Seroprevalence Survey of Aino Virus Infection in Dairy Cattle of Fukuoka, Japan in 1990

Kazuki ISHIBASHI, Hitomi SHIRAKAWA, Yukinori UCHINUNO, and Takashi OGAWA[1)]

Fukuoka Chuo Livestock Hygiene Service Center, 2–1–3 Isouda, Hakata, Fukuoka 816 and [1)]National Institute of Animal Health, 3–1–1 Kannondai, Tsukuba, Ibaraki 305, Japan

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ABSTRACT. The seroprevalence and seroconversion to Aino virus infection were epidemiologically analyzed in Fukuoka Prefecture, by a cohort study, in 1990. Serum samples (872) were taken from 436 cattle of 128 farms in five different districts at twice sampling with four month-intervals and were tested by a serum neutralizing test with Aino virus. In the first sampling (May-July), 38.3% of sera and 40.6% of farms tested were positive and 33.3% of cattle and 40.6% of farms were sero-converted during the period of September-November in 1990. Significant differences were found in seroprevalence of farms and seroconversion of cattle/farms among five districts; there was, however, a highly significant correlation between seroprevalence and seroconversion of farm. Consequently, five districts of Fukuoka Prefecture were classified into four epidemic areas according to seroprevalence and seroconversion factors of a principal component analysis.—KEY WORDS: Aino virus, epidemiology, principal component analysis, seroconversion, seroprevalence.

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Aino virus has been belonging a member of the Simbu group of the family Bunyaviridae of arboviruses, which has been isolated from mosquitoes (*Culex tritaeniorhynchus*) in 1964, in Nagasaki Prefecture of Japan [7]. After that, neutralizing antibody to Aino virus has been detected in precolostral serum from a small percentage of calves manifesting the arthrogryposis-hydranencephaly (AH) syndrome in Kagoshima Prefecture [3]. Aino virus may be one of the etiological viruses causing the AH syndrome as Akabane virus.

In Fukuoka Prefecture, we have isolated one strain of Aino virus from a dairy cattle with sudden astasia and leukopenia in September of 1990 [1]. Subsequently, during the period of December, 1990 to February, 1991, the clustering of congenital scoliosis suspected Aino virus infection was observed in 10 new born calves [2].

The purpose of this study is to determine retrospectively the seroprevalence of Aino virus infection and to investigate epidemiologically the seroprevalence and seroconversion clustering in Fukuoka Prefecture throughout 1990.

MATERIALS AND METHODS

Sampling: The target population was 500 dairy cattle in 130 farms of five districts i.e., the Chuo, Chikuho, Chikugo, Kita-Kyusyu and Ryochiku under the five livestock hygiene service centers in Fukuoka Prefecture in 1990.

One hundred thirty farms were selected retrospectively from data for brucellosis inspection program during the period of May-July in 1990. Within each farm, regarding hard size, sera of 2–8 cattle were sampled from the preservation serum stock. Each cattle and farm were sampled twice by a cohort study; the second sera were collected from same cattle at the first sampling during the period of September-November.

A total of 1,000 serum samples were collected from 500 cattle at twice sampling in four month-intervals. These samples were limited the cattle aged from less than 1 to 4. But, age distribution was not confounded with a particular district.

As serum samples which showed indistinct age or did not collect blood at the second sampling were excluded, 872 serum samples in 128 farms were consequently used for a serological test.

Serum neutralizing (SN) test: The serum samples were tested by the SN test against JaNAr28 strain of Aino virus [7]. All sera were inactivated at 56°C for 30 min. Serial two-fold serum dilutions were mixed with equal volumes of virus suspension containing about 200 $TCID_{50}$ per 0.025 m*l* in microplates. The mixtures were incubated at room temperature for 90 min, dropped into each duplicate wells per 0.05 m*l* and HmLu-1 cell (4×10^4/0.1 m*l*) was dropped into same wells.

After incubation at 34°C for 4 days in 5% CO_2 incubator, each dilution was examined for CPE and the titer was expressed as the reciprocal of serum dilution for 50% SN.

Epidemiological and statistical analysis: Seropositive cattle and seroprevalence farm were defined as more than twice in the SN titer and in geometric mean (GM) of the SN titer, respectively. On the other hand, seroconversion of cattle in paired sera was defined as following criteria; (1) Seropositive titer which turned from negative titer (<2) at the first sampling; (2) More than 4 times increasing of the SN titer. Also the criteria of seroconversion farm was more than twice increasing of GM of the SN titer in paired samples.

The statistical analysis was carried out using procedures of the Statistical Analysis Systems (SAS) Institute [6] with HP 9000/720 workstation of the MAFFIN (Ministry of Agriculture, Forestry and Fisheries Research Network) in Tsukuba, Japan.

Comparison of seroprevalence and seroconversion rates among the different districts was evaluated by Pearson's chi-square and Fisher's exact two-tailed tests because of

the small number of observation in one of the cells.

Principal Component Analysis (PCA) as described in previous paper [5] was calculated using correlation matrix (4 × 4 variables) derived from data of the seroprevalence and seroconversion rates of each cattle and farm.

