Epidemiology of bovine ephemeral fever virus infection in Taiwan

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Abstract

Sick animals with excessive nasal discharges and protruding tongue as a result of dyspnea were observed in the August of 1996. Eight strains of BEF virus were isolated from heparinized blood samples of the affected cattle. Most of the affected cattle were difficult to be treated and had a poor prognosis. A total of 516 farms in the 9 districts of Taiwan were affected in 1996. Among a population of 110,247 dairy cattle, 14,993 (13.6%) cattle were found to be clinically ill. During the epidemic, 1,685 (11.3%) affected cattle were culled or dead after the onset of the disease. Furthermore, a strain of Ibaraki virus was isolated from the blood sample of a sick cattle that showed pyrexia, labored respiration and solitary behavior in the affected farm. The cattle with Ibaraki virus infection had typical symptoms of BEF at the early stage of the disease, but neither stomatitis nor pharyngoesophageal paralysis was observed at the onset of the disease. The outbreak was presumably brought about by the low level or non-immune status of a large cattle population due to the negligence of BEF vaccination. Therefore, the disease easily recurred in Taiwan after a typhoon episode in the August of 1996, which resulted in the proliferation of biting midges in the field. No difference in the antigenicity was found between the new and the previous isolates of BEF virus. As analyzed by cross neutralization test, the isolated BEF viruses showed no relationship to the Kimberley and Berrimah viruses that were isolated from the blood of cattle and related to BEF virus in Australia. We have tried to advise farmers that they must vaccinate their cattle annually to prevent BEF outbreak in the future.

Key words : Bovine ephemeral fever, epidemiology, Ibaraki disease

Introduction

Bovine ephemeral faver (BEF) is a clinical disease of cattle. Outbreaks of BEF have been reported in

South Africa, Kenya, India, Australia and Japan since early 1900s (Burgess 1971, Davis et al. 1975, Inaba 1968, Morgan and Murray 1969, Murray 1970, Standfast et al. 1976). Ephemeral fever, three-day-sickness, stiff sickness, bovine epizootic fever and bovine influenza have been used to name this viral disease in the different countries at different times (Chiu 1986, Chiu and Lu 1986, Lin and Inoue 1969, St. George 1981). BEF could occur without noticeable clinical signs, but the features of the clinical disease were primarily the same as described in various countries, such as South Africa, Australia, Japan (Inaba1968). High fever, nasal discharges, salivation and lameness appear to the principal features of this disease (Snowdon 1970, St.George1985, Timoney *et al*. 1988, Tzipori 1975). BEF producted its main impact by disrupting the husbandry of beef cattle and dairy cows. It usually caused the bulls to be temporarily infertile (Burgess and Genoweth 1975). During fever, milk production almost ceased and the milk quality was poor. Lactation usually resumed on recovery, but the loss of milk production in a natural epizootic can be as high as 12% of the lactation in dairy cows (Davis *et al*. 1984). Under experimental conditions, the short-term milk loss was 50%. The loss of body weight during illness was apparent, but usually was not quantified.

It is believed that the disease, bovine influenza, has long existed in Taiwan. No description about BEF had been made until Lin (1969) and Otte (1968) first reported the outbreak of the disease in Kaohsiung district located in the southern Taiwan in 1967. Of the 4,441 dairy cattle investigated, 1,183 (26.6%) were infected, 62 (5.2%) either died or were culled and 31 dams with abortion. The disease was confirmed as BEF by the serological tests with serum samples sent to the National Institute of Animal Health, Ministry of Agriculture, Forestry and Fisheries, Japan. Later, no further study on this disease was reported in Taiwan until 1984 (Chiu 1986 ; Chiu and Lu 1986).

The causative virus of the disease was successfully isolated in Taiwan in 1984. At that time, the outbreak of BEF was observed in dairy cattle, yellow cattle and buffaloes. The incidence of BEF was 20% (5,650/28,117). About 6% of the affected cattle (340/5,650) were culled or dead after the onset of the disease. However, some deer with similar clinical signs were also found in the field, and then the affected animals were bled antibody detection. Neutralizing antibodies against BEF virus were found in sera of dairy cattle (74.5%), yellow cattle (44.0%), buffaloes (66.6%), goats (47.8%) and deer (8.5%). Although sheep were also positive for the antibodies to the virus, the prevalence was low (6.3%). All the sera of horses, pigs, chickens, wild birds and human were negative (Chiu and Lu 1986 ; 1987a). As the need of emergency policy, the effective way was to develop useful vaccine to control further spread of the disease. Thus, vaccines generated from the attenuated live virus and inactivated virus were developed and used as previously described (Chiu and Lu 1987b ; Inaba *et al.* 1973, 1974). After the emergency control program was executed, no further cases were found.

