AKABANE DISEASE: EPIDEMIC CONGENITAL ARTHROGRYPOSIS-HYDRANENCEPHALY SYNDROME IN CATTLE, SHEEP AND GOATS CAUSED BY AKABANE VIRUS

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Introduction

Cases of abnormal deliveries of unknown etiology were observed in epidemic proportions among cattle in the central and western parts of Japan during the summer through winter months of 1972 - 1973. The outbreak recurred in 1973 - 1974, although much limited in size and area. The abnormal deliveries consisted of abortions, stillbirths, premature births and calf deformities referred to as congenital arthrogryposis-hydranencephaly (AH) syndrome.

The central nervous system of affected fetuses and newborn calves showed various degrees of damage, and more importantly, inflammatory changes including perivascular cuffing, neuronophagia and glia cell proliferation were observed particularly in cases occurring in the early phases of the outbreaks. These findings, coupled with the seasonal occurrence in epidemic proportions and the geographical distribution of cases, suggested an infectious nature of the disease.

To explore this possibility we tested precolostral sera from calves with congenital AH syndrome for antibodies against several arboviruses and some other bovine viruses, and found antibodies to Akabane virus, a member of the Simbu group of Bunyaviruses, in a high percentage of the sera tested. Encouraged by this finding, we extended the serological survey and demonstrated the presence of a 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 epidemic areas during the summer months of 1972 and 1973. These findings strongly suggested Akabane virus to be 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 Akabane virus, which induced intrauterine infection of the fetuses and congenital abnormalities in infected fetuses as observed in natural cases of the congenital AH syndrome.

Seasonal occurrence of a congenital AH syndrome in cattle had also been reported in Australia and Israel. Furthermore, congenital AH syndrome was also observed in sheep in Australia and Israel and in goats in Israel. Recently, serological evidence has been obtained for the etiological role of Akabane virus in congenital AH syndrome in cattle in Australia and in cattle, sheep and goats in Israel. Furthermore, Akabane virus was isolated from naturally infected ovine fetuses, and congenital AH syndrome was induced by inoculation of pregnant sheep and of pregnant goats with Akabane virus, supporting the Akabane virus etiology of the disease in sheep and goats. We proposed therefore to designate the disease in cattle, sheep and goats as "Akabane disease."

In this paper we shall assemble the data so far accumulated on Akabane virus and Akabane disease, and arrange them for the proper understanding of the present status of knowledge and for the furtherance of the study on the virus and the disease.

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Characteristics of the virus

1   Taxonomy

Akabane virus was originally isolated from *Aedes vexans* and *Culex tritaeniorhynchus* mosquitoes collected in Japan in the summer of 1959. "Akabane" is the name of the village where the virus was first isolated. Subsequent serological studies enabled to classify Akabane virus in the Simbu group of arboviruses, one of the serological groups in the Bunyamwera Supergroup proposed in the *World Health Organization Technical Report* No. 369 (1967). In 1975 the International Committee on Taxonomy of Viruses approved the establishment of the new family Bunyaviridae which contains a single genus *Bunyavirus* comprising the Bunyamwera Supergroup viruses.

2   Physicochemical properties

Equilibrium centrifugation of infectious culture fluid in a CsCl density gradient gave a peak of infectivity and hemagglutination activity at a density of 1.22 g/ml. The peak fraction thus obtained contained numerous virus particles, roughly spherical, variable in size, 70 - 130nm in diameter, and mostly having a ragged, closely adherent envelope with projections, when examined following phosphotungstic acid negative staining, in an electron microscope.

In agreement with the electron microscopic observations, Akabane virus was readily filtered through membrane filters of 200- or 100-nm pore size, but not through 50-nm filters. The presence of an envelope was also indicated, since the virus was readily inactivated by ether and chloroform.

The replication of Akabane virus was not inhibited by 5-iodo-2'-deoxyuridine, indicating that the virus is a RNA virus. This finding should be further confirmed and extended by direct extraction and analysis of the nucleic acid from highly purified virions, including determination of whether it is single or double stranded, or segmented into small pieces.

