

# Bovine Epizootic Fever

YUJI INABA

Researcher, 1st Virus Research Section, 2nd Research  
Division, National Institute of Animal Health, Tokyo

Bovine epizootic fever is an acute febrile disease of cattle known in Japan since 1949. The disease shows a sudden onset of fever and recovers usually within two or three days. The fatality rate is considered to be less than one per cent. Outbreak of this disease begins usually in late summer and ends in late autumn or early winter. The occurrence of this disease is limited in the central and western parts of Japan.

Etiological studies have been restricted because of the lack of experimental hosts other than cattle. However, the causative virus was recently isolated using suckling mice, hamsters, and rats by intracerebral inoculation and using

BHK21 cells<sup>5),15)</sup>. Thus, the laboratory methods for primary virus isolation and serological tests have been accomplished and become feasible. These methods enable us to carry out more systematic studies on this disease.

In this paper there will be given some detail of the recent studies on this disease.

## Epizootiological findings

The disease has a seasonal incidence; outbreaks begin in late summer and terminate in late autumn or early winter. The disease has occurred only in the central and western parts of Japan, and it has never been reported in the northern parts, Tohoku and Hokkaido, Japan.

Table 1. Occurrence of "So-Called Cattle Influenza" in Japan

Years	Morbidity		Mortality		Season	Number of Prefectures
	Number	%*	Number	%**		
1949	161,967	10.5	835	0.5	Dec.	25
1950	464,631	20.7	6,247	1.3	Aug.-Dec.	39
1951	46,917	2.1	2,288	4.9	Aug.-Dec.	38
1952	160	0.0	15	10.0	Aug.-Nov.	9
1953	41	0.0	0	0.0	Aug.-Oct.	2
1955	4,140	0.6	65	1.6	Aug.-Nov.	10
1956	21,796	3.4	123	0.6	Aug.-Nov.	10
1958	54,459	17.2	206	0.4	Aug.-Nov.	3
1959	39,076	2.0	4,041	10.3	Aug.-Dec.	29
1960	4,550	1.1	560	12.3	Sep.-Dec.	7
1966	7,053	1.8	54	0.8	Oct.-Nov.	6

\* Percentage related to the bovine population in the infected prefecture.

\*\* Percentage related to the diseased cases.

Mortality includes slaughtered cases.

This indicate that the epizootics have never been observed in the districts which are in above 38° North latitude in Japan.

It should be mentioned here that the disease was once confused with another epizootic disease of cattle which is similar to bluetongue in the clinical and pathological findings: The outbreaks of the bluetongue-like disease in cattle were firstly recognized in 1959 and 1960, and a virus named as "Kaeishi virus or Ibaraki virus" was determined to be the etiologic agent of this disease by Omori et al. <sup>12)</sup>.

Although these two viruses, bovine epizootic fever and Kaeishi viruses, have quite different characteristics<sup>2), 4), 10)</sup>, the two types of epizootics have been controlled legally under the name of "so-called cattle influenza" in Japan. The statistical data shown in Table 1 refers to at least the two diseases.

However, it is well known that the mortality of the bluetongue-like disease in cattle caused by Kaeishi virus is remarkably higher than that of the bovine epizootic fever as seen in the epizootics in 1951, 1952, 1959 and 1960. These findings were coincidentally supported by the serological results by Omori<sup>10)</sup> indicating that the Kaeishi virus was more or less prevalent in 1949 and 1950, in addition to the bovine epizootic fever virus, and that the bovine epizootic fever virus was not prevalent in 1959 and 1960.

There are so many epizootiological problems still to be solved, for example, the infection may or may not occur by contact with affected animals. Omori et al.<sup>11)</sup> recently reported that, although the data was not enough, the contact infection of this disease was negative in the epizootic in 1966. On the other hand, the vector is still unknown, although the transmission of the disease by *Culicoides* or other small biting insects is suspected.

#### Clinical findings

The disease is clinically characterized by a sudden rise of body temperature of 41°C-42°C, which lasts from one to three days, together with anorexia, increased respiration accompanying with a temporary dyspnea, coughing, nasal discharge, increased salivation, lacrimation, muscle tremor, joint pain, decreased lactation, and remarkable leukopenia. In the experimental

cases, these symptoms appear usually within two to four days after the virus inoculation. In spite of the apparent severity of the symptoms, the prognosis of the disease is considerably favorable.

These clinical and epizootiological findings indicate that this disease has some resemblance to the ephemeral fever or three-day sickness which has occurred in Australia, South Africa and in the 'tropical countries'<sup>7), 8), 9), 14), 16), 18)</sup>. At the present time efforts are being made to carry out the studies on serological relationship between the bovine epizootic fever and the ephemeral fever viruses.