The third major epizootic of BEF erupted in the April of 1989. During the epizootic, 357cattle herds in 14 districts were affected. The morbidity was 14.5% (4,216/29,157). About 5% of the affected cattle

(210/4,216) were culled or dead. Eleven strains of BEF virus were isolates from defibrinated blood of infected cattle.These isolated were all neutralized by the antisera against BEF (Lu *et al*. 1992). BEF virus was an endemic pathogen and was commonly distributed in the field in Taiwan. Due to the enlargement of feeding lots, many breeders were imported from the United States or Canada. This resulted in reducing the antibody titers in the cattle population in the field. Consequently, most of the newborn calves were unable to resist the challenge of BEF virus in Taiwan.

After these epizootic, a BEF virus isolates was chosen and used for the development of inactivated virus vaccine for BEF prevention. The techniques of vaccine production were also transferred to private vaccine manufacturer for commercial production. Farmers could conveniently buy the inactivated BEF vaccine from veterinarian. In addition, we advised farmers to vaccinate their cattle annually. Furthermore. the commercial BEF vaccines were also inspected for the efficacy by an authorized institute with national standards. Due to the omission of vaccination in most of dairy farms, a fourth outbreak of BEF erupted again in Taiwan in the August of 1996. Eight strains of BEF virus were isolated from heparinized blood samples of sick cattle. The epizootiological investigation and serological relationship of isolates were described in this chapter.

Materials and methods

Clinical and epizootic features of natural infection. Most of the information relating to the occurrence of BEF was derived the Livestock Disease Control Center (LDCC) located in each district or city of Taiwan. The LDCC is primarily responsible for the collection of epizootic information, diagnosis and the enforcement of control measures for all animal diseases.

An acute febrile and hard respiratory syndrome among dairy herds was first reported in Hsinchu city, central Taiwan in the August of 1996. Clinical signs of pyrexia, extensive salivation, nasal discharges and labored respiration were observed in dairy cattle of several farms in Hsinchu city. Later, the disease spread to the neighboring districts in the north and the south of Taiwan. The disease was very contagious and virulent in most districts.

In epizootic farms, necropsy of dairy cattle that showed typical BEF clinical signs was conducted. Tissues including lung, liver, spleen, tonsil, mucosa of trachea, brain and spinal cord were collected for etiological and histopathological examinations.

Etiological examination. Collected nasal swabs and heparinized blood samples of the affected cattle were used for virus isolation. Furthermore, the tissues of sick cattle were also used for virus isolation. The homogenates of tissues or swabs were prepared in Eagle' s MEM (Gibco Co) containing streptomycine (0.1mg/ml) and gentamycine (0.1mg/ml). After centrifugation at 4℃ for 20 minutes, the supernatants of

individual homogenate were collected and stored at –70℃ for virus isolation. Heparinized blood samples were centrifuged at 1,000xg for 10 minutes, and the plasma was collected. The blood cell layer was then resuspended and washed in phosphate-buffer saline (PBS) for three times as previously described. After washing, the blood cell layer was again resuspended in PBS and stored at –70℃ for virus isolation.

After freezing and thawing, the blood samples and the supernatants of the homogenates were inoculated into cell cultures for virus isolation. The BHK-21 (baby hamster kidney continuous cell line), Vero (green monkey kidney continuous cell line) and HmLu-1 (hamster lung continuous cell line) cells were used for the virus inoculation. The monolayer cell culture, freshly prepared in $25cm^2$ flask, was individually inoculated with the samples. After incubation at 34℃ or 37℃ for 5~7 days, each culture fluid was inoculated into another freshly prepared cell culture. This culture passages were repeated at least three times. Virus isolation was determined by the appearance of a cytopathic effect (CPE) in the culture or immunofluorecent staining.

The virus isolated was further confirmed by the electron microscopy of cell cultures showing CPE. The sample collected from cell culture was centrifuged at 2,500xg for 20 minutes. The supernat was collected for ultracentrifugation by Airfuge (Beckman Co) at 90,000rpm for 10 minutes. The sediment was mounted on carbon-coated 400-mesh grids, stained with 2% potassium phosphotungstic acid, and examined with a transmission electron microscope (Hitach model H600, Japan).