RESULTS

Comparison of seroprevalence and seroconversion of cattle: As shown in Table 1, 167 of the 436 cattle (38.3%) in the first sampling (May-July) were positive by the SN test and 145 cattle were subsequently seroconverted (33.3%) during the period of September-November, 1990.

No overall significant difference was found among the five districts regarding the seroprevalence of cattle ($P>0.05$). The rates were revealed, ranging 29.4 to 45.2% (Table 1).

On the other hand, regarding the seroconversion, overall significant difference was found among the five districts ($P<0.01$). The rates were varied; particularly that of Kita-Kyusyu was the lowest (14.6%) across the five districts.

Comparison of seroprevalence and seroconversion of farm: As shown in Table 2, 52 of the 128 farms (40.6%) were positive in the GM of the SN titer and seroconversion was also same rate (40.6%). Overall significant difference was found on the both of seroprevalence and seroconversion among the five districts ($p<0.05$ and $p<0.01$).

Table 1. Aino virus seroprevalence and seroconversion of cattle population among districts

Dist.	Number of samples	Number of seropreva-lence	Prevalence (%)[a]	Number of seroconversion	Conversion (%)[b]
Chuo	113	40	35.4	48	42.5
Chikugo	93	42	45.2	35	37.6
Chikuho	51	15	29.4	21	41.2
Kita-Kyusyu	89	35	39.3	13	14.6
Ryochiku	90	35	38.9	28	31.1
Total	436	167	38.3	145	33.3

a) Chi-square value=4.01, d.f.=4, Prob.=0.404>0.05.
b) Chi-square value=20.71, d.f.=4, Prob.=0.00<0.01.

Table 2. Aino virus seroprevalence and seroconversion of farm among districts

Dist.	Number of farm	Number of seroposi-tive	Prevalence (%)[a]	Number of seroconversion	Conversion (%)[b]
Chuo	23	5	21.7	13	56.5
Chikugo	24	13	54.2	14	58.3
Chikuho	23	10	43.5	13	56.5
Kita-Kyusyu	29	8	27.6	3	10.3
Ryochiku	29	16	55.2	9	31.3
Total	128	52	40.6	52	40.6

a) Chi-square value=9.89, d.f.=4, Prob.=0.042<0.05.
b) Fisher's exact probability=2.45×10^{-4}<0.01.

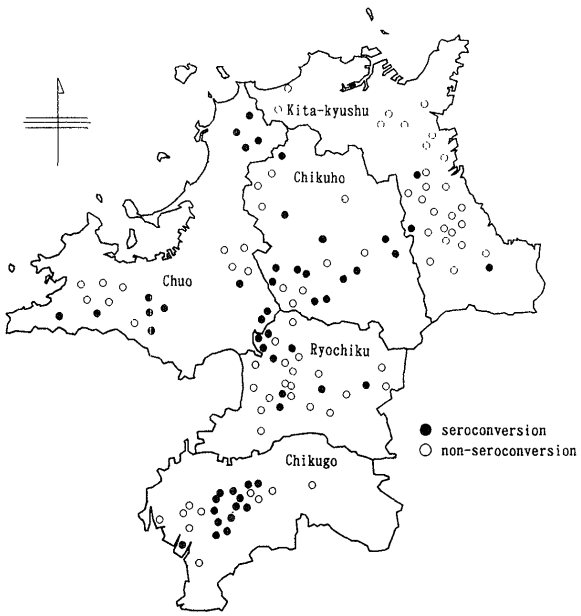

Fig. 1. Distribution of seroconversion farms to Aino viurs infection in Fukuoka Prefecture in 1990.

The seroprevalence rates were low in Chuo and Kita-Kyusyu districts (21.7 and 27.6%, respectively) and similarly the seroconversion rate was the lowest (10.3%) in Kita-Kyusyu district (Table 2). Locations of the seroconversion farms were plotted in a map of Fukuoka Prefecture as shown in Fig. 1.

Correlation matrix and PCA: The correlation matrix among the seroprevalence and seroconversion was shown in Table 3. The correlation coefficient between seroprevalence and seroconversion of farm was only extremely high (0.963) while the others were low.

As a result of the PCA (Table 4-a), eigenvalue of first principal component (PRIN1) was 2.1065 and that of second principal component (PRIN2) was 1.3301. Cumulative proportion of the PRIN1 and PRIN2 was 0.8591 reflecting a major variation observed among four variables. Eigenvectors (correlation coefficients between variable and principal component) were shown in Table 4-b. The seroconversions of cattle and farm contributed 0.6812 and 0.6639 to the PRIN1. On the other hand, the seroprevalences of cattle and farm contributed 0.6627 and 0.7412 to the PRIN2.

As results above, the PRIN1 could be interpreted as the factor relating to the seroconversion, namely, epidemic of Aino virus during the autumn season of 1990. Furthermore, the PRIN2 could be interpreted as the factor relating to the seroprevalence, namely, traces of the past epidemic.