The virus was readily inactivated by sodium deoxycholate, but not precipitated by protamine sulfate. The virus was shown to be very labile at pH 3 and to be readily inactivated by trypsin. Thermal degradation of Akabane virus was very rapid at 56°C, but rather slow at 37°C.

3   Hemagglutination and hemolysis

Early workers have experienced difficulty in demonstrating hemagglutination (HA) with Akabane virus.

Our early attempts resulted in erratic low HA titers. However, this difficulty was overcome, when we demonstrated enhanced HA with increased salt concentration in diluents. Furthermore, HA with Akabane virus was shown to be dependent on the pH of the diluent as well. The incubation temperature did not affect the HA titer, although the titer tended to be slightly lower at 4°C than at 37°C or room temperature. Akabane virus gave similar HA titers with duck and goose erythrocytes and somewhat higher titers with pigeon erythrocytes, but no HA with erythrocytes from sheep, cattle, man(0), guinea pigs and day-old chickens. Based on these observations an assay method for Akabane virus hemagglutinin was developed. HA with Akabane virus was specifically inhibited by antisera to the virus and an assay method for HA-inhibiting antibodies to Akabane virus was developed. These methods for HA and HA-inhibition were found to be applicable to other members of the Simbu group, in which it had also been reported that HA was difficult to demonstrate.

Analysis by CsCl equilibrium density gradient centrifugation indicated the hemagglutination activity to be structurally associated with the virion.

Enhanced HA with increased salt concentration in diluents has also been demonstrated with measles and rabies viruses.

Unlike antigens of the family Togaviridae viruses, which commonly have high HA titers after acetone extraction of infected mouse brains, many viruses in the family Bunyaviridae are poor agglutinators. However, Beaty *et al.* confirmed our findings with Akabane, Aino and Samford viruses in the Simbu group and extended them to many other Bunyaviridae viruses.

During the course of the studies on HA with Akabane virus, we noticed that the virus lysed as well as agglutinated pigeon erythrocytes and this hemolytic reaction was shown to be virus specific,

since it could be specifically inhibited by antisera to the virus. The viral hemolysis has been reported with mumps, Newcastle disease, parainfluenza, measles, eastern and western equine encephalomyelitis, Getah and rubella viruses.

We first noticed that Akabane virus antigens prepared by sucrose-acetone extraction of infected mouse brains not only agglutinated, but also lysed pigeon erythrocytes. The experiment was repeated with virus propagated in HmLu-1 cell cultures and concentrated by ultracentrifugation. The preparations demonstrated high HA titers, but no or weak hemolytic activity. However, it was soon found that repeated freeze-thawing enhanced markedly their hemolytic activity, whereas their HA titers remained unchanged. The hemolysis was found to be dependent on the NaCl molarity as well as the pH of the diluent as in the case of HA with the virus. On the other hand, the hemolysis was markedly affected by the incubation temperature, whereas HA was not; the hemolytic activity was highest at 37°C, somewhat lower at 25°C, very low at 4°C, and not present at 0°C. While pigeon erythrocytes were positive for both HA and hemolysis, goose erythrocytes were positive for HA but negative for hemolysis. Erythrocytes from cattle, sheep, rabbits, guinea pigs, mice and day-old chickens were all negative for hemolysis as well as for HA. A linear relationship was shown, in a wide range of the virus concentration, between the percent hemolysis and the virus concentration, as expressed in a logarithmic scale.

Based on these findings an assay method for Akabane virus hemolysin was developed. Analysis by CsCl equilibrium density gradient centrifugation indicated the hemolytic activity to be structurally associated with the virion. Scanning electron microscopy of pigeon erythrocytes undergoing hemolysis with the virus revealed the appearance of a depressed area with a hole on the cell surface. The hemolytic activity with Akabane virus was specifically inhibited by antisera to the virus and the hemolysis-inhibition test was developed.

4   Infection in pregnant hamsters, goat, sheep and cattle

Transplacental infection of hamster fetuses was produced by inoculation of pregnant hamsters with Akabane virus by either the intraperitoneal or the subcutaneous route. High-titered virus was detected first in the placenta and later in the fetus. Virus could also be readily isolated from blood, lung, spleen and liver of both pregnant and nonpregnant hamsters, but it reached higher titers and persisted longer in the placenta and fetus. Animals dying at birth had high-titered virus in the brain. Litter size was reduced by inoculation of the pregnant hamster at gestational day 11 or earlier, and survival of the newborn to one week of age was decreased by inoculation at gestational day 9 or later.

