Akabane Disease : An Epizootic Congenital Arthrogryposis-Hydranencephaly Syndrome in Cattle, Sheep and Goats Caused by Akabane Virus

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As early as in 1954, congenital deformities described as arthrogryposis-hydranencephaly (AH) syndrome affected cattle in epizootic proportions in Australia[1,24]. The syndrome was characterized by fixed flexion of joints, hydranencephaly and neurogenic skeletal muscular atrophy of calves. A similar syndrome in calves, lambs and kids was observed in Israel in 1971[15,17]. Epizootics in 1973–1975, involving more than 40,000 cases of abortion, premature birth, stillbirth and congenital AH syndrome, were reported in cattle in Japan[6,10,16].

Since the epizootic was first described by Blood[1] and Whittem[24], previous investigators suggested etiological agents ranging from genetic factors, teratogenic chemicals or toxins, vitamin or mineral deficiencies to infectious disease. On the other hand, these epizootics were seasonal and limited to definite geographic areas, indicating that an arbovirus infection could possibly be incriminated.

In 1974, Miura et al.[17] found a high incidence of neutralizing antibodies against Akabane virus, a member of Simbu group of the Bunyaviridae, in sera collected from calves with AH syndrome prior to their sucking of colostrum. Kurogi et al.[10] also demonstrated a close correlation between the occurrence of congenital AH syndrome of calves and antibodies against 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 of 1972 and 1973 in Japan. Antibodies against Akabane virus were also detected in precolostral sera from calves, lambs and kids with AH syndrome in Australia[4,5] and Israel[7].

In 1976, Kurogi et al.[11] reported the isolation of Akabane virus from the fetus and blood of a sentinel pregnant cow, and from a naturally aborted fetus. More recently experimental infections of pregnant cows, sheep and goats have resulted in vertical transmission of the virus and the production of the AH syndrome in calves, lambs and kids[12,13,20].

It is apparent that Akabane virus can seriously affect livestock production through transplacental infection which causes epizootics of abortion, premature birth, stillbirth, and congenital AH syndrome in calves, lambs and kids. Thus, Inaba et al.[6] proposed to designate the disease in cattle, sheep and goats as Akabane disease.

Epidemiology

Among the most conspicuous epidemiological features of Akabane disease figure the seasonal incidence and the distinctive geographical distribution of the disease limited to some areas. Cases of abortion, premature birth, stillbirth and congenital AH syndrome in cattle were observed in epizootic proportions in the central and western parts of Japan during the summer and winter months of 1972–1973, 1973–1974 and 1974–1975[6,10,17]. The total number of reported cases was 41,951 of which 37.3, 22.0 and 40.7% were cases of abortion (plus premature birth), stillbirth

and congenital AH syndrome, respectively.

The outbreak was mostly limited to Kyushu, Shikoku, Chugoku and Kanto districts in Japan, roughly south of 38 degrees north latitude (Fig. 1).

The distribution of clinical cases was in complete agreement with the distribution of antibodies against Akabane virus after the 1972–1973 outbreak (Fig. 2). Furthermore, higher altitude areas were free from infection of cattle with Akabane virus as indicated by serological test (Inaba et al. unpublished data).

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. 3). The outbreak subsided in May 1973. Epizootics recurred in 1973–1974 and 1974–1975, although limited in size and area. Cases occurring after the first epizootic tended to occur more commonly in adjoining areas than in areas severely affected by the previous epizootic. The dams were not affected in subsequent outbreaks. Similar outbreaks have also been observed in Japan in 1949–1950, 1959–1960 and 1966[6]. Those outbreaks resembled the latest outbreaks in epidemiological, clinical and pathological features, suggesting the same etiology. Elsewhere, seasonal occurrence of a congenital AH syndrome and hydranencephaly micrencephaly (HM) in calves, lambs and kids had been reported in Israel[8,15,17] and Australia[4,5].