Viruses and antisera. The standard virus of BEF (YHL strain), Kimberley virus (CS368 strain), and Berrimah virus (DPP63 strain) were provided by the National Institute of Animal Health, Tsukuba, Japan. These viruses were subcultured in BHK-21 cells. Then, these virus suspensions were measured by the simultaneous inoculation method and was adjusted to be $10^6 TCID_{50}$ / ml in the neutralization test, respectively.

Antisera to BEF virus were prepared from cattle that had been hyperimmunized with an inactivated BEF vaccine, and used for cross neutralization test. Other antisera against Ibaraki virus and BEF virus (YHL strain) were prepared from rabbits and were also used for neutralization test.

Seroloical examination. Sera were collected from cattle in the epizootic farms. For neutralization test, all serum samples were inactivated at 56℃ for 30 minutes before testing. Eight paired sera of the affected cattle were collected from the experimental farm of National Chung-Hsiung university, Taichung, during the outbreak of BEF. Furthermore, 472 sera of the affected cattle from seven districts were arbitrarily sampled during the onset of BEF, and then another 489 sera were collected after the onset of BEF. The neutralizing antibody titers of the paired sera against the isolates were examined to confirm the outbreak of the disease.

Neutralization tests were carried out in disposable flat-bottomed tissue-culture microtiter plates (Nunc Co). Test sera were diluted in MEM containing 0.2% bovine serum albumin (Sigma Co). Duplicated with a 2-fold serial dilution, From 1:2 to 1:256 in 0.05ml volumes, were prepared for each serum tested. An equal volume of virus suspension (containing 200 $TCID_{50}$) was added to each well. Cell and medium controls were included in each test and back titration of the virus was also carried out. The microtiter plates were covered with cellophane tapes, and were shaken for 30 seconds at room temperature. Subsequently, the microtiter plates with the mixture were incubated at 37℃ for 1 hour in a 5%CO_2 incubator.

After incubation, BHK-21 cell suspension containing 3 ×10^4 cells/0.1 ml was added to each well. The plates were further incubated for neutralization test at 37℃ in a 5% CO_2 incubator for 5 days. Tested plates were examinied daily by microscopy for the evidence of virus-specific CPE. Antibody titers were expressed as the reciprocal of the highest serum dilution in the serum / virus mixtures that neutralized virus at the 50% end point. Test sera were considered positive when titers were equal to or greater than 4.

Results

Clinical investigation

Pyrexia, respiratory disorders and lameness caused by articular pain were the main clinical signs observed in the natural cases of BEF. Body temperature usually went up to 41℃ and remained high for 2 to 3 days. Most of the infected animals showed a significant loss of appetite and depression during the course of the disease. Sick animals had a dry muzzle, excessive nasal discharges and a large amount of salivation. Animals with an open mouth and protruding tongue as a result of dyspnea were often seen. Other clinical signs included marked depression, solitary behavior and reduced movement in the exercise lot. With the onset of the disease, reduced milk yield and often only to one-fifth of the normal yield had been observed.Regardless the stage of gestation, abortion was observed in some cases. The course of the clinical disease in individual animals varies from a mild malaise and stiffness to death. All breeds of cattle appeared equally susceptible. Younger animals suffered less severely than older animals. Fat cows, steers, bulls and cows in heavy lactation exhibited more severe clinical signs and were more likely to die or show clinical complications.

Due to the serious clinical symptoms, most of the affected cattle were difficult to be treated and had a poor prognosis. A total of 516 farms in the 9 districts of Taiwan were affected in 1996 (Table 1). Among a population of 110,247 dairy cattle, 14,993 (13,6%) cattle were found to be clinically ill. During the lapse of the epizootic, 1,685 (11.3%) affected cattle were culled or dead and the incidence varied in affected districts.

Pathological findings

The post-mortem examination revealed severe pulmonary emphysema and hemorrhagic foci on the mucosa of trachea. Leukopenia was also observed in some sick cattle. Microscopically, most of the lymph nodes of sick cattle were congestive. Severe dilatation of alveoli and thin alveolar septa were the main characteristics of the disease. The alveolar and bronchiolous cavities were filled with fibrinous exudates containing gangrenous cells and chips, such as macrophages, erythrocytes, granular lymphocytes. No evident pathological change was found in the other organs.

