

THE SEROLOGICAL STUDY ON BOVINE EPHEMERAL FEVER IN TAIWAN IN 1984

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Serological investigations were conducted when Bovine Ephemeral Fever (BEF) outbreaked and prevailed in Taiwan in 1984. The neutralization antibody (NA) titer in the sera of ruminants, including cattle (Holsteins, Yellow Cattle and Hybrid Cattle), water buffaloes, goats, sheep and deer was measured. The sera of horses, pigs, chickens, ducks, wild birds (15 species) and humans were also tested.

High positive rate of NA titers was found in the sera of dairy (Holstein) cattle (74.5%), Yellow Cattle (44.0%), Hybrid Cattle (100.0%), water buffaloes (66.6%), goats (47.8%) and deer (38.5%). While that of sheep was also positive, though it was low (6.3%).

All of the sera of horses, pigs, chickens, ducks, wild birds and human were negative.

Serological investigations have been the most important means to identify the disease of BEF, before the causative virus was isolated and proved its pathogenicity. The detection of neutralization antibody (NA) titer in the pair sera has been the indispensable part of the diagnosis of BEF.^(1,6,10,12)

Only few serum samples were sent to the National Institute of Animal Health, Japan by Otte⁽¹¹⁾ for the identification of the BEF outbreak in Taiwan in 1967. Otherwise, no serological investigation has ever been made in Taiwan. Therefore, the epidemiology of BEF in Taiwan is obscure.

It has been reported that the ruminants, such as cattle, water buffaloes, goats and sheep maintained NA titers in the BEF epizootic area.^(1,3,6) But St. Gorge described (1978) that no NA was detected in sheep. From the epizootic point of view, not only the ruminants including sheep,

but also the other animals, such as horses, pigs, poultry, and human should be investigated serologically to clarify the situations. For these animals and human have been geographically closely distributed side by side with ruminants in the rural areas, in where the blood sucking vector insects swarm.

MATERIALS AND METHODS

1. Bovine Ephemeral Fever Virus

(1) *YHL strain*

This standard strain of BEF virus was given to the Provincial Research Institute for Animal Health by the National Institute of Animal Health, Ministry of Agriculture, Forestry and Fishery, Japan in 1979. After passaged once each with BHK 21 (baby hamster kidney origin) and HmLu-1 (hamster lung origin) cell lines, the virus was lyophilized and stored at

-80°C. The infection titer of the virus at the time of use was $10^{5.0-6.5}$ TCID₅₀/ml.

(2) *TLRI strain*

Using the defibrinated blood of the febrile (41.8°C) hybrid cow (No. 104) of Taiwan Livestock Research Institute (TLRI), this strain was isolated in mice with intracerebral inoculations on May 19, 1984. After 3 passages in mice, the strain was inoculated in BHK 21 cell line and cloned twice at 8th and 10th passages and then stored at -20°C after lyophilization.

(3) *Liu Yin strain*

From a dairy cow (Holstein, No. 073-232) of a private dairy farm in Liu Yin, Tainan, Liu Yin strain of BEF virus was isolated intracerebrally in mice on April 27, 1984. After 3 passages in mice, this virus strain was adapted in BHK 21 cell line and cloned with marginal dilution technics. The virus was then lyophilized

and stored at -80°C. The infection titer of the virus to BHK 21 cell was $10^{5.6-6.5}$ TCID₅₀/ml at the time of use.

2. Sera

All sera used in the NA tests were either collected and sent to this Institute by the Livestock Disease Control Centers of Prefectures or directly collected by this Institute. A total of 828 serum samples of dairy cattle (Holstein) from 8 prefectures collected in May 1982, i.e. before the outbreak of BEF, and a total of 1,383 serum samples from the same 8 prefectures in May 1984, i.e. at the height of prevalence, were collected.

As to the sera of the other ruminants, 25, 65, 42, 16 and 46 serum samples were respectively collected from the Yellow cattle, the Hybrid (beef) cattle, water buffaloes, sheep and goats. Likewise, 11, 110, 176 and 85 serum samples respectively

Table 1. Wild Bird Species Tested on the Neutralization Antibody Titre of Bovine Ephemeral Fever

