

## An epidemiological survey on seroprevalence of vector-mediated virus infection in cattle bred in a Japanese remote island, Okinawa

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## 일본의 오키나와섬에서 사육된 소의 벡터 매개성 바이러스감염에 대한 혈청학적 역학조사

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**초 록 :** 한국 및 일본을 포함한 동아시아 지역에 벡터 매개성 바이러스에 의한 소 질병으로 인하여 경제적 손실이 몇년을 주기로 극심하게 나타나고 있다. 그러나 본 질병의 정확한 역학에 대하여는 아직까지 확실한 연구가 되어 있지 않은 상태이다. 본 연구에서는 벡터 매개성 질병에 대한 역학을 좀더 명확하게 밝히기 위하여, 일본 열도중 가장 멀리 떨어져 있으며, 아열대 지역에 속하는 오키나와 섬에서 사육되고 있으면서 백신접종을 받지 않은 소(Japanese black cattle)를 대상으로 하여 1988년도부터 1992년도에 걸쳐 벡터 매개성 바이러스군에 대한 중화항체를 측정하고 분석하였다. 소의 유행성 바이러스(bovine

ephemeral virus)에 대한 항체 양성율은 연도 및 계절별로 크게 변화를 보였고, Ibaraki virus에 대한 항체 양성율은 그 어느 다른 계절보다도 5월에 높은 항체가를 보였으며, Akabane virus에 대한 항체 양성율은 이바라키 바이러스에 대한 항체 양성율과 유사한 수준으로 나타났다. 한편 Chuzan virus에 대한 항체 양성율은 계절적인 변화가 심하지 않은 것으로 나타났다.

**Key words** : vector-mediated virus, Ibaraki virus, Akabane virus, ephemeral fever virus, Chuzan virus.

## Introduction

Vector-mediated viruses cause diseases of Akabane, Ibaraki, Chuzan, or Ephemeral fever. Akabane virus belongs to *Bunyaviridae* and causes abortion and calf deformities. Ibaraki and Chuzan viruses belong to *Reoviridae* and cause pharyngo-laryngeal paralysis and congenital abnormalities, respectively. Ephemeral fever virus is grouped in *Rhabdoviridae* Vesiculovirus and causes fever, depression, respiratory signs, stiffness and lameness. An epidemic of these virus infections may cause serious economic loss to stock-breeder. The vectors that transmit such viruses have been identified<sup>7,8,10</sup>. However, ecology of the vector-mediated viruses and the virus-vector relationship in infectious cycle are presently unclear. This uncertainty has greatly complicated the prediction of vector-mediated virus epidemics and the establishment of preventive measures. To help clarify the epidemiology, we investigated seroprevalences of bovine ephemeral fever virus (BEFV), Ibaraki virus (IBAV), Akabane virus (AKV), and Chuzan virus (CHV) in Japanese black cattle bred in Okinawa, a subtropical island located south of Kyushu and close to Taiwan(Fig 1).

## Materials and Methods

**Serum** : Blood were collected from 647 unvaccinated Japanese black cattle bred in Okinawa island in Japan. Bloodings were performed in May, July, September and No-

vember for 5 years from 1988 through 1992. The blood were centrifuged and the sera were taken into new tubes and inactivated at 56°C for 30 minutes and then stored at -40°C until analyzed.

**Neutralization test** : The following viruses and cells were used : YHL strain and HmLu-1 cells for bovine ephemeral fever virus (BEFV), Ibaraki strain and HmLu-1 cells for Ibaraki virus (IBAV), OBE-1 strain and HmLu-1 cells for Akabane virus (AKV), and K-47 strain and Vero cells for Chuzan virus (CHV). For the neutralization test, the culture medium was Eagle's medium (Nissui Pharmaceutical Co. Ltd.) containing 10% of 2.98% tryptose phosphate broth (Difco), 10% of neutralizing antibody-negative inactivated bovine serum, 1% of 7.5% sodium bicarbonate, 100µl/ml of kanamycin, and 25µl/ml fungizone. Cells were added to this medium to obtain a suspension of 10<sup>5</sup> cells/ml. The neutralizing antibody titers against BEFV, IBAV, AKV, and CHV were measured by the microtiter method. Serum was prepared serially by two-fold dilution with the medium, Aliquots of 0.25ml were mixed with the equal volume of virus suspension (200 TCID<sub>50</sub>), and incubated at 25°C. This mixture of serum and virus was inoculated into microplates containing the cell suspension and incubated at 37°C for 5 days. Two wells were inoculated with each sample, and the maximum serum dilution that completely inhibited the cytopathic effect (CPE) in at least one well was determined to possess the neutralizing antibody. A neutralizing antibody titer at 1 : 2 and above dilution was determined as positive.

