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Serologic Evidence for Etiologic Role of Akabane Virus in Epizootic Abortion-Arthrogryposis-Hydranencephaly in Cattle in Japan, 1972—1974

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With 1 Figure

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Summary

In the outbreak of abortions, premature births, stillbirths and congenital arthrogryposis-hydranencephaly (AH) syndrome in Japan during the summer through winter of 1972—73 and 1973—74, precolostral sera from calves with congenital AH syndrome and normal calves were tested for neutralizing antibodies against some arboviruses, *i.e.* Akabane, Aino, Getah and Japanese encephalitis (JE) viruses. The incidence of antibody for Akabane virus was very high in calves with AH syndrome (49/59 or 83 per cent) as compared with normal calves (3/11 or 27 per cent), indicating an intimate correlation between the AH syndrome and precolostral anti-Akabane antibody. Three stillborn fetuses also had anti-Akabane antibody. On the other hand, no precolostral serum antibody for the other viruses was detected in any of the calves tested. The mothers of these calves, normal and with AH syndrome, had anti-Akabane antibody in high percentages (44/52 or 85 per cent and 7/8 or 88 per cent), whereas a few of the mothers had antibodies for the other viruses.

Serological surveys indicate a wide dissemination of Akabane virus in epizootic areas during the summer months of 1972 and 1973. Thus, 8 groups of cattle in epizootic areas showed high rates of seroconversion for Akabane virus during the 1972 or 1973 summer. Very high incidences of Akabane antibody were shown among cattle in epizootic areas but extremely low incidences in near-by non-epizootic areas. The geographic distribution of anti-Akabane antibody among cattle throughout the country in the 1973 spring generally agrees with the pattern of case distribution in the 1972—73 outbreak.

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All these findings strongly suggest that Akabane virus is the etiological agent of the outbreaks. Further studies are needed, particularly isolation of the virus, demonstration of infection with the virus in lesions by immunofluorescence and production of intrauterine infection by experimental infection of pregnant cows.

1. Introduction

Cases of abortion, premature birth, stillbirth and congenital arthrogryposis-hydranencephaly (AH) syndrome have been observed in epizootic proportions in the central and western parts of Japan during the summer through winter months in 1972—1973 (6, 7, 12, 16, 22—25, 31). The total number of reported cases amounted to 31,240 of which 36.2, 20.7 and 43.1 per cent were cases of abortion (plus premature birth), stillbirth and congenital AH syndrome, respectively (22).

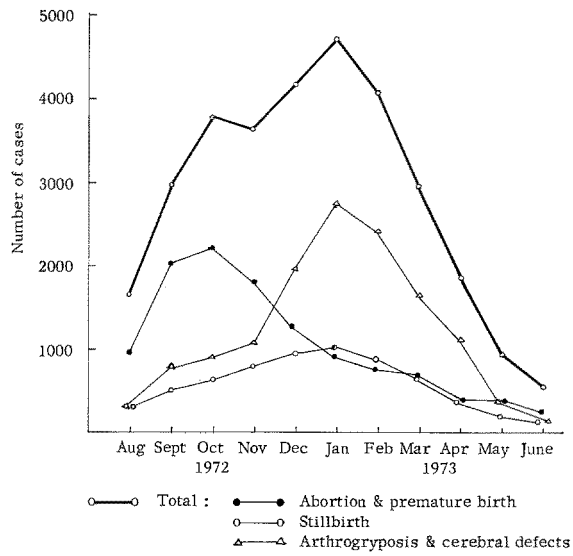

Fig. 1. Monthly numbers of reported cases (Bureau of Animal Industry, Ministry of Agriculture and Forestry)

The outbreak was mostly limited to Kyushu, Kanto, Chugoku and Shikoku districts (23). Dairy and beef cattle were affected likewise (23). No clinical abnormalities which could be related to those abnormal births have been recognized in the mother animals during their pregnancy (6, 7, 12). The monthly number of reported cases of abortion and premature birth increased rapidly in August and September of 1972, reached a peak in October, and then gradually declined, while the monthly number of AH syndrome cases showed a gradual rise in the early months of the outbreak and then a sharp rise in December, reaching a peak in January 1973. Stillbirths showed a gradual increase and decline with a peak in January (Fig. 1). The outbreak subsided in May 1973 (23).

