflammatory reactions such as cuffing or glial cell proliferation were observed occasionally at a low rate. These reactions were very mild. In regard to hypoplasia of cerebellum, the molecular and granular layers as well as the folial white matter were all thin. Purkinje's cells frequently disappeared and sometimes dislocated. Cavitation in the white matter was seen in some cases[6,7].

Experimental infection

Under an experimental infection to the calves, all the adult and pregnant cattle and the cattle inoculated intravenously with Chuzan virus showed leukopenia and viremia without any other clinical signs. Viremia could be detected in a long term such as 4 to 8 weeks after infection. The virus was recovered from the plasma only intermittently before the development of virus neutralizing antibody. It was however found persistently in erythrocyte fractions for several weeks. The virus was mostly detected in the purified erythrocyte fraction and sometimes in the thrombocyte fraction, but not in leukocyte fractions. On the other hand, the calves infected intracerebrally with the virus suffered a fatal infection with severe nervous symptoms. Chuzan virus was recovered mainly from central nervous system, but not from other organs except lymphatic tissues of the animal[9].

Antibody to Chuzan virus was observed in two

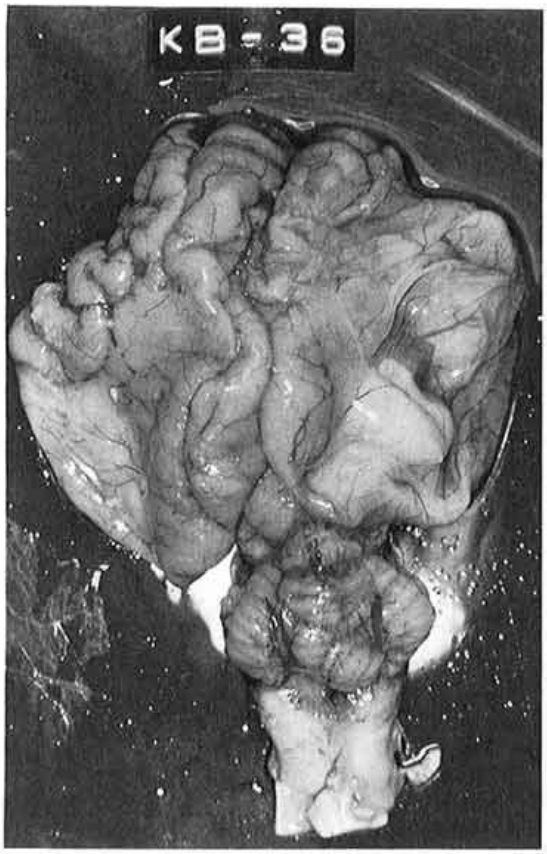

Plate 3. An affected calf with hydrocephalus with cerebellar hypoplasia

Plate 4. Hydranencephaly-cerebellar hypoplasia in a calf delivered from dam inoculated with Chuzan virus

weeks after inoculation by the NT and AGI test. The NT antibody titer ranged from 64 to 1,024 and maintained similar levels for 6 months after inoculation.

In the experimental infection of pregnant dams with Chuzan virus, HCH syndrome was produced in a dam inoculated in 120 days of gestation (Plate 4)[10]. Therefore, it is concluded that Chuzan virus has teratogenic effects on the fetus.

Epidemiology

1) Incidence: There has been no report on the incidences of HCH syndrome of calves in association with virus infections in Japan and in other countries as well. An epidemic of HCH syndrome of calves took place mainly in the Kyushu district, southwestern part of Japan, in the period November 1985 to April 1986. The number of suffered animals was about 2,400. Thereafter, however, that disease has not been found.

Delivery of calves with HCH syndrome was recognized in dams of any ages. It occurred mainly in beef breed, i.e. Japanese Black, and rarely in dairy breed of Holstain.

2) Serological investigation: a sero-epidemiological survey undertaken in 1986 indicated that antibody to Chuzan virus was present at a high rate of 30 to 87% in the case where an epizootic of HCH syndrome occurred during the period 1985 to 1986, and that the occurrences of HCH syndrome in Chugoku and Shikoku, both neighboring areas to Kyushu, were rare. The antibody was not found in the east and northeastern districts of Japan, such as Hokkaido, Tohoku, Kanto, Koshin-etsu, Hokuriku and Kinki[1].

Table 1. Comparison of Akabane disease and Chuzan disease

Findings	Akabane disease	Chuzan disease
Epidemiology		
Period of occurrence	Summer-Spring	Autumn-Spring
Breed of cattle	Any breed	Mainly Japanese beef cattle
Abortion, Stillbirth, Premature	+	−
Clinical findings		
Nervous symptoms	+	+
Weekness	+	+
Ability of suckling	±	−
Blind	+	+
Deformities in external appearance	+	−
Pathological findings		
Non-purulent encephalitis	+	+
Hydranencephaly	+	+
Cerebellar hypoplasia	−	+
Arthrogryposis	+	−
Polymyositis	+	−
Etiology	Akabane virus	Chuzan virus
Vectors	Mosquito Culicoides	Culicoides

+ : Positive, − : Negative, ± : Slightly positive.

The serologic evidence indicated that Chuzan virus had never been present in Kyushu before 1983. Chronological and geographical investigations on the prevalence of Chuzan virus in Japan showed that that virus had already been present in Ishigaki Island, the southernmost island in Japan, before 1981[1]. Furthermore, antibodies to the virus have been detected in Australian and Taiwanese cattle.

These data strongly suggest that Chuzan virus invaded the main islands of Japan through the islands in Okinawa from the Southeast Asian countries.

3) Vector: It may be concluded that the transmission is performed by the bites of arthropods, especially the genus *Culicoides,* as indicated by isolation and seasonal prevalence of Chuzan virus[1,8]. It is recognized that *C. oxystoma* from which Chuzan virus was isolated may act as a biological vector rather than as a mechanical one, since the virus was isolated from the midges after feeding them for 3 or 4 days to avoid the possibility of direct isolation of the virus from sucked blood.

Diagnosis

The presence of Chuzan disease could be suspected when an HCH syndrome takes place epidemically and endemically. A specific diagnosis of Chuzan disease needs laboratory tests. The presence of antibody to Chuzan virus in precolostral serum from abnormal calve indicated that a newborn had suffered vertical infection with the virus. For the serological diagnosis, the virus neutralization test or haemagglutination inhibition test could be applicable.

It may not be possible to isolate the virus from a calf with an HCH syndrome, because the affected fetus has already acquired antibody by itself before birth.

An HCH syndrome may have to be differentiated from the other diseases, especially from Akabane disease[5]. A comparison of Akabane disease and Chuzan disease is shown in Table 1. Differential diagnosis between these two diseases is not so difficult, if the clinical and pathological features are taken into consideration.

Prevention and control

Regarding the control of Chuzan disease, erradication of the related vector insect would be one of the most ideal methods, although the methods have not been well developed so far. At this time, application of vaccine is the most effective way in practice.

An evaluation test on the efficacy of a formalin-inactivated, aluminum phosphate gel-adsorbed vaccine revealed that the vaccine prevented development of leukopenia and viremia in immunized cattle after challenge inoculation[3]. An inactivated vaccine for cattle is scheduled for practical use in the field in 1990.

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