Etiological examination

Eight strains of BEF virus were isolated from heparinized blood samples of sick cattle, but no virus was isolated from tissue or nasal swab samples of the affected cattle. Prominent CPE was observed in BHK-21 cell cultures. Nevertheless, the isolation of virus from cell cultures with an incubation at 37℃ was not as sensitive as the method with an incubation at 34℃. The isolated viral particles showed a bulletshaped virion with a size of 75 ×150nm under electron microscope. These morphological features were consistent with those of BEF virus as described previously (Chiu 1986).

Furthermore, a strain of Ibaraki virus was also isolated from the blood sample of a sick cattle that showed pyrexia, labored respiration and solitary behavior in an epizootic farm of Changhua district. The cattle with Ibaraki virus infection had typical symptoms of BEF in the early stage of the disease by farmer's description. This affected cattle responded to treatment poorly and the clinical signs lasted more than one month till the animal was culled in the September of 1996. Neither stomatitis nor pharyngoesophageal paralysis was observed in this affected cattle.

Virus identification

Serological cross-reactivity tests were performed by using BEF sera and Ibaraki sera. From the results, the referred BEF sera had an identical antibody titer against the BEF virus which was isolated in 1996 (Table 2). However. significant variations were observed as the BEF sera was against Kimberley virus and Berrimah virus. Thus, the isolated viruses were similar in antigenicity to the BEF virus which was isolated from affected cattle in the previous epizootic. Furthermore, a strain (96–H–78) of Ibaraki virus was identified from an affected cattle in Changhua district. Consequently. BEF and Ibaraki viruses were successfully isolated during the epizootic.

Serological examination

Prominent antibody responses to BEF virus were found in the paired sera tested. Significant increase of the antibody titer against BEF virus was observed in the affected cattle after the onset of the disease (Table 3). According to the results of the paired sera, the infection with infectious bovine rhinotracheitis (IBR) virus

was not suspected in this epizootic.

In addition, sera collected from affected cattle were also examined with the isolated BEF virus. The antibody titers tended to be less than 8 during the outbreak in most affected cattle (Table 4). Later, the antibody titers shifted to higher level during the recovery period. The fluctuation of antibody titers indicated that most of the cattle were infected with BEF virus during the epizootic.

Disscussion

The outbreak of BEF was diagnosed by serological examination and virus isolation. From the epizootic investigation, no vaccination was regularly practiced in most of the affected farms in recent years. Therefore, serum samples showed low antibody titers at the early stage of the outbreak (Tables 3 and 4), possibly resulting from the high proportion of non-immunized cattle at that time and allowing the BEF virus to transmit and cause disease among cattle herds. Since the occurrence of BEF was mainly in the summer or rainy season, it was presumed that the outbreak of BEF was associated with the typhoon which hit Taiwan in the end of July of 1996 and resulted in the proliferation of bitting midges in the field. Consequently, the BEF virus was widely disseminated to the susceptible animals.

The Kimberley and Berrimah viruses were originally isolated from the blood of cattle which were under regular surveillance but did not shown any signs of disease in Australia (Cybinski and Zakrzewski 1983; Gard *et al*. 1983;1984). The immunofluorescence and complement fixation analyses could not differetiate the antigenicity to BEF virus. However. the neutralization test showed that both Kimberley and Berrimah viruses were not the BEF virus . Therefore, we performed the neutralization test to differentiate the Taiwanese isolates from those of the Australian isolates, and confirmed the isolated virus to be the BEF virus.

Although the Ibaraki virus was isolated from the affected cattle during the epizootic, we suspected that a mixed infection of BEF virus and Ibaraki virus might sporadically occur in clinical cases. Similarly, epizootic of BEF and Ibaraki disease were both present in the outbreak of 1950 and 1951 in Japan (Tanaka and Inaba 1986). Ibaraki disease had been reported to occur at the same period of time and in same area, and in the same manner as BEF in Japan (Omori *et al.* 1969).

BEF was a common disease in the summer and early autumn because it usually rains a lot during the summer months in Taiwan. The best preventive method for the disease is to inoculate cattle with inactivated virus vaccines that is most commonly used strategy for the prevention of other viral diseases. Furthermore, sentinel herds can be used in the surveillance of BEF virus and relative viruses. The serological survey of BEF can be regularly conducted in the sentinel animals. Isolation of the virus from insects, either collected in the field or used in the laboratory, can be useful to monitor the epizootiology of the disease. In addition, the pathogenesis of BEF disease and the transmission of different strains of BEF

virus should be further studied.