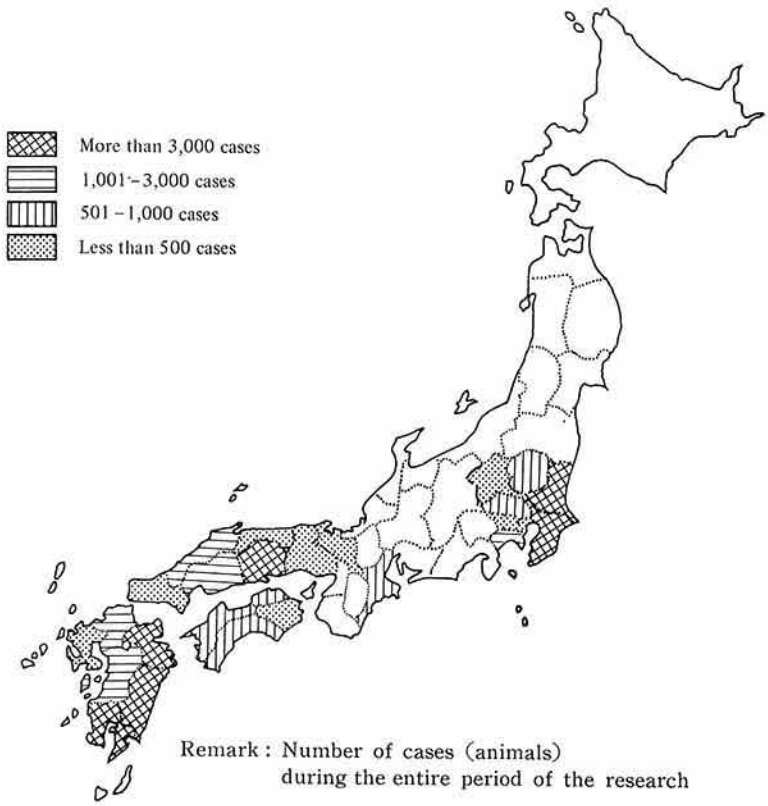

Fig. 1. Distribution of abnormal deliveries among cattle herds in Japan by prefectures in 1972–1974.

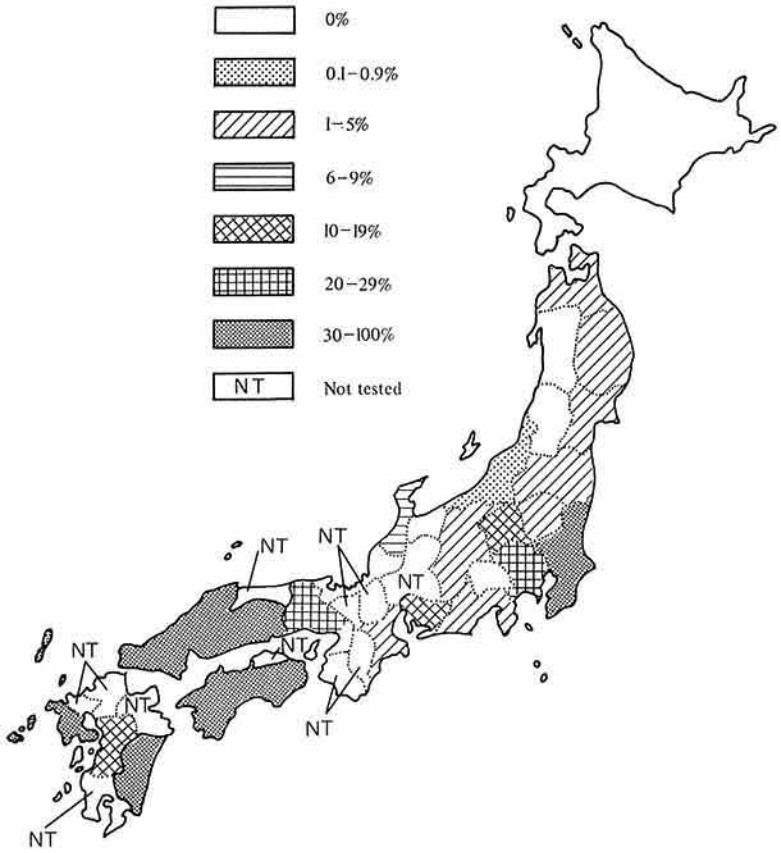

Fig. 2. Geographic distribution of neutralizing antibodies against Akabane virus.