**Meteorological data** : Meteorological data were obtained

from the Japan Meteorological Agency(1988~1992)<sup>6</sup>.

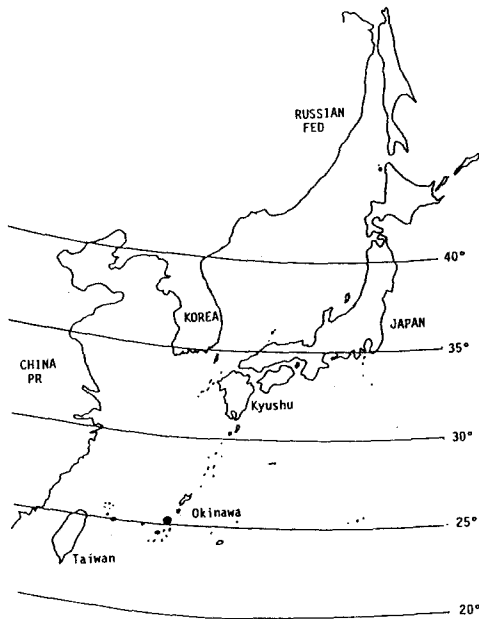


Fig 1. Sampling sites for the survey of antibody titers.

## Results and Discussion

Neutralizing antibody positive rate for BEFV varied widely from 0 to 73.3% with no seasonal or annual changes(Fig 2). The neutralizing antibody was first detected in May in 1990, 1991 and 1992. The positive rate was markedly high in May 1992. The seroprevalence of antibody to IBAV was high in May and decreased thereafter in each year(Fig 3). The seroprevalence of anti-AKV was usually > 30% and especially high (> 50%) in May(Fig 4). The seroprevalence of anti-CHV ranged from 0 to 20% (Fig 5).

Epidemiologically, the distribution area of *Culicoides oxystoma* (*C oxystoma*) was correlated with the occurrence of Akabane disease and Ibraki disease and the presence of anti-AKV and anti-IBAV neutralizing antibodies. In addition, AKV and IBAV have been isolated from *C oxystoma*<sup>5,8,9</sup>. This suggests that *C oxystoma* is one of insects that transmits AKV and IBAV. *C oxystoma* transmits AKV at about 18°C or higher temperature<sup>10</sup>. Since the temperature in the