Epidemiological characteristics of five districts: The component scores of five districts which induced from the PRIN1 and PRIN2 are presented in Fig. 2. The five

Table 3. Correlation matrix among seroprevalence and seroconversion to Aino virus

Variable		Seroprevalence of cattle	Seroprevalence of farm	Seroconversion of cattle	Seroconversion of farm
Seroprevalence of	cattle	1.000			
	farm	−.329	1.000		
Seroconversion of	cattle	0.334	0.170	1.000	
	farm	−.193	0.963	0.186	1.000

Table 4. Results of principal component analysis
a. Eigenvalues

Principal component	Eigenvalue	Proportion	Cumulative
PRIN1[a)]	2.1065	0.5266	0.5266
PRIN2[b)]	1.3301	0.3325	0.8591
PRIN3[c)]	0.5381	0.1345	0.9937
PRIN4[d)]	0.0253	0.0063	1.0000

a)–d) First to fourth principal components.

b. Eigenvectors

Variable		PRIN1[a)]	PRIN2[b)]
Cattle	seroprevalence	−.2785	0.6627
	seroconversion	0.6812	0.0254
Farm	seroprevalence	0.1324	0.7412
	seroconversion	0.6639	0.1041

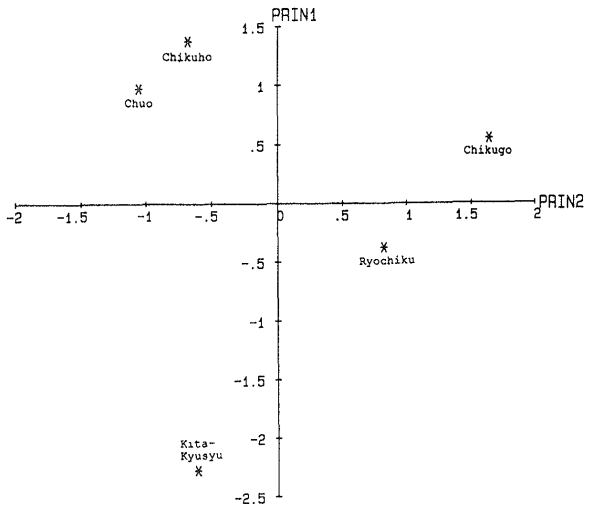

Fig. 2. Component scores of five districts to first and second components in principal component analysis.

districts could be classified into four groups by two dimensions of the axes of the PRIN1 and PRIN2 as follows:
(1) Chikugo district scored PRIN1>0 and PRIN2>0 was revealed well seroprevalence and seroconversion, i.e., was affected considerably with Aino virus infection in the past and subsequently was infected certainly in 1990.
(2) Ryochiku district scored PRIN1<0 and PRIN2>0 was affected certainly with Aino virus infection in the past, but less infected in 1990.
(3) Kita-Kyusyu district scored PRIN1<0 and PRIN2<0 was not revealed well seroprevalence and seroconversion, i.e., was affected slightly with Aino virus infection.
(4) Chuo and Chikuho districts scored PRIN1>0 and PRIN2<0 were less affected with Aino virus infection in the past, but were infected considerably in 1990.

DISCUSSION

Miura *et al.* [4] investigated the epizootic patterns of Aino virus in Kagoshima Prefecture of 1980, and indicated that prevalence of Aino virus was obvious once in several years. In 1990 in Fukuoka area in Japan, the Aino virus was isolated from the blood of a dairy cattle, which showed such symptoms as astasia and leukopenia [1]. Then, during December, 1990 and February 1991, 10 congenitally abnormal calves were found in the area, and some participation of the Aino virus was suspected [2]. The purpose of the present investigation was in that the whole picture of the prevalence of the Aino virus in 1990 should be more precisely understood, and sero-epidemiologic data were statistically analyzed. Seroprevalent cattle to the Aino virus before 1990 showed no distinct difference in the rate of seroprevalence in 5 districts, suggesting that the prevalence of the virus was widely spread in the whole Fukuoka area. In the retrospective investigation of the cohorts, significant difference in the seroconversion to the Aino virus among the 5 districts was found, suggesting that the spread of the Aino virus was so rapid and not even in the districts. Such facts may be verified by the map which showed situations of the seroprevalence farms (Fig. 1). For the purpose of analyzing the prevalence pictures more in detail, epidemiologic data of four variables of seroprevalence and seroconversion in cows and farms were analyzed by PCA. The cumulative proportion of the first and the second components was 0.8591,and prevalence of the Aino virus was classified into four patterns by two principal scores. Noteworthiness in the present investigation was that the rate of seroprevalence was not necessarily related to the prevalence of the virus in the two districts: PRIN1>0 and PRIN2>0 in Chikugo district and PRIN1<0 and PRIN2<0 in Kita-Kyusyu district.

Reviewing the correlation coefficients between seroprevalence and seroconversion of farm, significantly high correlations were found. Namely, it was explain that farms which were infected with the Aino virus in past showed new seroconversion.

The fact may imply that some particular farm may be accessible to the Aino virus. In other words, such farms are located in the geographically and meteorologically adequate area where growth of the sanguivorous insects is easy.

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