The outbreak recurred in 1973—1974, although it was limited in size und area. The outbreak tended to spare the areas severely affected by the previous out-

break and to move to adjoining areas. The dams affected in the last outbreak were spared in this outbreak.

The central nervous system of affected fetuses and newborn calves showed various degrees of damages, and more importantly some inflammatory changes including perivascular cuffing, neuronophagia and glia cell proliferation were observed particularly in cases occurring in the early stages of the outbreaks (16, 22). This finding, together with the seasonal occurrence and the geographical distribution of cases, suggests an infectious nature of etiology (24).

We have tested precolostral sera from calves with congenital AH syndrome for antibodies against several arboviruses and some other bovine viruses, and found antibody for Akabane virus, a member of the Simbu group of arboviruses, in a high percentage of sera tested (20). Encouraged by this finding, we extended the serological survey and proved an intimate correlation between the congenital AH syndrome of calves and antibody for Akabane virus in their precolostral sera, and obtained serological evidence for wide dissemination of Akabane virus among cattle in the epizootic areas during the summer months in 1972 and 1973.

2. Materials and Methods

2.1. Viruses

Akabane virus (JaGAr 39 strain) (26), and Aino virus (JaNAr 28 strain) (30) of Group Simbu of arboviruses, Getah virus (Haruna strain) (18) of Group A, and Japanese encephalitis (JE) virus (Nakayama strain) of Group B were used. Infected suckling mouse brains were homogenized with cell culture medium (see below) to make a 10 per cent suspension, and, after centrifugation at $700 \times g$ for 10 minutes, were stored at —80° C until use.

2.2. Cell Culture

Vero cells were grown at 37° C in Eagle's minimum essential medium containing 10 per cent tryptose phosphate broth, 10 per cent bovine serum, 100 μg/ml kanamycin and 2.5 μg/ml fungizone. The sera used were free of neutralizing antibodies for the viruses used in this study.

2.3. Sera

Bovine sera for the test were obtained from the jugular vein and stored at —20° C until use.

2.4. Neutralization (NT) Test

The test was performed by the serum dilution method using disposable flat-bottom microplates (Cook Engineering Co., U.S.A.). The cell culture medium described above was used for dilution of serum and virus as well as for cultivation of inoculated cell cultures.

For infectivity assay, serial decimal dilutions of the virus suspension were delivered in 0.05 ml amounts into wells, 3 wells per dilution, containing 0.1 ml of cell suspension, 5×10^5/ml. The plates were sealed with cellotape, incubated at 37° C for 5 days, and the $TCID_{50}$ titer was calculated on the basis of cytopathic effect.

For the NT test, 0.025 ml of each of serial twofold dilutions of the serum inactivated at 56° C for 30 minutes was mixed with 0.025 ml containing 200 $TCID_{50}$/0.05 ml of virus in wells of transfer plates. One well was used per serum dilution. The virus-serum mixtures were incubated at 25° C for 1 hour, transferred into wells of flat-bottom plates containing 0.1 ml of cell suspension, 5×10^5/ml, and incubated at 37° C for 5 days. The antibody titer was expressed as the reciprocal of the highest serum dilution inhibiting cytopathic effect. Titers of 4 or higher were taken as positive.