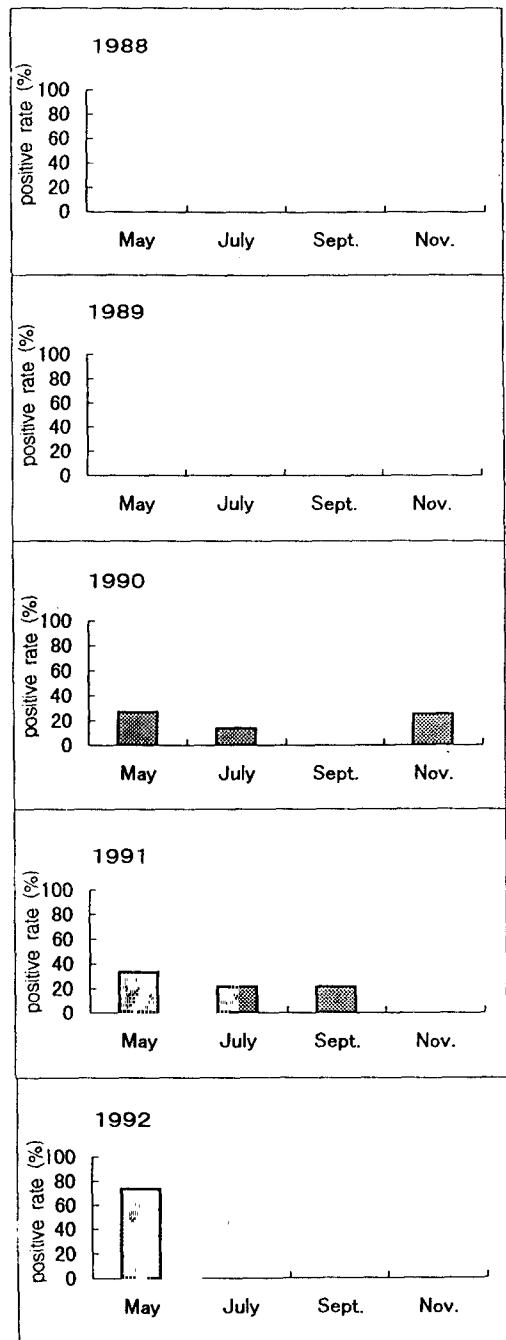


Fig 2. Variance of seropositive rate to bovine ephemeral fever virus in Okinawa cattle.

surveyed area in Okinawa from March to December was higher than 20°C every year, positive seroconversion of anti-

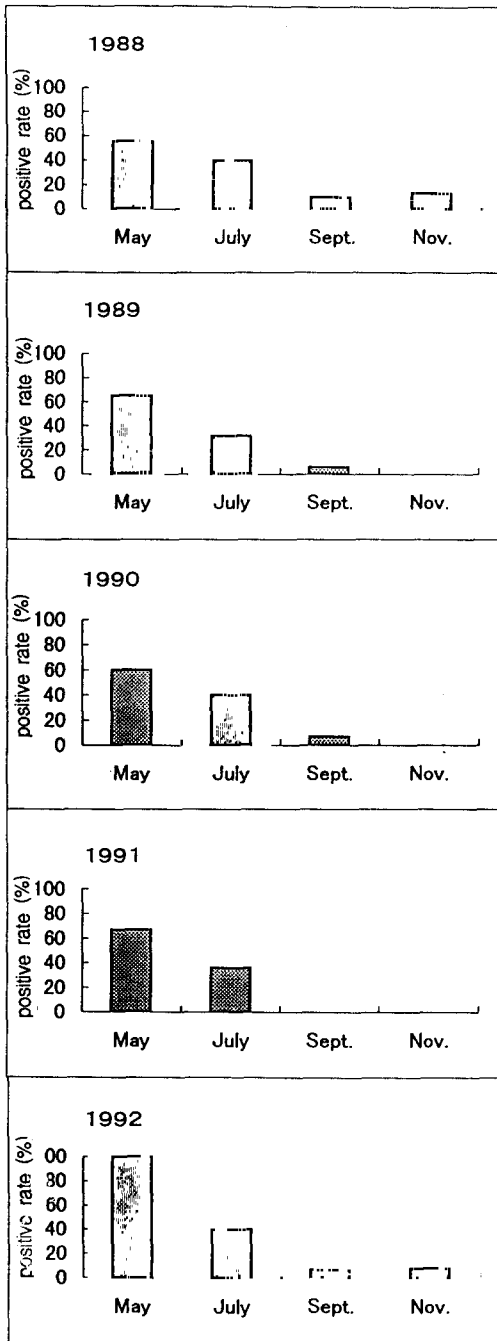


Fig 3. Variance of seropositive rate to Ibaraki virus in Okinawa cattle.