Chinese Name	Scientific Name	Number
1. 白頭鵪	<i>Grus monachus Temminck</i>	2
2. 赤足鵪	<i>Tringa incana brevipes (Vieillot)</i>	5
3. 小鶩	<i>Anas crecca crecca Linnaeus</i>	4
4. 小鶩	<i>Anas strepera strepera Linnaeus</i>	6
5. 赤頸鶩	<i>Anas penelope Linnaeus</i>	4
6. 班鳩	<i>Streptopelia chinensis formosa (Kuroda)</i>	34
7. 烏鶩	<i>Dicrurus macrocercus harterti (Stuart Baker)</i>	5
8. 白干鳥	<i>Charadrius alexandrinus dialbatus (Swinhone)</i>	1
9. 白鶩	<i>Egretta intermedia intermedia (Wagler)</i>	14
10. 褐色貓頭鷹	<i>Strix leptogrammica caligata (Swinhone)</i>	2
11. 野鴨	<i>Anas platyrhynchos platyrhynchos (Linnaeus)</i>	1
12. 海鳥		15
13. 文鳥	Java sparrow, <i>Padda oryzivora</i>	1
14. 鸚鵡	Love birds, <i>Agapornis Spp.</i>	2
15. 蒼鶩	<i>Nycticorax nycticorax nycticorax (Linnaeus)</i>	90
Total		186

from horses, pigs, chickens and ducks were also collected. In the field of wildlife, 13 serum samples of sika (*Cervus nippon* Taiouanus) and 186 samples from 15 species of wild birds found in the western sea coast of Central Taiwan were gathered (Table 1).

To widen the area of study, 103 serum samples of human sera from dairy farm families, workers and veterinarians were also tested.

3. Cells

The cell lines of BHK 21 (baby hamster kidney origin) and HmLu-1 (hamster lung origin) were used. Eagle's minimum essential medium (MEM), containing 10% tryptose, phosphate broth, 10% calf serum, 200 μ /ml of penicillin, 100 μ g/ml of streptomycin, 100 μ g/ml of kanamycin and 2.5 μ g/ml of fungizon, was the medium used. The serum in the medium was tested negative to BEF virus NA antibody titer.

4. Neutralization Antibody (NA) Test

The sera inactivated at 56°C for 30 minutes was sequentially diluted with MEM and used in NA tests. To each stage of diluted serum, an equal amount of cultured viral fluid which has a titer of 200 TCID₅₀/0.1 ml was added and left overnight at 4°C. An amount of 0.1 ml of this mixture was then added to 2 wells of microplate in which BHK 21 cells were grown. After an adsorption at 37°C for 60 minutes, 1 ml of MEM was added to each well and left at 34°C for 7 days. The NA titer was determined with the highest dilution fold of serum in which at least one of the two wells exhibited the inhibition of cytopathic effect (CPE).

RESULTS

1. The Neutralization Antibody Titer in Dairy Cattle

(1) *The seroconversion in natural infections:*

As shown in Table 2, majority of the NA titer of dairy cattle in the acute stage, i.e. at the beginning of epizootic was 2 or less, but in the convalescent stage, i.e. 7 or more days after the infection, it was more than 16. More than 4 fold increase in NA titer indicated clearly that seroconversion took place during this period.

(2) *The NA titer of dairy cattle before the epizootic:*

Of the 828 serum samples collected from 8 prefectures before the epizootic (May 1982), only 56 (6.8%) showed positive (4 or more) reactions with a highest titer of only 16 (Table 3).

(3) *The NA titer of dairy cattle during the epizootic:*

The results of NA titer testings of 1,383 serum samples collected from above mentioned 8 prefectures in May 1984, i.e. 2 months after the outbreak of BEF, showed majority of the samples holding high NA titer (Table 4). A total of 1,030 (74.5%) samples showed positive titer with more than 1/3 of them holding 16 or more NA titer. The highest titer was as high as 512.

2. The NA Titer of the Other Cattle

During July to September 1985, 25, 65 and 42 serum samples respectively from the Yellow, the Hybrid cattle and water buffaloes were collected from various prefectures and tested. The rate of positive reactors was 44.0%, 100.0% and 66.6% respectively for the Yellow, the Hybrid

Table 2. The Seroconversion of Dairy Cattle in the Natural Infection of Bovine Ephemeral Fever

Area	Cattle No.	Neutralization Antibody Titre		No. of Days between Acute and Convalescent Stages
		Acute Stage	Convalescent Stage	
Chiayi	2	<2*	>16	7
	9	<2	>16	7
	11	4	16	11
	14	<2	16	10
	15	<2	>16	7
	19	<2	>16	7
	114	16	64	7
Tainan	35	<2	>16	14
	436	<2	>16	14
	499	<2	>16	14
	470	<2	>16	14
	482	2	>16	14
	488	<2	8	14
	500	8	128	60

*: Neutralization test with serum dilution method; the value of 4 or more is observed as positive.