3. Results

3.1. Antibodies in Precolostral Sera of Calves with Congenital AH Syndrome and Sera of their Mothers

As shown in Table 1, neutralizing (NT) antibody for Akabane virus was detected in a high percentage (83.1 per cent) of 59 precolostral sera tested, whereas none of the sera tested was seropositive for Aino, Getah and JE viruses. All but 7 of their mothers were tested and a high percentage of 84.6 per cent were positive for anti-Akabane antibody, while antibody for any of the other viruses was found in only a few of the animals tested.

Table 1. *Incidences of Neutralizing Antibodies against Akabane, Aino, Getah and Japanese Encephalitis (JE) Viruses in Precolostral Sera of Calves with Congenital AH Syndrome and Sera of their Mothers*

	Akabane		Aino		Getah		JE	
Prefecture	M[a]	C[a]	M	C	M	C	M	C
Okayama	16/16[b]	18/18	1/15	0/18	0/15	0/18	5/16	0/18
Hyogo	3/3	8/8	0/3	0/8	0/3	0/8	1/3	0/8
Ishikawa	3/3	3/3	0/3	0/3	1/3	0/3	0/3	0/3
Chiba	17/22	16/22	1/22	0/22	3/22	0/22	3/22	0/22
Ibaraki	4/4	4/4	0/4	0/4	1/4	0/4	0/4	0/4
Tokyo	1/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
Total	44/52	49/59	2/52	0/59	5/52	0/59	9/52	0/59
(%)	(84.6)	(83.1)	(3.8)	(0)	(9.6)	(0)	(17.3)	(0)

[a] Precolostral sera from calves with congenital AH syndrome (C) and sera of their mothers (M) (Holstein and Japanese black beef cattle) were collected during the winter months, i.e. from November 1972 to March 1973 and from November 1973 to March 1974. The mothers of 2 and 5 calves in Okayama and Hyogo Prefectures, respectively, were not tested.

[b] Number of positives / number tested.

Of those calves tested, both Holstein and Japanese black cattle, 16 had hydranencephaly, 40 had arthrogryposis and 3 had both. These three groups of animals showed no significant difference in the incidence of anti-Akabane antibody in precolostral serum, the positive rates being 14/16 (85.7 per cent), 33/40 (82.5 per cent) and 2/3, respectively.

Correlation of NT antibody titers for Akabane virus in individual pairs of calf and mother is shown in Table 2. All the mothers of positive calves were also positive, whilst the calves of positive mothers were not always positive, *i.e.* 2 of 44 calves from positive mothers were negative. Of 42 pairs of positive mother and positive calf, 13 pairs or 31 per cent showed significantly higher titers, 4-fold or greater, in mothers than in calves, and only in one pair a 4-fold lower titer was shown in mother. In the remaining 28 pairs (67 per cent) no or only twofold titer difference was shown between mother and calf.

3.2. Antibodies in Precolostral Sera of Normal Calves and their Mothers in Epizootic Areas

As shown in Table 3, NT antibody for Akabane virus was detected in only 3 (27 per cent) of 11 precolostral serum samples of normal calves, this incidence is significantly lower than that (83 per cent) of calves with congenital AH syndrome. In contrast, the incidence of anti-Akabane antibody in the mothers of normal calves was very high (88 per cent), as observed in the mothers of calves with congenital AH syndrome. All the precolostral serum samples of normal calves were negative for antibodies against Aino, Getah and JE viruses, whereas a few of their mothers had antibodies for Getah and/or JE viruses.

Table 2. *Correlation of Anti-Akabane Antibody Titer between Precolostral Sera of Calves with AH Syndrome and Sera of their Mothers*

		Antibody titer of calf							
		<4	4	8	16	32	64	128	Total
Antibody titer of mother	<4	8							8
	4			1	1				2
	8				1				1
	16			1	3	2			6
	32	1		1	2	7	4		15
	64			1	4	2	1	2	10
	128	1		2	1	2	1	1	8
	256					1			1
	512			1					1
	Total	10	0	7	12	14	6	3	52