#### Pathological findings

Microscopically, the lesion was mainly observed in the lung. The affected lung was characterized by a congestion, edema and emphysema. This was, in general, the extent of the pathologic changes found in those cases which showed a rapid and uneventful recovery. However, in the more severe cases, particularly those that terminated fatally, the pulmonary emphysema became more intensive and diffusive, extending into the bronchus, trachea, pericardium, mediastinum, submaxillar and the cervical areas. Localized lobular collaps was usually found in an affected lung. Slight cloudy swelling was occasionally observed in the pulmonary and mediastinal lymph nodes. Organs other than the lung were normal in naked eye appearance.

Microscopically, infiltration of round cells and neutrophils in acinus, peribronchitis and inter-alveolitis were observed in experimental and natural cases of the disease. Hepatic necrosis, nephrosis, and activation of reticuloendothelial cells in liver, lymph nodes and spleen were also observed<sup>6)</sup>. From these facts it was suspected that the agent might be pantropic in character.

#### Virus isolation

Since the bovine epizootic fever was recognized in 1949, several viral strains have been established from natural cases of the bovine epizootic fever by serial transfer through cattle<sup>3), 5), 17), 19)</sup>. Those strains were shown by cross protection tests using cattle to be related antigenically to each other <sup>3), 5), 17)</sup>.

The lack of experimental hosts other than

cattle against those strains has greatly hampered the etiological study of the disease. However, Sasaki et al.<sup>15)</sup> recently isolated the virus using suckling mice by intracerebral inoculation and Inaba et al.<sup>4)</sup> also could succeed in propagating the virus using suckling hamsters, mice and rats by intracerebral inoculation, and using BHK21 cells derived from a baby hamster kidney<sup>20)</sup>. These findings have provided laboratory tools whereby the virus can be readily grown, and assayed, and serological tests against the virus become feasible. Our results of the virus isolation are described below in more detail.

Defibrinated blood obtained from a cattle at the 2nd bovine passage of the Strain Yamaguchi, established in 1966<sup>5)</sup>, was inoculated intracerebrally into the suckling hamsters, which were within 24 hours after birth, in a litter in an amount of 0.01 ml. The inoculated hamsters showed weakness, emaciation and sluggish staggering movement for seven days after inoculation. Some of them were found dead and the remaining animals were moribund for 10 days after the inoculation and they were sacrificed on the eleventh day after the inoculation (Fig.

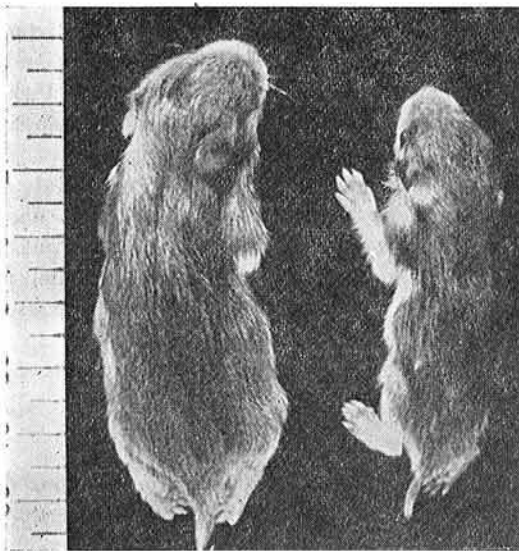


Fig. 1. Right hamster is inoculated with hamster passage strain of bovine epizootic fever virus. 10 days after inoculation. Left hamster is an uninoculated control.

1). Ten per cent suspensions were made from the brains of the dead and sacrificed hamsters, respectively. All the inoculated hamsters were taken ill on the 5th or 7th day after the inoculation and showed clinical manifestations similar to those observed in the initial passage hamsters. Thus, further serial passages were readily accomplished by intracerebral inoculation of 10 per cent brain emulsion into the suckling hamsters (YH line). The inoculated animals were usually moribund or dead on the fifth or sixth day after the inoculation.

The YH line was also readily passed in day-old mice and rats (YHM and YHR lines), which developed systemic convulsion and paralysis of the hind part of the body and were dead two or three days after the intracerebral inoculation. Ten per cent emulsion of their brains contained  $10^6$ – $10^8$  LD<sub>50</sub>/0.01 ml. of virus. Mice and rats tended to be less sensitive to the virus in accompany with an increase of age.

The YH line grew well showing cytopathic changes in BHK21 cell cultures at 34°C and the serial passages were readily accomplished by the inoculation of infected tissue culture fluids (YHK line). The cytopathic effect was detectable two or three days after the inoculation. The changes consisted of rounding, clumping, disintegration and detachment of the affected cells from a glass wall of culture tubes.