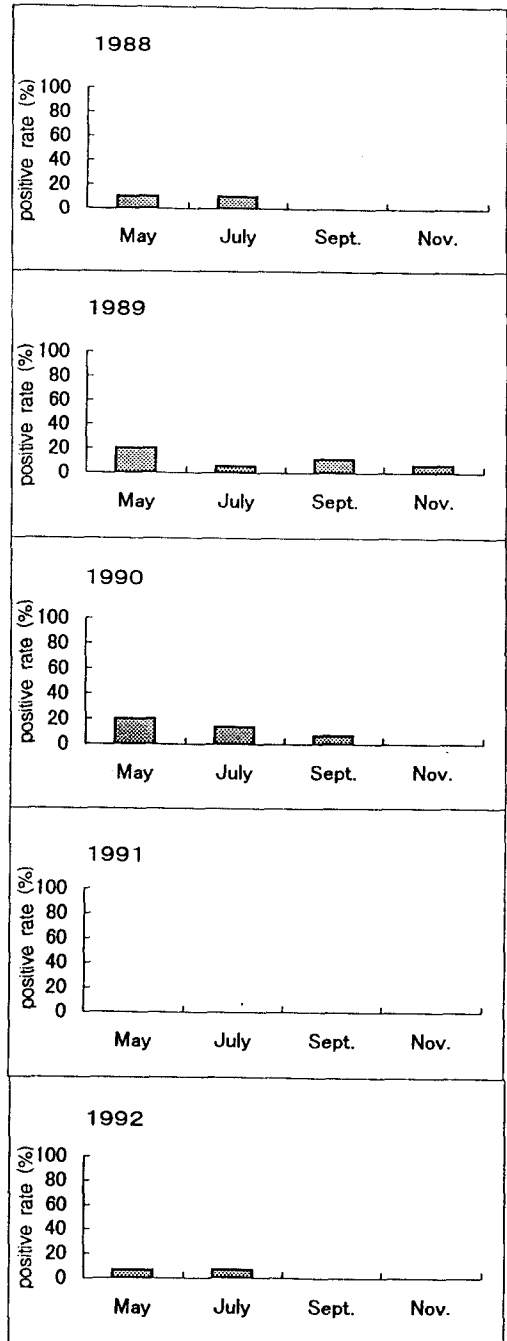


Fig 4. Variance of seropositive rate to Akavane virus in Okinawa cattle.

AKV antibody can occur from March onwards. Our study showed a high anti-AKV antibody positive rate in May.

Many vector-mediated bovine viruses have been reported in Japan. Kyushu is a warm region where cattle are exposed

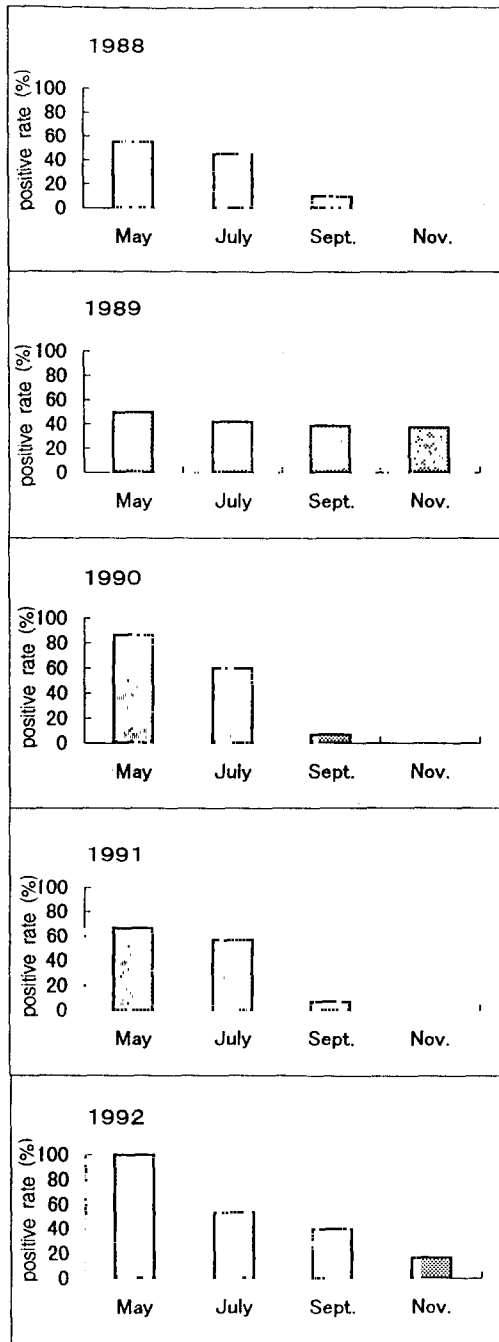


Fig 5. Variance of seropositive rate to Chuzan virus in Okinawa cattle.

to vectors that transmit these viruses throughout a year. The annual incidence of vector-mediated bovine virus infections

in Kyushu is high<sup>2-5,7-14</sup>. In Kagoshima, in the southern part of Kyushu, large outbreaks of epizootic abortion and congenital arthrogryposis-hydranencephaly syndrome due to AKV in cattle have been reported<sup>4,9</sup>. In this region, AKV epidemics have a cycle of 5-7 years<sup>3</sup> and occur in August-October<sup>9</sup>. Therefore, it is likely that the antibody rate is already high in May in Okinawa, which is located south of Kyushu with higher temperatures.