Table 3. *Comparison of Incidences of NT Antibodies to Akabane, Aino, Getah, and JE Viruses in Precolostral Sera of Normal Calves and Calves with Congenital AH Syndrome, and in Sera of their Mothers*

	Akabane		Aino		Getah		JE	
Group[a]	M[b]	C[b]	M	C	M	C	M	C
Normal	7/8[c] (88%)	3/11 (27%)	0/8	0/11	2/8 (25%)	0/11	3/8 (38%)	0/11
AH syndrome	44/52 (85%)	49/59 (83%)	2/52	0/59	5/52 (10%)	0/59	9/52 (17%)	0/59

[a] Precolostral sera of normal calves and sera of their mothers were collected in epizootic areas (Okayama, Ishikawa and Chiba) during winter months as were the serum samples used in the tests shown in Table 1. The data for the AH syndrome group are taken from Table 1.

[b] M Mothers, C Calves.

[c] Number of positives / Number tested.

3.3. Antibodies in Stillborn Fetuses

Three stillborn fetuses, 9 months of gestation, were obtained in Kagoshima Prefecture in October 1972. Two of them had histological lesions of encephalomyelitis, and the last one showed ankylosis of the neck and forelegs. Heart blood and/or pericardial, pleural, cerebrospinal or peritoneal fluids were tested for antibody against Akabane and Aino viruses. One of the fetuses contained anti-Akabane antibody in blood and peritoneal fluid, another one in blood, and the last one in pericardial fluid. The titers ranged from 1:10 to 1:80. None of the fetuses had antibody for Aino virus.

3.4. Seroconversion for Akabane Virus of Cattle in Epizootic Areas in 1972 and 1973

Table 4 summarizes the results of NT tests on paired serum samples obtained from individual normal cattle, 1 to 10 years of age, in epizootic areas at intervals of several months with the 1972 or 1973 summer in between. Initially seropositive animals were rare, and initially seronegative animals showed high rates of seroconversion for Akabane virus.

Three groups of initially seronegative cattle in epizootic areas were tested for anti-Akabane antibody monthly over several months in 1972 or 1973 (Table 5). Most of the animals in Hyogo Prefecture seroconverted in August. In the Kumamoto group seroconversion occurred in many animals in September. The Kagoshima group showed seroconversion in some animals in August, and then in many animals in September. Furthermore, in another group of cattle in Kagoshima

Table 4. *Seroconversion for Akabane Virus of Cattle in Epizootic Areas*

Prefecture	Time of bleeding		No. tested	Initially positives		Seroconverted	
	Pre	Post		No.	%	No.	%[a]
Okayama	April 1973	April 1974	141	9	6.4	65	49.2
Hyogo June-Sept. 1973		May 1974	64	1	1.6	53	84.1
Chiba (A)	Nov. 1971	Feb. 1972	16	0	0	15	93.8
Chiba (B)	June 1972	Jan. 1973	19	1	5.3	11	61.1
Chiba (C)	June 1972	Aug. 1973	163	7	4.3	62	39.7

[a] No. of seroconverted animals / No. of initially negative animals.

Table 5. *Seroconversion for Akabane Virus of Cattle in Epizootic Areas*

Prefecture	No. tested[a]	Age (years)	Year tested	Positives (%) in			
				July	Aug.	Sept.	Oct.
Hyogo	13	5—9	1973	0	92	100	
Kumamoto	24	1—2	1972	0	0	63	82
Kagoshima	13	1	1972	0	8	46	63

[a] All these animals were seronegative when tested monthly for several months before July.

Prefecture in October 1971, 4-year-old or younger animals were rarely positive for Akabane antibody while 5-year-old or older animals were positive in high rates (Table 6).

