Report

THE INCIDENCE OF ANTIBODY TO AINO VIRUS IN ANIMALS AND HUMANS IN FUKUOKA

SHIGENORI FUKUYOSHI, YUHEI TAKEHARA, KATSUMI TAKAHASHI and RYOICHI MORI*

Fukuoka Environmental Research Center, Dazaifu, Fukuoka 818-01 and *Department of Microbiology, School of Medicine, Kyushu University, Fukuoka 812

(Received, October 27, 1980. Accepted, December 12, 1980)

Aino virus was isolated from Culex tritaeniorhynchus in Aino town, Nagasaki Prefecture, Japan, and has been reported to be a member of the Simbu group of Bunyavirus (Takahashi et al., 1968). Miura et al. (1978) showed that this virus is serologically identical with Samford virus, which was isolated from Culicoides brevitarsis in Australia (Doherty et al., 1972). We now report a sero-epidemiologic study of Aino virus infection in apparently healthy animals and humans in Fukuoka Prefecture.

Prototype Aino virus, JaNAr 28 strain, passaged 15 times by intracerebral inoculation into suckling mice was used. The infected brains were emulsified and made up to a 10% suspension with 0.75% bovine serum albumin (Fraction V from bovine plasma) in phosphate buffered saline, pH 7.4 (PBS). The suspension was centrifuged at 10,000 rpm for 20 min, and the supernatant was stored at -70°C until use. The titers of the virus samples were $1 \times 10^{5.5}$ TCID$_{50}$/0.025 ml as measured in Vero cells. Cells were grown in 96 wells in micro tissue culture plates with Eagle’s minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS) free of anti-Aino antibody. MEM supplemented with 2% FBS was used as maintenance medium for virus titration and for neutralizing antibody assay.

For titration of neutralizing antibody, a fourfold dilution of the serum was inactivated at 56°C for 30 min. Serial twofold dilutions of the inactivated serum were made and 0.025 ml of each was dispensed into a well in a transfer plate. To each well, 100 TCID$_{50}$ of Aino virus in 0.025 ml was added. The mixtures were incubated at 4°C for 90 min and transferred to wells containing confluent monolayers of Vero cells. After 7 days’ incubation at 37°C in a 5% CO$_2$ atmosphere, the cultures were examined for cytopathic effect (CPE) under a light microscope. The antibody titer was expressed as the reciprocal of the highest
The incidence and titer of antibody to Aino virus in animals and humans who had lived in Fukuoka Prefecture for more than one year

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of sera tested</th>
<th>Number of individuals with neutralizing antibody at titer of:</th>
<th>Number positive/number tested (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;4</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Horse</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cow</td>
<td>101</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>Swine</td>
<td>143</td>
<td>141</td>
<td>2</td>
</tr>
<tr>
<td>Domestic fowl</td>
<td>193</td>
<td>193</td>
<td>0</td>
</tr>
<tr>
<td>Human</td>
<td>171</td>
<td>168</td>
<td>5</td>
</tr>
</tbody>
</table>

dilution of serum completely preventing CPE.

The serum samples were obtained from apparently healthy 1–3 years old animals and from 1–74 years old humans who had lived in Fukuoka Prefecture for more than one year. All serum samples were stored at -20°C and thawed just before the neutralizing test.

The results of the study are shown in Table I. If the neutralizing antibody titer of 4 or higher is taken as positive, approximately 25% of the cows and horses had the antibody to Aino virus at the highest titers. Little or no antibody was found in swine or domestic fowls. Of the 171 humans tested, 4.7% were found to have antibody to Aino virus.

Since Akabane virus is one of the members of Simbu group of Bunyavirus, the antigenic relationship between Aino and Akabane viruses was considered. Cross-neutralization tests were carried out on the mouse antisera with Aino and Akabane viruses to see if there is any antigenic relationship between them. Hyper-immune sera against Aino and Akabane viruses used for the cross-neutralization test were prepared in 4-week-old mice with five ip injections of each living virus. Seven days after the last dose, serum was obtained. The neutralization tests were performed by the microplate method using Vero cells as described above. No cross-neutralization was observed between Aino and Akabane viruses.

To our knowledge, there have been no seroepidemiologic studies on Aino virus in healthy animals or humans in Japan. Miura et al. (1974) and Kurogi et al. (1975) studied the incidence of neutralizing antibody to Aino virus in calves and cows with congenital arthrogryposis-hydranencephaly (AH) syndrome and reported that 2 of 20 (Miura et al., 1978) and 2 of 52 (Kurogi et al., 1975) calves had antibody to the virus.

On the other hand, the incidence of antibody for Akabane virus was very high in calves and cows with AH syndrome (83%) as compared with normal calves and cows (27%), indicating an intimate correlation between AH syndrome and precolostral anti-Akabane antibody. Akabane virus was strongly suggested to be the etiologic agent of epizootic abortion and congenital AH
syndrome in cattle (Miura et al., 1974; Kurogi et al., 1975). Incidence of antibody to Akabane virus in normal calves and cows is not different from our data on Aino virus. Pathogenicity of Aino virus to animals or humans is not known at present.

Doherty et al. (1972, 1973) studied the incidence of antibody to Samford virus, which is serologically identical to Aino virus, in cattle, horses, domestic fowls and humans at the time of an endemic of bovine ephemeral fever in Australia. Cattle had the highest titers and the highest incidence of antibody. However, the incidence of neutralizing antibody to Samford virus in cattle varied from district to district.

In Fukuoka Prefecture, which is not far from Aino town, cows and horses showed a higher incidence of antibodies and higher titers of antibody to Aino virus than did swine and domestic fowls. Moreover, some humans had antibody to Aino virus. The fact may suggest that humans are infected with Aino virus although no etiological relationship of this virus to any disease is known.

**Acknowledgement**

The authors are grateful to Dr. A. Oya, Department of Virology and Rickettsiology, National Institute of Health, for his kind advice to this study and to Dr. Mary Louise Robbins for reviewing the manuscript.

**References**