The destruction of the cell sheet was usually complete on the fifth day after the inoculation. Virus titer reached about  $10^5$  TCID<sub>50</sub>/0.1 ml. (Fig. 2, 3). The YHK line also multiplied

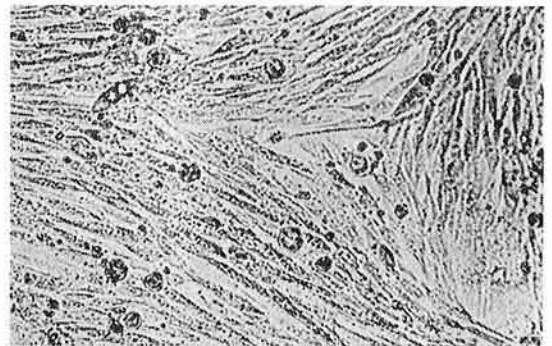


Fig. 2. Normal BHK21 cells in 7-day culture. Unstained,  $\times 100$ .

in the cell cultures of the calf kidney and testis without cytopathogenic effect, and of bovine embryo kidney with cytopathogenic effect. Better results of the virus growth in cell cultures were obtained using incubation at 30°C than at 34°C or 37°C.

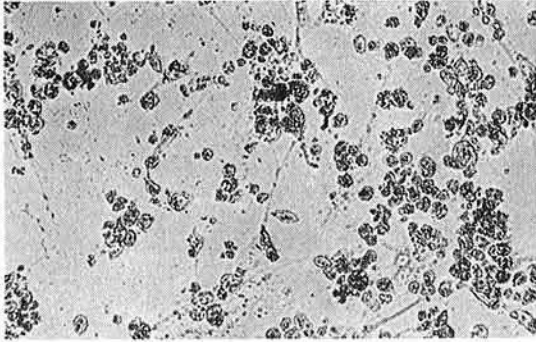


Fig. 3. Infected BHK21 cells 4-days after inoculation of YHK strain of bovine epizootic fever virus. Unstained,  $\times 100$ .

The relative sensitivity of the suckling hamster, mouse and rat, and BHK21 cell culture for primary isolation of the virus is to be evaluated to establish the practical effective procedure in the near future.

#### Characteristics of the virus

Cattle can be infected experimentally by inoculation of the virus by only intravenous route, but subcutaneous, intracutaneous, intramuscular, intracerebral, intratracheal and intraperitoneal inoculation of the virus are negative to transmit the disease. The transmission of the disease is confirmed by the appearance of neutralizing and complement fixing antibodies in the serum of the affected or inoculated cattle.

There are no results which proved the transmission of the disease by contact with the infected cattle; this may be explained by the absence of the virus in the feces, urine, saliva, etc.—of the infected cattle. The virus is not pathogenic for guinea pig, rabbit, goat and sheep. These animals do not produce antibodies against the virus.

The pathogenicity of YH, YHM and YHK lines for calves were tested. All the calves inoculated with these lines at the early passage levels, namely the 3rd, 4th and 8th passage levels of YHM, YH and YHK lines, respectively,

developed an acute illness which was essentially the same as that observed in natural cases of the bovine epizootic fever. In all cases, small amounts of virus were recovered from the defibrinated blood at the febrile stage by intracerebral inoculation using suckling hamster or mouse and or BHK21 cell cultures.

All the calves were initially negative for serum neutralizing antibodies and showed significant antibody rises after infection. Complement fixing antibodies for the YHM, YHR and YHK lines were also developed after infection in all calves. The tests were carried out by the complement dilution method of Omori et al.<sup>13)</sup> using antigens prepared from infected mouse or rat brains by the acetone-ether extraction method of Casals<sup>1)</sup>, and also prepared from infected BHK21 cell culture fluids.

The calves were found to be resistant for challenging the virus of Strain Kitasato, which had been used as a prototype strain of bovine passage virus of the bovine epizootic fever, 2 or 3 weeks after the first infection. Inoculation of the virus into calves at the further passage levels, the 4th, 6th and 10th passage levels of YHM, YH and YHK lines, respectively, failed to produce either clinical evidence of disease or a specific immunological response.

However, after repeated inoculations of these viral strains, antibodies being comparable to those in calves recovered from natural attacks of the bovine epizootic fever were developed and the calves were found to be resistant for challenging the bovine passage virus. The extremely rapid modification and the loss of pathogenicity of the isolated virus for calves is surprising, but a similar observation by Van der Westhuizen has been made with the adaptation of ephemeral fever virus in mice<sup>21)</sup>. According to his paper, the BF1 strain of mouse adapted ephemeral fever virus lost its capacity to produce the disease and also to develop neutralizing antibodies in cattle as early as the 3rd passage in the suckling mice.