Table 6. *Age Distribution of Anti-Akabane Antibody among Cattle in Kagoshima in October 1971*

Age in years	No. tested	Positives	
		No.	%
0	18	1	6
1	18	1	6
2	18	0	0
3	17	2	12
4	17	1	6
5	17	7	41
6	17	10	59
7	17	13	76
8	18	17	94
9	18	16	89
10	18	16	89
≥11	18	17	94

3.5. Antibody for Akabane Virus among Cattle in Epizootic and Near-by Non-Epizootic Areas

Sera were collected in the spring of 1974 from normal cattle, 1 to 10 years of age, in an epizootic and a non-epizootic area in the same prefectures. As shown in Table 7, in the epizootic areas NT antibody for Akabane virus was detected in high rates of the serum samples tested, whereas it was found in a few or none of the serum samples from the non-epizootic areas.

Table 7. *Comparison of Positive Rate for Anti-Akabane NT Antibody among Adult Cattle in an Epizootic and a Non-Epizootic Area within the same Prefectures*

Prefecture	Area	Time of bleeding	No. tested	Positive	
				No.	%
Hyogo	Epizootic	May 1974	64	53	82.8
	Non-epizootic	May 1974	134	3	2.2
Ishikawa	Epizootic	April 1974	43	38	88.4
	Non-epizootic	May 1974	20	0	0

3.6. Geographic Distribution of Antibody for Akabane Virus in Cattle throughout Japan

Serum samples were collected from 2301 normal cattle, 1 to 10 years of age, in 35 prefectures during the months from April to June of 1973. The results of NT tests with Akabane virus in these serum samples are summarized in Table 8.

Table 8. *Geographical Distribution of Anti-Akabane NT Antibody in Sera Collected from Adult Cattle in April—June 1973*

Prefecture	No. tested	Positives No.	Positives %	Prefecture	No. tested	Positives No.	Positives %
Hokkaido	160	0	0	Chubu			
				Ishikawa	35	3	8.6
Tohoku				Fukui	20	0	0
Aomori	20	1	5.0	Aichi	60	10	16.7
Iwate	56	1	1.8				
Miyagi	120	2	1.7	Kinki			
Akita	60	0	0	Mie	20	1	5.0
Yamagata	80	0	0	Osaka	20	0	0
Fukushima	120	7	5.8	Hyogo	150	37	24.7
Kanto				Chugoku			
Tochigi	60	3	5.0	Shimane	80	30	37.5
Gunma	23	3	13.0	Okayama	141	65	46.1
Saitama	80	17	21.3	Hiroshima	100	77	77.0
Tokyo	20	3	15.0	Yamaguchi	6	5	83.3
Kanagawa	112	27	24.1				
Ibaraki	80	63	78.8	Shikoku			
Chiba	163	62	38.8	Tokushima	20	6	30.0
				Ehime	10	6	60.0
Chubu				Kochi	4	3	75.0
Niigata	140	1	0.7				
Nagano	90	3	3.3	Kyushu			
Yamanashi	40	0	0	Nagasaki	16	15	93.8
Shizuoka	80	2	2.5	Kumamoto	44	6	13.6
Toyama	40	0	0	Myiazaki	31	31	100

4. Discussion

The passive transfer of maternal antibodies into the fetus does not usually occur in pregnant cows, since the newborn acquires maternal antibodies by ingesting colostrum (2, 29). On the other hand, bovine fetuses develop the ability to produce antibody upon antigenic stimulation early in gestation (27, 29). These observations enable us to diagnose intrauterine infections of the fetus by testing fetal or precolostral sera from newborn calves. By this means, intrauterine infections in cattle have been diagnosed for bovine diarrhea virus (9, 10, 13—15), bovine rhinotracheitis virus (15), parainfluenza virus type 3 (5, 15), bovine enteroviruses (5) and bluetongue virus (19).