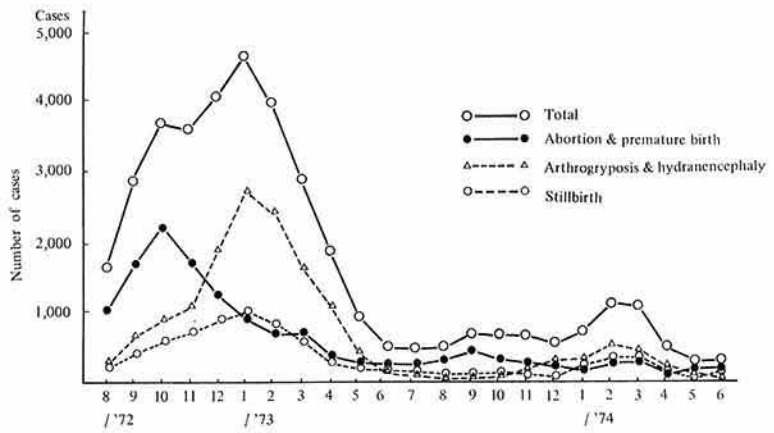

Fig. 3. Monthly number of reported cases in 1972–1974.

Clinical and pathological features

Akabane disease affects all age groups and breeds of beef and dairy cattle and crossbreds, as well as sheep and goats, and occurs in calves, lambs and kids from both natural and artificial mating.

It has been reported that different clinical and pathological entities are observed and that the form of expression of the disease depends on the stage of gestation at which it is contracted.

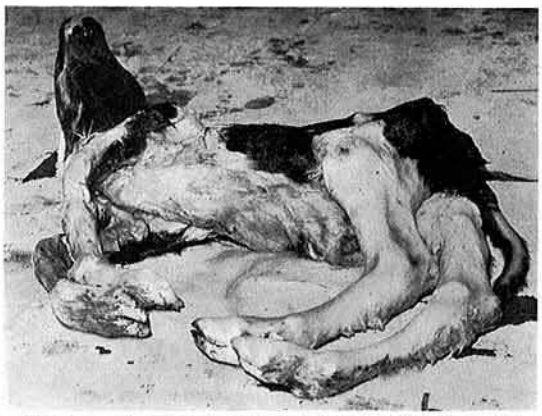

Plate 1. Arthrogryposis with severe involvement of both fore-and hind-limbs and cervical scoliosis.

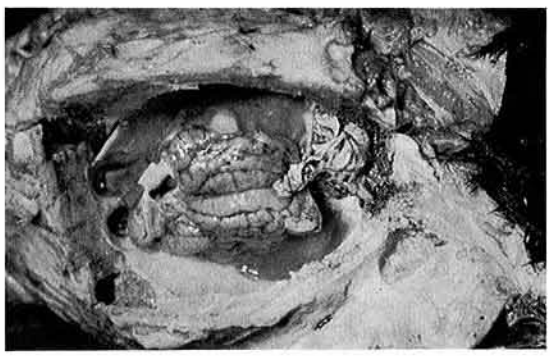

Plate 2. Dorsal view of the content of the cranial cavity of a calf with hydranencephaly.

The disease consists of abortion, premature birth, stillbirth, arthrogryposis, hydranencephaly and micrencephaly with mild inflammatory lesions in some calves (Plates 1, 2). In Israel and Australia outbreaks, arthrogryposis and hydranencephaly were also observed in lambs and kids[4,5,8,15,18]. Abortions and stillbirths have been recorded as a major feature of the disease in Japan and it has been previously suggested that they might possibly be related to the outbreaks in Australia, although Blood[1] could not find any evidence of increased rate of abortions in the 1951 and 1955 epizootics. Recently Shepherd et al.[21], however, have reported the evidence of abortions and stillbirths in Australia.

There is a marked increase in the number of abortions and stillbirths at the outset of Akabane disease. These losses are not associated with dystocia, and macroscopic examination of organs is also negative. Histological examination of the aborted fetus at an early stage disclosed the presence of encephalitic changes in the central nervous system. Nerve cells in the nuclei of the brain stem underwent degenerative changes, with some of the degenerated cells being surrounded by an accumulation of glia-cells[5,9].