It was recently found that the inoculated calves with the virus after alternating passage using BHK21 cell and bovine embryo kidney cell cultures showed only very slight fever and transient leukopenia, but neutralizing antibodies developed to the great extent as seen in cattle

**Table 2.** Development of neutralizing and complement fixing antibodies against the YHM and YHK lines of virus in sera of cattle infected with the bovine passage strains of bovine epizootic fever virus.

Virus strain		Cattle No.	Antibody titers against							
Name (recovered)	Bovine Passage No.		Pre-infection NT*	YHM CF**	Post-infection NT	CF	Pre-infection NT	CF	YHK Post-infection NT	CF
Kitasato (1951)	253	364	< 2		≥128			< 2		64
	254	359	< 2	0	≥128	4.7		< 2		128
	255	409	< 2	0	32	2.2		< 2		256
	255	410	< 2					< 2		256
	255	412	< 2		≥ 32			< 2		≥256
Ishikawa (1955)	2	Suzukaze						< 2		1024
	4	2	< 2	0	32	1.4		< 2		256
Chiba (1956)	3	Harukaze						< 2		128
	2	5	< 2	0	32	2.9		< 2		512
Miyazaki (1958)	1	41	< 2		128			< 2		1024
Yamaguchi (1966)	1	372	< 2	0.1	128	2.9		< 2		128
	1	375	< 2	0.1	32	1.3		< 2		256
	2	389						< 2		512
	2	392						< 2		256
	2	5	< 2		64			< 2		128
	2	6	< 2		64			< 2		128
Nagasaki (1966)	1	382	< 2		≥ 32			< 2		256
	1	383						< 2		≥512
	1	384						< 2		≥512
	2	413						< 2		256

\* The reciprocal of the highest serum dilution that showed neutralization against 100 LD<sub>50</sub> of the YHM line or 100 TCID<sub>50</sub> of the YHK line.

\*\* Complement dilution method was employed. Titers equal to, or higher than 1.2, are positive.

inoculated with the bovine passage virus and also showed resistance for challenge.

Table 2. summarized the results of neutralization and complement fixation tests using the YHM and YHK lines of the virus in calves infected with six viral strains which were derived natural cases of the bovine epizootic fever and were maintained by bovine passage. All the strains produced neutralizing and or complement fixing antibodies being reactive with both these viral strains.

The physico-chemical properties of the virus are described as follows: Replication of the

virus is not inhibited by 5-iodo-2'-deoxyuridine, and this fact indicates that the virus is an RNA virus. The virus is sensitive to ether, chloroform, sodium deoxycholate and trypsin, and is labile at pH 3.0, and readily inactivated at 56°C for 10 minutes. The virus passes through a 220 m $\mu$  Millipore filter without any infectivity loss. The resulting filtrates hardly lose infectivity by filtration through a 100 m $\mu$  Millipore filter, and no infectivity is recovered after 50 m $\mu$  filtration. The infectious particles of the virus is sedimented by ultracentrifugation at 73,000  $\times$  g for one hour.

These biological and physico-chemical properties of the virus indicate it to be hitherto undescribed. Determination whether this is a new or known virus, and elucidation of its classification, require more detailed comparative studies.

### Prophylaxis and treatment

For the prophylaxis of the disease, there are two methods; one of them is that a susceptible cattle should be avoided from an insect suspected as a vector and the other one is that of using killed or attenuated virus vaccine. The killed virus vaccine prepared from the defibrinated blood of the infected cattle has been practically used for this purpose, but the effectiveness of this vaccine is doubtful. Therefore, more effective vaccine should be expected in the near future.

Treatment using hyperimmune cattle serum is considerably effective to protect the cattle from infection and to aid the recovery of the affected cattle<sup>17)</sup>. This procedure, however, is not useful from the standpoint of economy. It is a common practice for veterinarians to administer various chemotherapeutic agents such as antibiotics and sulfonamides to select, severely affected individuals. This is done in an attempt to combat secondary infection with microorganisms which may gain access to the body through the lesions in various organs of the affected cattle.

### Addendum

More recently the following results were obtained by the author and his associates: 1) Bovine epizootic fever virus was antigenically related to ephemeral fever virus by neutralization test. 2) the buoyant density of bovine epizootic fever virus was 1.196 g/ml. 3) The electronphotomicrographs of negatively stained bovine epizootic fever preparations showed the agent to be a bullet form, about 80 × 140 m $\mu$ , with an envelope.

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