In the present study, 83 per cent of calves with congenital AH syndrome had NT antibody for Akabane virus in their precolostral sera (Table 1). Stillborn calves also contained antibody for Akabane virus. These findings suggest that intrauterine infection with Akabane virus had occurred in those calves. On the other hand, of 11 normal healthy control calves only 3 calves or 27 per cent had anti-Akabane NT antibody in their precolostral sera (Table 3). The difference in the antibody incidence between the calves with congenital AH syndrome and the normal control calves was shown to be statistically highly significant by the chi-square test, indicating a close correlation between the disease and precolostral

anti-Akabane antibody. This finding strongly suggests that Akabane virus is the cause of the disease. Akabane virus, a member of the Simbu group of arboviruses, was originally isolated from mosquitoes, *Aedes vexans* and *Culex tritaeniorhynchus*, in Japan in 1959 (26), but its etiological role in man or any other animals in nature has not yet been known. In Australia, Akabane virus has been isolated from *Culicoides brevitarsis* (3) and serological evidence for its spread among cattle has been reported (3, 4).

For the other viruses tested, *i.e.* Aino, Getah and JE viruses, no precolostral serum antibody was detected in any of the calves examined (Tables 1, 3). The mothers of these calves, normal and with AH syndrome, had NT antibody for Akabane virus in high percentages (Tables 1, 3). In the AH syndrome group, of 44 seropositive mothers 42 had calves with precolostral antibody. These 42 mothers are considered to have acquired antibody by infection with Akabane virus during pregnancy, most probably during the summer months, and the virus further invaded the fetus. The calves of the 2 remaining mothers were seronegative, probably because the mothers had no infection during the pregnancy and their antibody was merely the result of a past infection, or because the mothers were actually infected but the virus failed to infect the fetus. The serological data on the normal calves and their mothers can also be interpreted in the same manner.

Antibody for any one of Aino, Getah and JE viruses was present in only a few of the mothers in both groups (Tables 1, 3). A total of 17 mothers had antibodies for at least one of these viruses, but none of their calves had antibodies for any of these viruses. However, anti-Akabane antibody was present in both mother and calf in 14 of these 17 pairs. In the remaining 3 pairs the mothers had antibody for Akabane virus, but their calves did not contain antibody in their precolostral sera. These calves were tested after ingestion of colostrum and all became seropositive for all viruses against which their mothers had antibodies. These findings are not in line with a passive transfer of material antibodies to the fetus during pregnancy, but support the acquisition of maternal antibodies through colostrum by the newborn.

Ten of the 59 tested calves with congenital AH syndrome were negative for anti-Akabane antibody (Table 2). The congenital malformation in these 10 calves might be caused by some other unknown agent, although there still remains the possibility that the fetuses acquired infection with Akabane virus resulting in AH syndrome, but failed to produce antibody for one or another reason. The presence of anti-Akabane antibody in precolostral sera of 3 normal control calves (Table 3) could indicate that fetal infection with Akabane virus does not always result in clinically recognizable abnormalities.

The serological data summarized in Tables 4—8 indicate a wide dissemination of Akabane virus in epizootic areas during the summer months of 1972 and 1973. Thus, the seroconversion rate for Akabane virus was very high among cattle in Okayama, Hyogo and Chiba Prefectures during periods of several months with the 1972 or 1973 summer in between (Table 4). Positives were rare in the first serum samples. Since Akabane virus is mosquito-borne and spreads during warm months, these data are interpreted as indicating that the virus was disseminated widely in epizootic areas during the summer months in 1972 or 1973, after some quiet years. This interpretation is also compatible with the fact that Chiba Pre-

fecture was severely affected by the outbreak in 1972—73, whilst some areas of Okayama and Hyogo Prefectures were not affected in 1972—73 but in 1973—74. The data presented in Tables 5 and 6 may be likewise interpreted.

A further support for the close relation between the outbreaks and the dissemination of Akabane virus is provided by the finding that very high incidences of Akabane antibody were shown among cattle in epizootic areas in Hyogo and Ishikawa prefectures in the 1974 spring, whereas the antibody incidence was extremely low in non-epizootic areas in the same prefectures (Table 7).