The lesions in the central nervous system of calves with AH syndrome have been classed into 5 groups, as described below, which possibly approximate to the gestational age of the fetus at the time of the initial attack[5]. However, as there is often some overlapping of the lesions, the groups have been placed in the order in which they were observed in the field during the epizootics. Group 1: Calves showing signs of incoordination at birth with microscopic examination revealing a generalised mild to moderate nonsuppurative acute encephalomyelitis. Group 2: calves showing signs of incoordination at birth or mild arthrogryposis with microscopically, a mild to moderate severe acute Wallerian type degeneration of all zones of the spinal cord except the dorsal funiculi. Group 3: Calves born with arthrogryposis with, microscopically, a severe diffuse loss of myelinated fibres in the lateral and ventral funiculi of affected areas of the spinal cord along with a moderate to severe atrophy of the skeletal musculature. Group 4: Calves with hydranencephaly, sometimes associated with arthrogryposis, with

almost complete replacement of both cerebral hemispheres by a fluid-filled cavity. Group 5: Calves born with micrencephaly and hydranencephaly and sometimes arthrogryposis and loss of anterior and mid-brain-stem and cerebellar cavitation.

Isolation of Akabane virus

Akabane virus was originally isolated from mosquitoes, *Aedes vexans* and *Culex tritaeniorhynchus*, in Japan in 1959[10], but its etiological role in man or any other animals in nature has not yet been elucidated. In Australia, Akabane virus has been isolated from *Culicoides brevitarsis* and serological evidence for its spread among cattle has been reported[2,3].

Since Akabane virus was suspected to be the cause of the outbreaks from the results of serologic surveys, Kurogi et al.[11] made efforts to find pregnant cows recently infected with the virus by applying neutralization test. Thus, they located 200 pregnant cows without Akabane neutralizing antibodies in 20 prefectures where the 1972–1974 outbreaks were severe and tested them each week for serum neutralizing antibodies to Akabane virus, during the period extending from June through October in 1974. Seroconversion was shown in only two cows and all the other sentinel cows remained negative throughout the observation period. Akabane virus was successfully isolated from the fetus and from the blood of the two cows in which serological tests suggested a recent infection with the virus as well as from an aborted fetus in an epizootic of the disease. The former fetus had histological changes consisting of encephalomyelitis and polymyositis, and specific antigens of Akabane virus were demonstrated by the immunofluorescence technique in brain tissues as well as in skeletal muscles and fetal placenta.

Recently Akabane virus has also been isolated from the blood of a sentinel normal bull

Table 1. Relative sensitivity of suckling mice, HmLu-1 and Vero cell cultures for primary isolation of Akabane virus

Specimen	Infective titer determined by: Inoculation of baby mice	Inoculation of culture: HmLu-1	Inoculation of culture: Vero	Plaque count
OBE-1				
Brain	2.5[a] (3.5[b])	3.2[c] (3.5[d])	1.8[c] (3.5[d])	3.4[e]
Cerebral fluid	2.0 (3.5)	3.5 (3.5)	2.5 (2.5)	3.5
Spinal cord	1.2 (2.0)	2.2 (2.2)	— (1.2)	2.4
Visceral pool[f]	≤0.8 (≤0.8)	—(≤0.8)	— (—)	—
Muscles	1.5 (2.3)	2.2 (2.5)	— (2.2)	2.3
Intestines	— (—)	— (—)	— (—)	1.0
Fetal placenta	1.5 (2.0)	2.8 (3.2)	1.5 (1.5)	2.6
Amnion	1.3 (1.8)	1.5 (1.5)	1.2 (1.8)	1.0
Amniotic fluid	— (—)	— (—)	— (—)	—
Blood	— (—)	— (—)	— (—)	—

[a] log(LD_{50}/0.01 g or ml)
[b] log(ID_{50}/0.01 g or ml); a mouse was taken as infected when it died of encephalitis or produced antibodies against the virus
[c] log($TCID_{50}$/0.1 g or ml); based on cytopathic effect
[d] Same as "c" except that all the negative cultures were passaged and were taken as infected when cytopathic effect was produced in the passage cultures
[e] log(PFU/0.2 g or ml)
[f] Pool of lung, liver, spleen and kidney tissues
"—" sign means negative with 10 per cent tissue suspension or undiluted fluid specimens

and from a pool of mosquitoes, *Anopheles funestus*, in Australia[22] and Kenya[16], respectively.