Table 3. The Neutralization Antibody Titre in Dairy Cattle Sera in Pre-epidemic Stage of Bovine Ephemeral Fever in Taiwan

Area	Date of collection	No.	Neutralization Antibody Titre										
			<2	2	4	8	16	32	64	128	256	>512	
	1982	*											
Taipei	5	60	60	0	0	0	0	0	0	0	0	0	0
Miaoli	5	63	45	10	6	2	0	0	0	0	0	0	0
Taichung	5	112	93	16	3	0	0	0	0	0	0	0	0
Changhua	5	138	130	8	0	0	0	0	0	0	0	0	0
Chiayi	5	121	98	12	8	3	0	0	0	0	0	0	0
Tainan	5	144	129	8	6	1	0	0	0	0	0	0	0
Kaohsiung	5	87	68	14	4	1	0	0	0	0	0	0	0
Pingtung	5	103	62	19	17	3	2	0	0	0	0	0	0
Total (%)		828	685 (82.7)	87 (10.5)	44 (5.3)	10 (1.2)	2 (0.2)	0	0	0	0	0	0
Number Positive (%)										56 (6.8)			

*: Number of serum samples

Table 4. The Neutralization Antibody Titre in Dairy Cattle Sera in Mid-epidemic Stage of Bovine Ephemeral Fever in Taiwan

Area	Date of collection	No.	Neutralization Antibody Titre										
			<2	2	4	8	16	32	64	128	256	>512	
	1984	*											
Taipei	5	80	22	30	18	10	0	0	0	0	0	0	0
Miaoli	5	84	5	13	20	18	16	6	4	2	0	0	0
Taichung	5	90	9	23	22	20	11	5	0	0	0	0	0
Changhua	5	227	66	58	23	30	19	11	13	4	3	0	0
Chiayi	5	330	5	18	60	72	67	44	22	19	14	9	9
Tainan	5	266	8	20	44	57	63	32	18	13	9	2	2
Kaohsiung	5	180	15	19	38	33	41	17	9	5	3	0	0
Pingtung	5	126	18	24	19	33	15	13	3	1	0	0	0
Total (%)		1,383	148 (10.7)	205 (14.8)	244 (17.6)	273 (19.7)	232 (16.8)	128 (9.3)	69 (5.0)	44 (3.2)	29 (2.1)	11 (0.8)	
Number Positive (%)													1,030 (74.5)

*: Number of serum samples

Table 5. The Distribution of the Neutralization Antibody Titre of Bovine Ephemeral Fever in Ruminants

Animal	* Positive or Negative	period	Area					Total (%)	
			Taipei	Miaoli	Yunglin	Chiayi	Kaohsiung		Pingtung
Water Buffalo	Positive	1985 7~9		17/26	6/10	5/6			28/42 (66.6)
	Suspect			3/26	1/10	1/6			5/42 (12.0)
	Negative			6/26	3/10	0/6			9/42 (21.4)
Yellow Cattle	Positive	1985 9			1/5	10/20			11/25 (44.0)
	Suspect				2/5	2/20			4/25 (16.0)
	Negative				2/5	8/20			10/25 (40.0)
Hybrid Cattle	Positive	1985 7		15/15		50/50			65/65 (100.0)
	Suspect			0/15		0/50			0/65 (0.0)
	Negative			0/15		0/50			0/65 (0.0)
Sheep	Positive	1985 9	1/3					0/13	1/16 (6.3)
	Suspect		2/3					1/13	3/16 (18.8)
	Negative		0/3					12/13	12/16 (75.0)
Goat	Positive	1985 9			12/26	10/20			22/46 (47.8)
	Suspect				3/26	1/20			4/46 (8.7)
	Negative				11/26	9/20			20/46 (43.5)
Deer	Positive	1985 9	0/5			3/6	2/2		5/13 (38.5)
	Suspect		4/5			0/6	0/2		4/13 (30.8)
	Negative		1/5			3/6	0/2		4/13 (30.8)

*: Positive : Neutralization antibody titre; 4 or more
 Suspect : Neutralization antibody titre; 2
 Negative: Neutralization antibody titre; less than 2

Table 6. The Distribution of the Neutralization Antibody Titre of Bovine Ephemeral Fever in Human, Animals and Birds