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Table 1. Epizootic investigations on bovine ephemeral fever in Taiwan 1996

District	No. of farm affected	No. of cattle affected	No. died or culled	Duration of disease
Hsinchu	21	897	67	8.20 ~ 9.19
Nantou	16	732	70	8.22 ~ 10.5
Changhua	52	952	216	8.26 ~ 10.7
Taichung	29	896	40	8.29 ~ 10.7
Chiayi	46	1883	303	8.29 ~ 10.7
Miaoli	64	4058	424	8.30 ~ 10.7
Yunlin	29	750	177	9.4 ~ 10.5
Penghu*	200	252	0	9.9 ~ 10.11
Kaohsiung	59	4553	388	9.21 ~11.18
Total	516	14,993	1,685	

*: Only buffaloes were reared for labor.

Table 2. Serological relationships between new isolates, bovine ephemeral fever and Ibaraki viruses revealed by cross neutralization test

vitus	Immune		sera	
(strain)	BEF (YHL)	BEF (TW)*	96-H-81	Ibaraki
New isolate				
96-H-50	512**	1048	512	<2
96-H-81	256	512	512	<2
96-H-84	256	512	512	<2
96-H-78	<2	<2	<2	256
BEF (TW)*	256	1048	512	<2
BEF (YHL)	512	512	512	<2
Kimberley	<2	4	<2	<2
Berrimah	2	4	2	<2
Ibaraki (No. 686)	<2	<2	<2	512

*: The TW strain of BEF virus was used for the inactivated BEF vaccine in Taiwan.

**: Neutraling antibody titer.

Table 3. Neutralizing antibody titers of paired sera from affected cattle in the experimental farm of National Chung-Hsiung university, Taichung

Cattle No.	First samples (1996, 8, 21)		Second samples (1996, 9, 11)	
	Titer to BEFV*	Titer to IBRV*	Titer to BEFV	Titer to IBRV
61	<2	<2	128	<2
62	<2	8	64	16
63	<2	16	<256	16
9	<2	16	64	2
20	16	<2	128	16
24	16	<2	128	2
29	<2	<2	<256	16
44	16	8	64	16

*: BEFV = Bovine ephemeral fever virus.

*: IBRV = Infectious bovine rhinotracheitis virus.

Table 4. Seroepizootic investigations of affected cattle during the epidemic of bovine ephemeral fever 1996

District	During onset		After onset	
	No. of sera tested	Titer*	No. of sera tested	Titer*
Hsinchu	40	4	16	13.9
Miaoli	32	4.3	46	32
Nantou	18	3.0	30	27.8
Chiayi	46	4.3	18	22.6
Tainan	24	8	65	13
Kaohsiung	112	3.0	98	22.6
Pintung	200	8	216	24.3
Total	472	4.9	489	22.3

*: Geometric mean of neutralizing antibody titer.

台灣地區牛流行熱病毒感染流行病學

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摘要

民國 85 年 8 月間，本省乳牛群顯現急性發燒與呼吸困難的流行熱病症。經統計，共有 9 個縣（市），516 戶酪農之 14,993 頭乳牛發病，發生率佔當時乳牛總數 110,247 的 13.6%。在發病牛中有 1685 頭（11.3%）因為發病死亡或被淘汰。在該疫情期間，我們由發病牛樣品共分離出 8 株牛流行熱病毒，以及一株茨城病病毒。發現茨城病病毒的牛隻病程是較其他發病牛長，且有呼吸困難及離群現象；該病牛早期呈流行熱發熱及喘的症狀，但沒有喉頭麻痺症狀。分離之流行熱病毒經以流行熱不活化疫苗之免疫血清，進行交叉中和試驗，結果新分離株皆可為免疫血清中和，顯示流行熱不活化疫苗刺激產生的抗體仍可保護牛隻耐過野外病毒的攻擊。由疫情調查發現，因為近年來沒有疫情發生，農民皆疏於預防接種。故發生流行熱的疫情是因為牛群抗體低弱，又逢七月間颱風過後，媒介蚊蟲孳生，致牛流行熱病毒再流行。以分離病毒經試驗證實，該次流行的病毒不是澳洲報告之 Kimberley 或 Berimah 病毒。在牛流行熱防疫檢討，現階段仍要求農民能每年定期實施疫苗接種，以確保牛隻安全。

關鍵詞：牛流行熱，流行病學，茨城病