The intracranial inoculation of suckling mice is most sensitive for primary isolation of Akabane virus[11]. Cultures of HmLu-l cell, a continuous cell line from hamster lung, seem also very sensitive while plaque formation on HmLu-l cell monolayers is also a sensitive method. Vero cell cultures appear to be somewhat inferior to HmLu-l cell cultures as Vero cell cultures failed to yield virus from the spinal cord and muscles, while these specimens yielded virus in HmLu-l cell cultures (Table 1). Similar experiments were also carried out by inoculation of the cerebral fluid from naturally infected fetus[11] into various cell cultures. Among the cell cultures tested, HmLu-l cells were the most sensitive followed by Vero, BHK21-W12, PK-15, BEK-1*, BTR*, and PK-13 cells. All the other cell cultures tested (primary cultures of calf kidney and testicle cells and swine kidney and testicle cells, as well as HeLa, HEp-2 and L cells) yielded no virus.

Physicochemical and biological properties of Akabane virus

Oya et al.[19] in their report on the discovery of Akabane virus, stated that the virus was readily inactivated by sodium deoxycholate, but was not precipitated by protamine sulfate. Takahashi et al.[23] confirmed these results, and further demonstrated that the virus was extremely labile at pH 3 and was readily inactivated by trypsin. Thermal degradation of the virus is very rapid at 56°C, but rather slow at 37°C, i.e., showing about a three log-unit decline in titer in 12 hours.

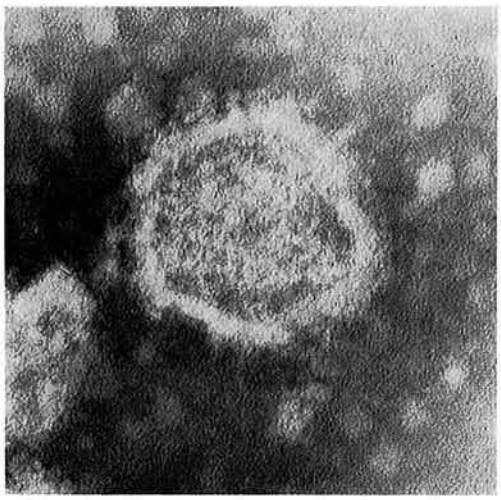

Plate 3. Electron micrograph of negatively stained Akabane virus. ×30,000

The replication of Akabane virus is not inhibited by 5-iodo-2′-deoxyuridine, indicating that the virus is a RNA virus. The virus is considered to have an envelope, as it is readily

Table 2. The effect of NaCl concentration on hemagglutination with Akabane, Aino, Samford and Japanese encephalitis (JE) viruses

Virus	pH of VAD	NaCl Molarity of VAD with 0.2 M phosphate buffer						
		0.1	0.15	0.2	0.25	0.3	0.35	0.4
Akabane								
JaGAr39	6.2	<2*	4	16	16	32	32	32
OBE-1	6.2	<2	<2	4	8	16	32	32
NBE-9	6.2	<2	8	32	64	64	64	64
R 7949	6.2	<2	<2	2	16	64	128	128
B 8935	6.2	<2	2	16	16	32	32	32
Aino	6.0	<2	<2	128	512	512	512	512
Samford	6.0	<2	<2	32	64	128	128	128
JE	6.4	2,560	2,560	2,560	2,560	2,560	2,560	2,560

* Reciprocal of HA titer.

* BEK-1 : Continuous cell line from bovine embryo kidney
BTR : Continuous cell line from bovine thymus

inactivated by ether and chloroform.

Akabane virus is easily filtered through membrane filters with a 200 or 100 nm pore size, but not through 50 nm filters.

These results are confirmed by electron microscopy in negatively-stained preparations. Numerous viral particles (Plate 3), roughly spherical, variable in size, about 70–130 nm in diameter, are observed in the CsCl density gradient fractions coinciding with the peak of infectivity and hemagglutination (HA). The buoyant density of the virion is estimated to be 1.22 g/ml.