Intrauterine infection of the fetus was produced by inoculation of pregnant sheep and pregnant goats. The inoculated sheep and goats showed no clinical signs of infection, but developed neutralizing antibodies to Akabane virus. Viremia and leukopenia were often observed. Virus was recovered from various tissues, particularly cerebral and muscular tissues of the fetuses, and nonpurulent encephalomyelitis and polymyositis were observed in the affected fetuses. Congenital AH syndrome was also observed in some of the fetuses and the newborn animals, and the occurrence of intrauterine infection was shown in the newborn animals with or without AH syndrome by the presence of neutralizing antibodies to Akabane virus in their precolostral sera. The pathogenic effects of Akabane virus seemed to be related to the gestational age at which the fetus was infected.

We inoculated Akabane virus intravenously into seronegative pregnant cows. All the inoculated cows developed viremia and neutralizing antibodies for the virus, indicating that the cows were actually infected with the virus, although fever or any other clinical abnormalities were not noted. The virus further infected the fetuses. This was proved by virus isolation or by demonstration of neutralizing antibodies to the virus in their precolostral sera. Some of the *in utero* infected calves displayed congenital abnormalities such as cerebral defects, hydranencephaly and arthrogryposis.

Clinical features, pathogenesis and diagnosis

1   Clinical features

Akabane disease is caused by intrauterine infection of fetuses with Akabane virus in pregnant cattle, sheep and goats. The intrauterine infection of fetuses results in abnormal deliveries such as abortion, stillbirth, premature birth, and deformities referred to as congenital arthrogryposis-hydranencephaly (AH) syndrome.

No clinical abnormalities that could be related to the abnormal deliveries were recognized in the dams during their pregnancy.

The AH syndrome as observed in affected calves and fetuses during the outbreaks comprised various pathological conditions which occurred as separate entities or simultaneously in the same calves. These pathological changes have been described by many authors.

Arthrogryposis was characterized by pathological changes in the muscles of the limbs manifested by flexion or extension of various joints. The affected joint was in a state of contracture and could not be straightened out even by the application of force. Any joint of any limb could be affected, the carpal and tarsal joints were involved in most cases. The fore limbs were more often involved than the hind limbs. Most arthrogrypotic calves were unable to rise themselves. The birth of most arthrogrypotic calves was associated with dystocia. Most of such cases were slaughtered or died shortly after birth as a result of additional involvement of the nervous system or insufficient care on the part of the owner.

Torticollis, scoliosis and kyphosis occurred as a result of pathological changes in the muscles of the vertebral column. These lesions could co-exist with arthrogryposis. Fate of the calves of this group was similar to that of the arthrogrypotic calves.

In these deformities of the limbs and the spinal column no changes were detected in bones, but the muscles of affected limbs or spinal column showed a considerable reduction in volume and often appeared to be absent altogether, being replaced by tissue of a pinkish-gray or grayish-white color and hard tensile consistency. Besides muscular hypoplasia, fatty dystrophy or fibrosis of muscle tissues was also observed.

Hydranencephaly was characterized by thin cerebral cortex with an internal surface lined by dysplastic ependyma and thickened leptomeninges.

In the cerebellum there were lesions similar to those occurring in the spinal cord and cerebrum, such as edema and degeneration of Purkinje cells. The striking lesion in this organ was cerebellar hypoplasia, affecting the molecular and granular layers and the Purkinje cells.

Seasonal occurrence of similar congenital AH syndrome has also been reported in sheep and in goats.

2   Pathogenesis

Akabane disease is caused by *in utero* infection with Akabane virus, which results in abortion, premature birth, stillbirth and congenital AH syndrome. No clinical signs of infection are noticed in the dams infected with the virus. Although the site of initial infection is not known, viremia seems to be a constant feature of the infection. These findings, together with the results indicating the occurrence of infection of the placenta in infected cows, suggest that Akabane virus may infect the fetus through hematogenous infection of the placenta. Serological data on precolostral sera from newborn calves and sera from their mothers in the epidemic areas suggest that in about one-third of infected pregnant cows the virus may invade the fetus.

