Hydranencephaly-Cerebellar Hypoplasia in a Newborn Calf after Infection of its Dam with Chuzan Virus

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ABSTRACT. Chuzan virus at 2 to 3 passage levels in cell cultures after isolation was inoculated intravenously into 15 seronegative pregnant cows at 89 to 150 days of gestation. All of the cows developed viremia a few days after inoculation and antibodies 2 weeks after inoculation. No clinical signs, except leukopenia, were observed throughout the experimental period. These 15 cows delivered 15 calves after normal gestation. One of the calves which was born to a dam inoculated at 120 days of gestation, showed impairment of movement, and the remaining 14 were healthy. Postmortem examination revealed that this calf had hydranencephaly-cerebellar hypoplasia (HCH) syndrome and that the remaining calves were normal. Two of the 15 calves, including the one that had HCH syndrome, had antibody to Chuzan virus in their precolostral sera. These findings provide additional evidence that Chuzan virus is the etiological agent of an epizootic of congenital abnormalities with HCH syndrome of calves in Japan, 1985 to 1986. We propose to name the HCH syndrome caused by Chuzan virus infection Chuzan disease.—KEY WORDS: cerebellar hypoplasia, Chuzan disease, Chuzan virus, congenital abnormality, hydranencephaly.

An epizootic of congenital abnormalities with hydranencephaly-cerebellar hypoplasia (HCH) syndrome of calves was observed in the Kyushu district of Japan from November 1985 through April 1986 [1, 3]. Although an etiologic agent was not isolated from the calves, results of serotests of precolostral serum samples strongly indicated that Chuzan virus, which was isolated in the Kyushu district from the blood of healthy calves and Culicoides oxystoma in 1985 [5] and identified as a new virus belonging to the Palyam subgroup of the Orbivirus genus, had a very close relationship to the occurrence of the HCH syndrome [2]. Seroepidemiological surveys of the disease also revealed that there was an inseparable relationship between the occurrence of the HCH syndrome and prevalence of Chuzan virus [1].

In the present study pregnant cows were inoculated with the virus to confirm the etiological role of Chuzan virus in congenital abnormalities with HCH syndrome of calves.

MATERIALS AND METHODS

Viruses: Nine strains of Chuzan virus were used. The K-47 (prototype strain), K-20 and K-21 strains were isolated from the blood of two sentinel calves kept in our institute, and C-4 and C-8 strains were isolated from Culicoides oxystoma collected in the same place [5]. Strains 31, 181, 165 and 422 were isolated from the blood of 4 calves in Oita, Miyazaki, Nagasaki and Nagasaki Prefectures, respectively, of the Kyushu district in October and November of 1985. Viruses subcultured once or twice after their isolation in HmLu-1 (hamster lung cell line) cell cultures were used for
Table 1. Results of inoculation of pregnant cows with Chuzan virus

<table>
<thead>
<tr>
<th>Cow No.</th>
<th>Days of gestation when inoculated</th>
<th>Strain used</th>
<th>Dose and infectivity of inoculum*</th>
<th>Clinical signs</th>
<th>Duration of pregnancy (days)</th>
<th>Pathological changes in newborn calves</th>
<th>Antibody titers of precolostral sera in calves</th>
<th>Vertical infection</th>
<th>Virus recovery from calves</th>
</tr>
</thead>
<tbody>
<tr>
<td>969</td>
<td>150</td>
<td>K-47</td>
<td>5(10^6.0)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>1024 (512)</td>
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</tr>
<tr>
<td>967</td>
<td>149</td>
<td>K-47</td>
<td>5(10^6.2)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (256)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>970</td>
<td>141</td>
<td>K-47</td>
<td>5(10^6.5)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (256)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>971</td>
<td>140</td>
<td>K-47</td>
<td>5(10^6.5)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (512)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>987</td>
<td>137</td>
<td>pool</td>
<td>20(10^6.3)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (256)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>986</td>
<td>131</td>
<td>pool</td>
<td>20(10^6.3)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (64)</td>
<td>-</td>
<td>-</td>
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<tr>
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<td>130</td>
<td>K-47</td>
<td>5(10^6.5)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>4b (256)</td>
<td>-</td>
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</tr>
<tr>
<td>975</td>
<td>130</td>
<td>K-47</td>
<td>5(10^6.9)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (256)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>973</td>
<td>120</td>
<td>K-47</td>
<td>5(10^6.7)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (512)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>966</td>
<td>120</td>
<td>K-47</td>
<td>5(10^6.7)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>HCH syndrome 512 (1024)</td>
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<td>-</td>
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<tr>
<td>985</td>
<td>120</td>
<td>pool</td>
<td>20(10^6.5)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (64)</td>
<td>-</td>
<td>N.D. f)</td>
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<tr>
<td>984</td>
<td>109</td>
<td>pool</td>
<td>20(10^6.5)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (128)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>988</td>
<td>108</td>
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<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (256)</td>
<td>-</td>
<td>N.D.</td>
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<tr>
<td>991</td>
<td>90</td>
<td>pool</td>
<td>20(10^6.3)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (128)</td>
<td>-</td>
<td>N.D.</td>
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<tr>
<td>992</td>
<td>89</td>
<td>pool</td>
<td>20(10^6.3)</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>&lt;2 (256)</td>
<td>-</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