The geographic distribution of anti-Akabane antibody among cattle throughout the country in the 1973 spring (Table 8) generally agrees with the pattern of case distribution in the 1972—73 outbreak. Thus, in Hokkaido and Tohoku districts that were not involved in the outbreak, only rare serum samples were positive. In Kanto, the most severely affected Ibaraki and Chiba prefectures had high positive rates of 79 and 39 per cent, respectively, whilst the other prefectures, from which no or only small numbers of cases were reported, showed reduced rates of positives. In Chubu and Kinki districts that were largely spared by the outbreak, much reduced positive rates were obtained except Hyogo Prefecture which had a positive rate of 25 per cent. This elevated percentage, however, could be caused by the fact that only a small number of cases was reported. In Chugoku, Shikoku and Kyushu districts high incidences of antibody were generally obtained, in accordance with the fact that these areas were severely affected by the outbreak. The exceptional relatively low antibody incidence for Kumamoto Prefecture might be readily explained, for the serum samples tested were collected from various places where the severity of the outbreak varied markedly, particularly the mountainous areas being more or less spared. As discussed earlier, a high degree of virus dissemination is obvious in epizootic areas of this prefecture (Table 5). In this survey the antibody incidence was low for Ishikawa Prefecture, while a very high antibody incidence was shown in an epizootic area of the prefecture in April 1974 (Table 7). These divergent results agree with the fact that the prefecture was spared by the 1972—73 outbreak but suffered from the next outbreak.

All these considerations support the hypothesis that Akabane virus is the etiological agent of the outbreaks. As discussed above, it seems reasonable to think that an abrupt wide dissemination of Akabane virus occurred in the 1972 and 1973 summer among bovine populations consisting largely of non-immune animals after a poor virus dissemination in the previous years, and many cattle acquired infection in the epizootic areas. Most of the infected animals remained apparently healthly although some might have had mild not reognized febrile illness (6, 7, 12). In some, probably about one-third of infected pregnant cows the virus invaded the fetus, as judged from the serological data obtained from normal control calves (Table 3). Fetuses severely affected by the infection might soon result in abortion, premature birth or stillbirth. Of fetuses surviving the infection some might recover with little sequelae, some might gradually develop more or less brain lesions such as hydranencephaly or brains of spongy appearance and marked reduction in number of motor neurons in the spinal anterior horn (16, 22), depending upon the severity and the distribution of the primary damages of nervous tissues by the infection and the stage of gestation when infection

occurred. Arthrogryposis might ensure from these damages in the central nervous system (16, 22). Those fetuses might be born in term or prematurely but some might result in stillbirth. This course of events readily explains the contrasting time course for cases of abortion and premature birth, those of stillbirth and those of congenital AH syndrome as shown in Figure 1 (23), and also the fact that some inflammatory lesions were found in the central nervous system in cases occurring during the early stages of the outbreaks (16, 22).

Further investigations are needed to prove the etiological role of Akabane virus in these pathological conditions, particularly by isolation and identification of Akabane virus from natural cases, demonstration of Akabane virus antigen in lesions by immunofluorescence technique, and production of *in utero* infection of fetuses by experimental infection of pregnant cows with the virus.

Similar outbreaks have also been observed in 1949—50, 1959—60 and 1966 in Japan (11, 23, 24). Those outbreaks resembled the 1972—73 and 1973—74 outbreaks investigated in this study both in epidemiological, clinical and pathological features, suggesting the same etiology. It is of interest that the serological data presented in Table 6 indicate a wide dissemination of Akabane virus in the 1966 summer in Kagoshima Prefecture that was affected by the 1966 outbreak.

Seasonal occurrence of congenital AH syndrome in cattle has also been reported in Israel (17, 21) and Australia (1, 8, 32, 33). In Australia Akabane virus has been reported to spread among cattle (3, 4). The possible etiological role of Akabane virus in those pathological conditions seems worthy to be investigated.

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