Histological examination of the fetuses from which Akabane virus was isolated revealed pathological changes consisting of encephalomyelitis and polymyositis of skeletal muscles. Immunofluorescence staining demonstrated specific antigens of Akabane virus in skeletal muscle cells and cells in cerebral tissues. Relatively high-titered virus was recovered from the brain, cerebral fluid, spinal cord, skeletal muscles and fetal placenta of a naturally infected fetus.

These observations, together with those obtained in experimental infection of pregnant dams with Akabane virus seem to indicate that the primary pathological lesions in the infected fetus

consist of encephalomyelitis and polymyositis. When fetuses are severely affected by the infection, abortion, premature birth, or stillbirth may take place. Among the fetuses surviving the infection some may gradually develop a range of brain lesions such as hydrocephalus, spongy degeneration and marked reduction in the number of motor neurons in the spinal anterior horn, depending upon the severity and the distribution of the primary damage of nervous tissues by the infection and the gestational stages at which the infection occurs.

Arthrogryposis may result from damage in the central nervous system. Affected fetuses may be born at term or prematurely, but, in some cases stillbirth is recorded. This course of events readily explains the contrasting time course in cases of abortion, premature birth, stillbirth and congenital AH syndrome as discussed later, and also the fact that some inflammatory lesions could be found in the central nervous system particularly in cases occurring during the early phase of the outbreaks.

Polymyositis was observed in naturally and experimentally infected fetuses, suggesting that it may be an important cause of the congenital deformities and muscular changes observed in spontaneous cases, although these changes could also be sequelae of the central nervous system involvement, as discussed above.

Further virological, histopathological investigations are needed to fully understand the pathogenesis of the disease. The exact relationship between the type of lesion and fetal gestational age at infection must await detailed experimental observations. It will also be necessary to elucidate the mechanism of transplacental spread of the infection.

3 Diagnosis

The etiological diagnosis of Akabane disease requires laboratory tests. However, a tentative diagnosis of the disease may be suggested on clinical, pathological and epidemiological grounds. Epidemiological information on the previous or current occurrence of the disease in the area is helpful in the diagnosis.

For the isolation of Akabane virus the clinical materials are inoculated into suckling mice by the intracerebral route or into cell cultures of primary baby hamster kidney or continuous hamster cell lines, HmLu-1 or BHK21. The clinical materials selected for virus isolation are tissues of the central nervous system, skeletal muscles of the fetus and fetal placenta. Fresh specimens from aborted fetuses occurring in late summer and early fall may give a better chance of successful virus isolation. Of the inoculated suckling mice, some may die from encephalitis but some may recover after showing mild clinical illness. Serial passage can be easily made from brain to brain in suckling mice. In the inoculated cell cultures Akabane virus readily replicates with cytopathic effect and serial passage can be obtained without difficulty. The isolated virus can be identified by serological tests with known antisera to Akabane virus. The etiological diagnosis can also be made if specific antigens of Akabane virus are demonstrated in cerebral or muscular tissues of aborted fetuses by the immunofluorescence staining technique.

For the etiological diagnosis by serological means, sera are collected from newborn animals before these suck colostrum or from fetuses. In cattle, sheep and goats the passive transfer of maternal antibodies through the placenta to the fetus does not occur, and the newborn acquires maternal antibodies by ingesting colostrum. On the other hand, their fetuses develop the ability to produce antibodies upon antigenic stimulation early in gestation. Therefore, the presence of antibodies to Akabane virus in precolostral or fetal serum indicates that the newborn or fetus had acquired intrauterine infection with Akabane virus or antigenically related virus. By this method intrauterine infection with bovine diarrhea virus, bovine rhinotracheitis virus, parainfluenza type 3 virus, bovine enterovirus and bluetongue virus has also been diagnosed.

Influence of geography on disease
1 Global distribution of virus and disease

Akabane virus was first isolated in Japan from mosquitoes, *Aedes vexans* and *Culex*

tritaeniorhynchus, in 1959 and later in Australia from biting midges, *Culicoides brevitarsis*. During the outbreaks of Akabane disease in Japan in 1972-1974, we isolated Akabane virus from naturally affected bovine fetuses and from the blood of sentinel cows. The virus was also recovered from the blood of sentinel cattle and sentinel sheep in Australia in 1977.