Goto et al.[7] found HA with Akabane virus to be dependent not only on the pH but also on NaCl concentration and showed that these findings could be applicable to other viruses in the Simbu group, Aino and Samford, which had been incriminated in HA (Table 2). The incubation temperature does not affect the HA titer, although the titer tended to be slightly lower at 4°C than at 37°C or at room temperature (22–25°C). Akabane virus gives similar HA titers with duck, goose and pigeon erythrocytes, but no HA with erythrocytes from sheep, cattle, humans (O), guinea pigs and day-old chicken. The HA-inhibition test along with neutralization and complement fixation tests appear to be useful in studies of antibody responses in cattle and other animals infected with Akabane virus.

Some information on host range of Akabane virus in cell cultures is supplied in the Section "Isolation of Akabane virus".

Experimental production of congenital abnormalities by Akabane virus

Kurogi et al.[13] reported that the OBE-1 strain, a fresh isolate of Akabane virus from a naturally infected bovine fetus, induced intrauterine infection of fetuses when inoculated intravenously into seronegative pregnant cows and goats, and that some of the in utero-infected calves and kids from these dams showed congenital abnormalities similar to those observed in natural cases of congenital AH syndrome (Plates 4, 5).

Personson et al.[20] also reported that pregnant sheep infected with the B8935 strain of Akabane virus isolated from *Culicoides brevitarsis* produced deformed lambs with arthrogryposis, hydranencephaly, kyphosis, scoliosis, brachygnathia and micrencephaly.

All the inoculated pregnant cows, sheep and goats are considered to be actually infected with Akabane virus, since they developed viremia and neutralizing antibodies against the virus. The virus further infected the fetuses, as proved by virus isolation carried out in some of these fetuses, and by demonstration of neutralizing antibodies against Akabane virus in the precolostral sera from

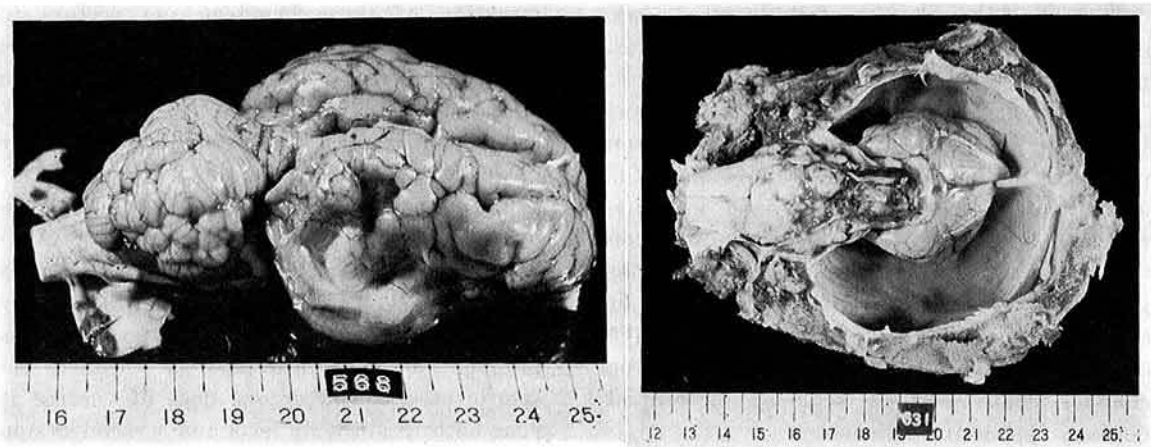

Plate 4. Congenitally deformed calves produced by inoculation of pregnant cows with Akabane virus. (Left) cerebral defect; (Right) hydranencephaly.