* a) Pregnant cows were inoculated intravenously. Dose is expressed in milliliters and infectivity (shown in parenthesis) in TCID_{50}/ml.

b) Number in parentheses shows neutralizing antibody titer of dams at delivery.

c) A pool of 9 strains of Chuzan virus.

d) Maternal antibody titer. Sucking of colostrum was confirmed by postmortem examination.

e) Examined 10 to 20 months after birth.

f) Not done.
inoculation into cows.

_Cow inoculation tests:_ Cows of the Japanese Black breed were used since the abnormal calves were seen mainly in that breed during the 1985–86 epizootic [1]. Fifteen pregnant cows seronegative to Chuzan virus at 89 to 150 days of gestation were inoculated intravenously with 5 to 200 ml of virus suspension (10^{6.0}–10^{6.7}TCID_{50}/ml) of K-47 strain or a pool of 9 strains of Chuzan virus. They were kept individually in isolated insect-free pens until their delivery. Details are shown in Table 1.

They were observed for clinical signs daily and body temperature was taken twice a day. Blood was obtained to investigate differential blood cell counts, viremia and antibodies daily until 2 weeks after inoculation, once a week between 3 and 10 weeks after inoculation, and once every two weeks thereafter.

_Virus recovery:_ Tests for recovery of the virus from the plasma and washed erythrocyte suspensions of cows were performed in HmLu-1 and/or BHK-21 (baby hamster kidney cell line) cell cultures as described previously [6]. The method described previously were used for isolation of the virus from the blood and tissues of newborn calves [2].

_Virus neutralization test:_ This test was performed with the K-47 strain in tube cultures of HmLu-1 cells as described previously [5]. The antibody titer was expressed as the reciprocal of the highest serum dilution that inhibited the cytopathic effect of the virus.

RESULTS

_Observations in cows:_ None of the cows inoculated showed any clinical signs except mild leukopenia which was observed for 1 to 4 days between 2 and 7 days after inoculation. The virus was detected in the erythrocyte fraction and in the plasma of all cows. However, it was recovered more frequently and in higher titers from the erythrocyte fractions than from the plasma.

The virus appeared 2 to 4 days after the inoculation. Thereafter, it was recovered persistently until 2 weeks and intermittently between 3 and 8 weeks from the erythrocyte fractions. However, it could not be recovered thereafter. In contrast, the virus was isolated sporadically from the plasma 3 to 12 days after inoculation. The highest infective titer of the erythrocytes ranged from 10^{2.5} to 10^{3.5} TCID_{50}/ml in individual cows, and that of the plasma was 10^{1.5} TCID_{50} or equal to or less than 10^{0.7} TCID_{50}/ml.

Neutralizing antibody was detected 2 weeks after inoculation and the titer at delivery was 64 to 1,024. Details of virus propagation and antibody response in cattle

Fig. 1. Macroscopic findings of hydranencephaly-cerebellar hypoplasia in a calf delivered by a dam inoculated with the K-47 strain of Chuzan virus.
were given in a previous paper [6].

Observations in newborn calves: Fifteen calves were born after 277 to 298 days (Ave. 286.4 days) of gestation. After birth, these calves were tested clinically and precolostral serum was collected. Then, 11 of them were sacrificed for etiological and pathological examinations. Summarized results are shown in Table 1.

Of the 15 calves, one (No. 966) showed ataxia, slight tremor and intermittent opisthotonos. This calf was born after 285 days of gestation, and had no retarded growth or external malformations. The other 14 calves showed no clinical abnormalities. Two (Nos. 966 and 969) of the 15 calves, including the one that showed clinical abnormalities, had high titers (512 and 1,024) of precolostral antibody to Chuzan virus. Another one (No. 968) also had a low titer (4) of the antibody, but this antibody was thought to be a maternal one since sucking of colostrum was confirmed by the postmortem examination. The remaining 12 calves had no antibody to Chuzan virus.

Eleven of the calves, including the 2 antibody-positive ones, were examined etiologically and pathologically soon after birth. No virus or bacteria were isolated from the brain, lung, liver, spleen, kidney, or lymph nodes of any of the calves. The remaining 4 calves were sacrificed 10 to 20 months after birth.