Metselaar and Robin (1976) isolated a virus from a pool of *Anopheles funestus* mosquitoes collected in a forest in the coastal area of Kenya. The isolated virus was indistinguishable from Akabane virus by the complement fixation test, but there was a slight one-way difference in the neutralization test. Recently Akabane virus has been isolated in South Africa from moribund lambs, 3-7 days of age, and biting midges.

The geographical distribution of antibodies to Akabane virus in Japanese bovine populations was studied. High incidence of neutralizing antibodies was shown in the central and western parts of Japan where Akabane disease epidemics had occurred in the previous year, whereas few animals had antibodies in the non-epidemic northern parts of the country. Furthermore, cattle in epidemic areas showed a high rate of seroconversion for Akabane virus, indicating a wide dissemination of Akabane virus in the epidemic areas during the summer months of 1972 and 1973.

Other species found to have antibodies to Akabane virus were horses, goats and sheep, but no antibodies were found in domestic chickens, pigs and man. HI antibodies were detected in a few humans.

In Australia high antibody incidence was found among cattle in northern Australia (Western Australia, Queensland and Northern Territory), and antibodies were distributed southwards and eastwards whereas at high latitudes such as Victoria and Tasmania no antibodies were found. Other species found to have antibodies to Akabane virus in Australia were buffaloes, horses, camels and sheep, but no antibodies were detected in domestic chickens, ducks, wallabies or man.

Antibodies to Akabane virus were found in pigs and monkeys in Indonesia, in monkeys in Malaya and the Philippines, in pigs in Taiwan, and in horses in Thailand. Antibodies to Akabane virus were also found in sera collected upon arrival from cattle imported to Japan from Australia (29/48, 56%), Indonesia (11/13, 85%), Korea (11/50, 22%) and the Netherlands (1/15, 7%), but no antibodies were found in cattle, horses, pigs and sheep imported from Canada, England, France, New Zealand, the United States and Venezuela. In Israel where outbreaks of congenital AH syndrome were observed in cattle, sheep and goats in 1969, antibodies to Akabane virus were detected in these animals. Antibodies to Akabane virus were found in cattle and sheep in Cyprus and South Africa.

These results of virus isolation and serological tests, although fragmentary, indicate a wide distribution of Akabane virus among cattle and other domestic animals in the tropical and temperate areas.

As discussed later, in Australia Akabane virus is believed to survive in transmission cycles involving cattle, sheep and horses as vertebrate hosts, and *C. brevitarsis* as vector. Doherty speculates that Akabane virus may have been introduced into Australia together with its vector and vertebrate host from India or South Africa during the period of importation of veterinary animals in the nineteenth century.

The occurrence of Akabane disease has so far been demonstrated in Japan, Australia and Israel. However, in view of the wide distribution of Akabane virus in the tropical and temperate areas of the world as discussed above, Akabane disease seems to occur in many other countries. This view may be strengthened when we consider the fact that in Akabane disease as observed in Japan, intrauterine infection with the virus results in various pathological manifestations which show contrasting time courses over a period of half a year. This situation may make it difficult to consider these different pathological conditions to be caused by the same agent. In the tropical or subtropical areas sporadic, isolated cases may occur all the year round, because vectors may be active, and hence active viral transmission may occur throughout the year. This situation may also make the recognition of the disease difficult.

Diseases suspected to be Akabane disease have been recorded in Argentina, Rhodesia and South Africa.

2 Life cycle

The biting midge *Culicoides brevitarsis* is the only insect from which Akabane virus has been isolated in Australia. There was a good correlation between the presence of antibodies to Akabane virus in cattle and the distribution of *C. brevitarsis* in Australia. The development of Akabane antibodies in cattle coincided with the periods when *C. brevitarsis* was detected. These findings suggest that *C. brevitarsis* may indeed be the principal vector of Akabane virus in Australia, although there were some data suggestive of the presence of other vectors as well. *C. brevitarsis* feeds not only on cattle but also on sheep and horses, and antibodies to Akabane virus were found in these animal species, giving further support to the theory that *C. brevitarsis* is the principal vector. The failure to demonstrate Akabane antibodies in chickens, ducks, wallabies and man may be due to the failure of the suspected vector *C. brevitarsis* to attack them or to the failure of Akabane virus to multiply in these species.