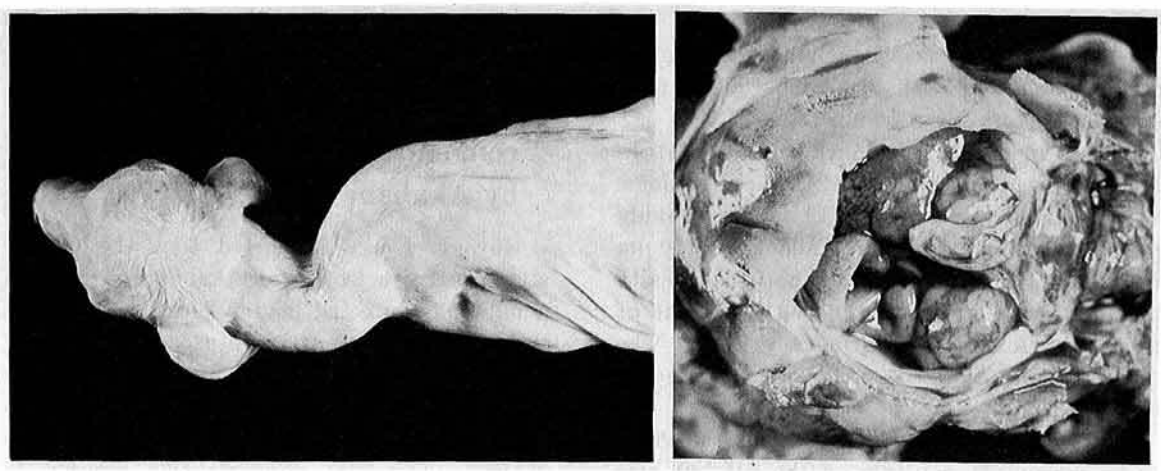

Plate 5. Congenitally deformed fetus produced by inoculation of pregnant goat with Akabane virus. (Left) torticollis ; (Right) hydranencephaly.

calves, lambs and kids born live. These results indicate that in utero infection with Akabane virus took place in these calves, lambs and kids, since the passive transfer of maternal antibodies into the fetus does not occur in cows, sheep and goats, and the fetus develops the ability to produce antibodies upon antigenic stimulation early in gestation.

The dams infected with Akabane virus developed viremia. This observation, together with the previous results indicating the occurrence of viremia and infection of the placenta in naturally infected cows[16], suggests that Akabane virus may infect the fetus through hematogenous infection of the placenta.

In none of the experimentally infected cows, sheep and goats is fever or any other clinical abnormalities noted.

Polymyositis, as observed in the fetuses from cow infected experimentally, had been previously noted in a naturally infected fetus and proved by virus isolation and immunofluorescent staining to result from infection of muscle cells. These findings suggest that polymyositis may be an important cause of congenital deformities and muscular damage observed in natural cases, although these changes could also be sequelae of central nervous system involvement.

These findings provide additional evidence for assuming that Akabane virus is the etiological agent of epizootic abortion and congenital AH syndrome in cattle, sheep and goats.

Further studies are needed to elucidate the pathogenesis of fetal infection with Akabane virus.

Prophylaxis

The following methods can be considered for the prophylaxis of Akabane disease. In specific terms, in view of the fact that some vectors play a role in transmitting the virus from one animal to another, it will be necessary to take indirect measures for the extermination of these blood-sucking vectors in using pesticides or insect-proof nets or to take such direct measures as the application of inactivated or attenuated live virus vaccine, as commonly used for prophylaxis of other viral diseases.

A formalin-inactivated, aluminum phosphate gel-adsorbed vaccine prepared with Akabane virus grown in HmLu-1 cell cultures produces specific neutralizing antibodies by intramuscular inoculation in cattle as well as in mice and in guinea pigs[14]. Antibody response of cattle after one dose of vaccine is poor both in antibody level and seroconversion rate. However, a second dose given at intervals of 3 or more weeks induces a rapid and

Table 3. Resistance of vaccinated cattle to challenge with virulent virus

Group	Cattle No.	Clinical findings: Fever	Clinical findings: Leukopenia	Viremia	Neutralizing antibody titer: At challenge inoculation	Neutralizing antibody titer: 4 weeks after challenge inoculation
Vaccinated	626	No	No	No	2	8
	627	No	No	No	8	4
	628	No	No	No	4	16
Control (Unvaccinated)	629	No	Yes	Yes (For three days)	<1	16
	630	No	Yes	Yes (ditto)	<1	32
	632	No	No	Yes (ditto)	<1	32

Three head of cattle were vaccinated with 2 doses of 3 ml each of vaccine at 4-week interval and together with 3 unvaccinated head of cattle, they were challenged with virulent virus 4 weeks after the administration of the last dose of vaccine.