Human and animal	* Positive or Negative	Period	Area						Total (%)
			Taipei	Miaoli	Yunlin	Chiayi	Tainan	Pingtung	
Human	Positive	1985 7~9	0/6		0/97				0/103 (0.0)
	Suspect		0/6		0/97				0/103 (0.0)
	Negative		6/6		97/97				103/103 (100.0)
Horse	Positive	"	0/4					0/7	0/11 (0.0)
	Suspect		0/4				0/7	0/11 (0.0)	
	Negative		4/4				7/7	11/11 (100.0)	
Pig	Positive	"	0/30		0/10	0/30	0/20	0/20	0/110 (0.0)
	Suspect		0/30		0/10	0/30	0/20	0/20	0/110 (0.0)
	Negative		30/30		10/10	30/30	20/20	20/20	110/110 (100.0)
Chicken	Positive	"	0/100		0/6	0/20	0/50		0/176 (0.0)
	Suspect		0/100		0/6	0/20	0/50		0/176 (0.0)
	Negative		100/100		6/6	20/20	50/50		176/176 (100.0)
Duck	Positive	"	0/10		0/25	0/20	0/30		0/85 (0.0)
	Suspect		0/10		0/25	0/20	0/30		0/85 (0.0)
	Negative		10/10		25/25	20/20	30/30		85/85 (100.0)
Wild Bird	Positive	"			0/184	0/2			0/186 (0.0)
	Suspect				0/184	0/2			0/186 (0.0)
	Negative				184/184	2/2			186/186 (100.0)

*: Same as Table 5

cattle and water buffaloes (Table 5).

3. The NA Titer of the Other Animals, Poultry and Human

The ruminants other than cattle, i.e. goats and sheep, showed positive NA titer of 47.8% (22/46) and 6.3% (1/16) respectively (Table 5). However, the sera of the other animals and poultry, including horses (11 samples), pigs (110), chickens (176) and ducks (85) were all tested negative (Table 6). All human sera (103) also showed negative result (Table 6).

4. The NA Titer of Wild Life

Of the 13 serum samples collected from the deer in the north (5), the central (4) and the south (4) Taiwan, 5 samples

(38.5%, 4 from the central and 1 from the south) showed positive result (Table 5). But all of the 186 serum samples of 15 wild bird species (Table 1) showed negative result (Table 6).

DISCUSSION

1. Comparing the NA titer of BEF in the pair sera of dairy cattle in acute and convalescent stages, it was found that there was a significant (4 fold) increase. All titer in the sera of convalescent stage showed 16 or more, while that of initial acute stage showed less than 2. The seroconversion indicated that the cattle infected BEF.

2. The low NA titer in dairy cattle before the epizootic provided a good pre-

disposition for the rapid prevalence when it outbreaked in 1984. Only 6.8% of 828 sera samples in pre-epizootic stage showed positive value of only 4-16. The infection of BEF could be explained by high rate (74.5%) of positive NA titers (4-512) in 1,383 mid-epizootic sera samples.

3. The high NA titer of BEF in the Hybrid (100.0%), the Yellow (44.0%) and water buffaloes (66.6%) meant that these animals were infected and might have helped each other in accelerating the prevalence of the disease. Cattle and water buffaloes are evenly distributed in the rural area in Taiwan.

4. The existence of NA titer in goats (47.0%), sheep (6.3%) and deer (38.5%) indicated that all ruminants in Taiwan were susceptible to BEF. This finding coincided with the report of Hall et al.⁽³⁾ that BEF virus multiplied well in sheep, but was contrary to Matsumoto's description⁽⁷⁾ that goats were unsusceptible.

5. No NA titer of BEF in the sera of hoeses, pigs, chickens, ducks, wild birds and human. Doherty et al.⁽²⁾ also reported that no BEF NA titer was found in the sera of hoeses, chickens and human. Nevertheless, it might be necessary to further investigate it to clarify the role of different animals in the epizootic of BEF.

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牛流行熱的血清學調查

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1984 年在台灣乳牛發生牛流行熱時，對各種家畜實施血清學調查。

在牛流行熱發生前，即 1982 年 5 月採取的乳牛血清 828 例的中和抗體價陽性（4 倍或 4 倍以上）者僅 6.8%（56 例），而在流行期，即 1984 年 5 月採取的 1383 例乳牛血清的中和抗體價陽性者高達 74.5%（1,030 例）。牛流行熱發生時的配對（Pair）血清中和抗體價為發生初期在 2 倍以下，而 7~14 日以後，均在 16 倍以上。

各種牛隻在牛流行熱流行後的中和抗體價

陽性率分別為雜種（肉用）牛 100%（65 / 65），黃牛為 44.0%（11 / 25），水牛為 66.6%（28 / 42），而山羊的陽性率為 47.8%（22 / 46），綿羊為 6.3%（1 / 16）。

農家飼養的鹿亦有很高的陽性率，38.5%（5 / 13）。

其他家畜，如豬（110 例），馬（11 例），鷄（176 例），鴨（85 例），野鳥（15 種 186 例）的血清則均呈陰性。

人的血清 103 例檢查結果亦為陰性。