Pathological changes were found in the calf with impairment of movement. Macroscopically, lesions were observed in the central nervous system and they were the same as those seen in cases of natural infection. Most of the cerebrum was of membranous thickness and contained cerebrospinal fluid in the ventricle. Structures such as the basal ganglia, hippocampus, and

Fig. 2. A: Thin membranous cortex. Ependymal cells are lining the ventricle. Brown pigment-laden macrophages infiltrate into the leptomeninges. B: Cerebellum. A large cavity is seen in the medulla. Purkinje's cells and neurons in the granular layer are decreased in number. The molecular layer is thinner than normal. HE staining, ×20.
thalamus remained intact (Fig. 1). Part of the cerebellum was defective and transparent fluid was retained. The medulla oblongata, spinal cord and other visceral organs had no lesions.

Histologically, the walls of the thin cerebral hemispheres consisted of remaining nervous tissues with adherent leptomeninges and ciliated ependymal cells (Fig. 2-A). Hemosiderin-laden macrophages infiltrated into the leptomeninges. The cerebellum was hypoplastic. Degeneration and cavitation of white matter were seen in those parts where the cyst was seen grossly (Fig. 2-B). Degeneration and disappearance of some Purkinje's cells and granular cells were observed. Calcification, which was frequently observed in natural cases, was not observed. Other organs had no lesions.

DISCUSSION

A role of Chuzan virus in the congenital HCH syndrome has been strongly suspected because 44 of 46 (96%) abnormal calves with the syndrome had high titers of precolostral antibody to the virus [2] and the occurrence of the disease coincided well geographically and chronologically with prevalence of the virus [1]. As the result of inoculation of Chuzan virus into pregnant cows, vertical infection of the virus was proved indirectly, since two calves had precolostral antibody to the virus. It is generally accepted that the presence of precolostral antibody is evidence of infection with the corresponding agent at some fetal stage. Maternal antibody does not pass the placenta in the ruminant and a bovine fetus produces an antibody to the heterologous protein throughout the middle and late fetal stages [8]. Furthermore, one of the precolostral antibody-positive calves demonstrated congenital abnormalities like those observed macroscopically and microscopically in natural cases of congenital HCH syndrome. Although the virus could not be recovered from the calf, just as in the case of naturally infected abnormal calves [2], it was believed that the virus was excluded during the fetal stage by the host defense mechanism. This finding provides strong additional evidence that Chuzan virus is the etiological agent of epizootic congenital abnormalities with HCH syndrome. Therefore, we propose to name the congenital HCH syndrome caused by Chuzan virus Chuzan disease.

The high-risk period of pregnancy for placental transmission of Chuzan virus is roughly known since vertical infection was observed when cows were inoculated at 120 and 150 days of pregnancy. This period coincides well with the seroepidemiological estimation that infection with Chuzan virus in utero develops at around 130 days of gestation [1, 2]. Of 11 cows infected with the virus between 120 and 150 days of pregnancy, one cow delivered a calf with HCH syndrome. This rate is similar to the rate of occurrence of congenital abnormalities in Kagoshima Prefecture during prevalence of the disease [1].

Viremia may play an important role in establishing vertical infection. Both cell-free and erythrocyte-associated viremia was observed in every cow in this experiment. Although the degree of viremia differed slightly among individuals, the difference did not seem to be directly correlated with in utero infection. Transmission of the virus to the fetus may depend on the developmental stage of the placenta as the fetal infection with the virus occurred in a limited gestation period, but the mechanism remains to be elucidated.

It was found that Chuzan virus causes subclinical vertical infection in the bovine fetus as commonly shown in other teratogenic virus infections [4, 7]. Since we had only 2 cases of fetal infection with Chuzan virus, it is difficult to determine the rela-
relationship between fetal age at infection and development of pathological changes. Further investigations are needed to disclose the pathogenesis of HCH syndrome.

REFERENCES


要 約

チュウザンウイルス接種妊娠牛から生まれた子牛の水無脳-小脳低形成症：三浦康男・久保正法・後藤義之1)・甲野雄次2)（家畜衛生試験場九州支場、2)家畜衛生試験場）——妊娠89-150日の15頭の抗体陰性牛の静脈内にチュウザンウイルスを接種した。接種2、3日後から2週後にかけて、すべての牛はウイルス血症を示したが、軽い白血球減少症以外の臨床症状は示さなかった。これらの牛は正常な妊娠期間の後に出産した。15頭の子牛のうち妊娠120日に接種された母牛から生まれた1頭が運動障害を示し、剖検の結果、病理組織学的に自然例の病変と区別できない、水無脳-小脳低形成症が認められた。他の14頭は健康で、なんらの病理変化も認められなかった。水無脳-小脳低形成症牛および妊娠150日に接種を受けた母牛から生まれた1頭は初乳摂取前血清中にチュウザンウイルスに対する抗体を保有していた。これらの結果は、1985-86年にかけて九州地区で流行した子牛の水無脳-小脳低形成症がチュウザンウイルスにより起こったことを実験的に示したものと思われる。チュウザンウイルスにより起こる水無脳-小脳低形成症をチュウザン病と呼称することを提唱したい。