Table 4. Resistance of vaccinated pregnant goats to challenge with virulent virus

Group	Pregnant goat No.	Clinical findings: Fever	Clinical findings: Leukopenia	Viremia	Fetuses*2: Recovery of virus	Fetuses*2: Neutralizing antibody in sera from umbilical cord	Neutralizing antibody titer in serum from dam: At challenge inoculation	Neutralizing antibody titer in serum from dam: Cesarean section
Vaccinated	20	No	No	No	No	<1	2	16
	21	No	No	No	No No	<1 <1	8	16
	22	No	No	No	No No	<1 <1	2	16
	23	No	No	No	No	<1	4	32
Control (Unvaccinated)	16	No	Yes	Yes (For one day)	Yes (Fetal placenta)	<1	<1	128
	17	No	No	Yes (For two days)	Yes (ditto)	<1	<1	64
	18	No	Yes	Yes (ditto)	Yes (Muscle and fetal placenta)	<1	<1	64
	19	No	Yes	Yes (ditto)	Yes (Muscle) Yes (ditto) Yes (Fetal placenta)	<1 <1 <1	<1	128

Four pregnant goats were vaccinated with 2 doses of 3 ml each of vaccine at 4-week interval and together with 4 unvaccinated pregnant goats, they were challenged with virulent virus 4 weeks after the administration of the last dose of vaccine. Fetuses were removed by cesarean section 10 days after challenge inoculation.

high-titered antibody formation in almost all animals. Based on these findings as well as for reasons of convenience, in practice, the administration of 2 doses of 3 ml each at intervals of 4 weeks has been adopted as the standard schedule.

The antibody level attained in pregnant cows after vaccination by the standard schedule declines rather rapidly. However, a booster dose given one year later induces rapid antibody response.

Considering this pattern of neutralizing antibody response and duration in cows after the administration of the initial doses and the booster inoculation of the vaccine as well as the seasonal incidence of Akabane disease, it seems possible to assume that the immunization with initial two doses or a booster dose given in late spring or early summer just before the epizootic season would be adequate.

The protection tests in vaccinated cattle and pregnant goats showed the effectiveness of the vaccine in preventing the disease (Table 3, 4). No adverse effects were observed in pregnant cows, and milk production was unaffected.

In view of these results, this vaccine is currently being made commercially available in Japan for prophylaxis of the disease.

It is, however, generally hoped that an attenuated live virus vaccine will be developed in conformity with the general tendency of prophylaxis techniques of viral diseases. Thus, an attenuated strain of the virus developed by the authors might be safe enough to be used as live virus vaccine for prevention of the disease.

Summary

Serological studies on the epizootics of abortion, premature birth, stillbirth and congenital AH syndrome observed among cattle in Japan in 1972–1975, strongly suggested that Akabane virus, a member of Simbu group of the Bunyaviridae, was the etiologic agent of the outbreaks. This view was further corroborated by the isolation of Akabane virus from naturally affected fetuses and by the experimental inoculation of pregnant cows with the virus, which induced intrauterine infection of the fetuses and congenital deformities in infected fetuses as observed in natural cases of the congenital AH syndrome. Elsewhere, seasonal occurrence of a congenital AH syndrome in calves, lambs and kids had been reported in Australia and Israel, and serologic evidence for the etiologic role of Akabane virus had also been obtained in these countries. Furthermore, congenital AH syndrome was also reproduced by inoculation of pregnant sheep and pregnant goats with Akabane virus.

Akabane disease in cattle, sheep and goats provides an interesting model of a congenital infection, about which very little is known with respect to how the virus causes congenital abnormalities and how long the virus persists in the fetus. Studies of the disease in these animals could also have much wider implications in determining the causes of mental retardation and congenital deformities in humans and various animals.

These findings add Akabane virus to the growing list of teratogenic viruses, and further studies are needed to elucidate the mechanism by which it causes congenital defects.

The formalin inactivated, aluminum phosphate gel-adsorbed vaccine prepared with Akabane virus grown in cultures of hamster lung HmLu-l cells is currently being made commercially available in Japan for prophylaxis of Akabane disease.

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