In the far northern part of Australia *C. brevitarsis* adults are active throughout the year, and Akabane virus seems likely to survive in transmission cycles involving cattle, sheep and horses as vertebrate hosts, and *C. brevitarsis* as vector. The breeding (in cow dung) and feeding habits (restricted to cattle, sheep and horses) of the vector make this survival cycle more likely. However, in southern Australia there is little, if any, activity of *C. brevitarsis* in the winter. This situation precludes continuous active transmission of Akabane virus and therefore investigations are required to explain the apparent endemicity of the virus through winter. Several possibilities may be considered. One may assume either that Akabane virus does not remain there through winter, or that the virus persists there through winter. Under the former assumption, the apparent endemicity may be explained by assuming repetitive annual arrival of infected vertebrate or arthropod hosts traveling from truly endemic far northern areas just prior to or at the same time as conditions favoring active transmission. Under the second assumption, there may be several possibilities, for instance, the overwintering survival of the virus in arthropod hosts including *C. brevitarsis* or in vertebrate hosts which can serve as a long-term reservoir.

The hypothesis, that *C. brevitarsis* is the principal vector of Akabane virus, has been put forward on the basis of epidemiological findings. It then becomes necessary to verify the hypothesis by the experimental demonstration of the multiplication of Akabane virus in *C. brevitarsis* and the virus transmission by feeding infected midges on susceptible animals.

Akabane virus has been isolated from *Aedes vexans* and *Culex tritaeniorhynchus* mosquitoes in Japan and from *Anopheles funestus* mosquitoes in Kenya. In Japan the geographical distribution and seasonal occurrence of the disease as well as active viral transmission in the summer suggests the presence of vectors. However, information on the vectors is lacking and the mechanism of transmission and survival of Akabane virus in nature awaits elucidation. These problems should also be investigated in the other countries where Akabane virus has been identified so far.

3 Epidemic and endemic behavior

The outstanding epidemiological features of Akabane disease are the seasonal occurrence and the geographical distribution. The 1972 - 1973 outbreak in Japan was mostly limited to Kyushu, Chugoku, Shikoku and Kanto districts, and dairy and beef cattle were affected likewise. Prevalence of abortions was first recognized in southern Kyushu in August, somewhat later in Chugoku and Shikoku districts, and in southern Kanto areas in September. The monthly number of reported cases of abortions and premature births 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 increase in the early months of the outbreak and a sharp rise in December, reaching a peak in January 1973. Stillbirths showed a gradual increase and decline with a peak in January 1973. The outbreak subsided in May 1973.

The epidemic recurred in 1973 - 1974. It resembled the preceding outbreak in the geographical distribution, although it was much limited in size and area. The outbreak tended to spare the areas severely affected by the previous outbreak and to move to adjoining areas. The dams affected in the previous outbreak were spared in this outbreak. A small outbreak was observed in 1974 - 1975, with cases being recognized in the prefectures bordering the Japan Sea as well as the western parts of the country. The number of reported cases in these epidemics, in the period 1972 - 1975, amounted to 42,000, of which 37, 22 and 41% were cases of abortion and premature birth, of stillbirth and of congenital AH syndrome, respectively.

In the 1974 outbreak of Akabane disease in New South Wales in Australia, Shepherd *et al.* performed field observations on the clinical entities, their time of appearance, distribution and incidence in an attempt to define an epidemiological pattern. The neurological entities observed covered different time spans in the epidemic, the order of appearance being encephalomyelitis, arthrogryposis, hydranencephaly and microcephaly. The probable period of infection correlated well with the likely presence of *C. brevitarsis* in the epidemic area, and the distribution and incidence of neurologic cases likewise correlated well with the expected geographical and climatic distribution of *C. brevitarsis* in the period. It seems likely that these epidemics occur when conditions enable a movement of virus-infected *C. brevitarsis* from an endemic area into non-endemic areas, where insects feed on susceptible pregnant animals. The possibility that infected midges may move with the wind is worth considering as a windborne spread was proposed for bluetongue, African horse sickness, and bovine ephemeral fever. A small percentage (about 20%) of animals in the endemic area, northern Australia, were found not to possess antibodies to Akabane virus. A number of these susceptible animals could become infected with Akabane virus and produce the sporadic and isolated cases which were observed in this area. In support of this view, antibodies to Akabane virus have been shown to develop in cattle in northern Australia throughout the year.