In this survey area, the cattle-vector-cattle infection cycle for AKV and IBAV might be existed. The multi-year cycle of the epidemic of vector-mediated virus infection may reflect the antibody prevalence rate in cattle and the state of the vector population. In Okinawa, the seroprevalences of BEFV and CHV were low. It is possible that the vector, infected with the virus, may have traveled from China to Okinawa via the jet stream or moved north along the island chain<sup>2,13</sup> and finally invaded Okinawa. This possibility is supported by the fact that neutralizing antibodies against those vector-mediated viruses are prevalent in Korea even though Korea is remote from China with Demilitarized Zone (DMZ) between North and South Korea<sup>1</sup>.

## Conclusion

Vector-mediated viruses in the East Asian countries including Japan and Korea have caused great economic damages with multi-year circulations of disease outbreaks. However, epidemiology of these viruses were not clearly illustrated. To clarify the epidemiology of these vector-mediated viruses, neutralizing antibody titers against these viruses were measured to unvaccinated cattle bred in a Japanese remote island, Okinawa with a subtropical weather. The serological survey was conducted from 1988 to 1992 and the changes of antibody positive rate were analyzed. The prevalence of anti-bovine ephemeral fever virus antibody varied markedly both annually and seasonally. The anti-Ibaraki virus antibody titer was higher in May than in other months and this pattern was similar in anti-Akabane virus antibody. The prevalence of anti-Chuzan virus antibody showed narrow seasonal variation.

## References

1. Cho IS. Prevention and control of mosquito transmissible diseases. *J Kor Vet Med Assoc*, 32:418-425, 1996.
2. Goto Y, Miura Y, Kono Y. Epidemiological survey of an epidemic of congenital abnormalities with hydranencephaly-cerebellar hypoplasia syndrome of calves occurring in 1985/86 and seroepidemiological investigations on *Chuzan virus*, a putative causal agent of the disease, in Japan. *Jpn J Vet Sci*, 50:405-413, 1988.
3. Hashimura K, Mimura Y, Tsuruta Y. Epizootiology of Akabane virus infection in cattle. *Jpn Vet Med Assoc*, 33:122-125, 1980.
4. Inaba Y, Kurogi H, Omori T. Akabane disease : epizootic abortion, premature birth, stillbirth and congenital arthrogryposis-hydranencephaly in cattle, sheep and goats caused by Akabane virus. *Aust Vet J*, 51:584-585, 1975.
5. Iwasaki S, Goto Y, Mimura Y, *et al.* An outbreak of Ibaraki disease of cattle in Miyazaki prefecture. *Jpn Vet Med Assoc*, 43:244-248, 1990.
6. Japan Meteorological Agency. Meteorological observations. In : *Annual Report of the Japan Meteorological Agency*. Japan Meteorological Agency, Tokyo, Japan 1988-1992.
7. Kurogi H, Akiba K, Inaba Y, *et al.* Isolation of Akabane virus from the biting midge *Culicoides oxystoma* in Japan. *Vet Microbial*, 15:243-248, 1986.
8. Kurogi H, Akiba K, Kubo M. Isolation of Akabane virus from *Culicoides oxystoma* in Kagoshima, Japan in 1984. *Jpn Vet Med Assoc*, 39:166-170, 1987.
9. Kurogi H, Inaba Y, Goto Y, *et al.* Serologic evidence for etiologic role of Akabane virus epizootic abortion-arthrogryposis-hydranencephaly in cattle in Japan, 1972-1974. *Arch Virol*, 47:71-83, 1975.
10. Miura Y, Goto Y, Kubo M. *et al.* Isolation of virus, a new member of the Palyam subgroup of the genus Orbivirus, from cattle and *Culicoides oxystoma* in Japan. *Am J Vet Res*, 49:2022-2025, 1988.
11. Miura Y, Inaba Y, Hayashi S, *et al.* A survey of antibodies to arthropod-borne viruses in Japanese cattle. *Vet Microbial*, 5:277-282, 1980.
12. Miura Y, Kubo M, Goto Y, *et al.* Chuzan disease as congenital hydraencephaly-cerebellar hypoplasia syndrome in calves. *Jpn Agr Res Quar*, 25:55-60, 1991.
13. Ogawa T. Epidemiological investigation of bovine ephemeral fever outbreaks in Kyushu Island in Japan during the fall of 1988. *Prev Vet Med*, 14:69-76, 1992.
14. Omori Y. Bovine ephemeral fever. *Jpn Vet Med Assoc*, 24:2-7, 1971.