The epidemiological pattern of Akabane disease in Japanese cattle, as discussed earlier, might also be explained by the geographical and climatic distribution of the vector. However, as discussed in the preceding section, the problem of the vector in Japan has not yet been solved.

In the Japanese outbreaks of 1972 - 1974 the disease was first shown to be caused by Akabane virus. Similar outbreaks had also been recorded in 1949 - 1950, 1959 - 1960 and 1966 in Japan. Those outbreaks resembled the 1972 - 1974 outbreaks in their epidemiological, clinical and pathological features, suggesting the same etiology. Serological evidence was obtained for a wide dissemination of Akabane virus in the 1966 summer in Kagoshima Prefecture that was affected by the 1966 outbreak. Repeated occurrence of similar outbreaks has also been recorded in Australia.

4   Control and prevention

There are two main approaches to the control of Akabane disease. One is aimed at the vector and the other at protection of individual animals by vaccination. The former method includes the elimination or modification of breeding sites or the direct attack on the adult arthropods by use of insecticidal substances or other means, and the avoidance of exposure to vector bites by screening of houses or by use of insect repellent substances. These vector control measures seem impractical at the present time, and the knowledge concerning the vector is still largely inadequate in many countries. Therefore, vaccination becomes the chief means of control.

A formalin-inactivated, aluminium phosphate gel-adsorbed vaccine has been developed with Akabane virus propagated in hamster lung cell cultures. The vaccine produced neutralizing antibodies to Akabane virus by intramuscular inoculation in cattle as well as goats, guinea pigs and mice. High-titered antibodies were produced in nearly all the cattle inoculated with two doses of the vaccine given at 4-week intervals. The antibody levels thus attained declined rather rapidly in several months, hence a booster dose given one year later induced a rapid antibody response. The vaccination prevented the development of viremia and infection of the fetus in calves and pregnant goats challenged with virulent Akabane virus. The vaccination exerted little side effects in cattle. Cows vaccinated during pregnancy gave birth to healthy full-term calves. All these findings indicate

efficacy and safety of the vaccine, although the final evaluation rests on large-scale field trials. The vaccine has been licensed for general use in Japan.

An attenuated strain of Akabane virus was developed by serial passage at a low temperature of 30°C in hamster lung cell cultures. The strain did not induce pyrexia, leukopenia or viremia, but produced neutralizing antibodies when inoculated into calves and pregnant cows by the intracerebral, intravenous or subcutaneous route. No virus was recovered from various organs and fetuses of the inoculated animals. These findings seem to indicate that the strain could be used for production of live virus vaccine.

Discussion

Snowdon, W. (Australia): Would you like to speculate on the possible arthropod vector(s) of Akabane virus in Japan?

Answer: In Japan, the virus was originally isolated from mosquitoes, but it may also be harboured by midges as in Australia.

Snowdon, W. (Australia) Comment: In Australia, members of the Simbu virus group may be incriminated in fetal defects in cattle.

Oya, A. (Japan) Comment: I believe that it is very important to clarify which are the vectors of Akabane virus in Japan.

Snowdon, W. (Australia): What is the critical stage of gestation in cattle when the infection will result in fetuses affected with arthrogryposis and/or hydranencephaly?

Answer: In our experiments, bovine fetuses less than 3 months of gestation are more likely to develop congenital arthrogryposis and/or hydranencephaly.

Snowdon, W. (Australia) Comment: In sheep the most critical period is 30 to 40 days of gestation. Under experimental conditions, sheep are more sensitive to the virus than cattle. Under field conditions, it is more prevalent in cattle as a disease entity as *C. brevitarsis* appears to prefer cattle and horses to sheep.