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EPIDEMIOLOGICAL STUDIES ON DUCK VIRAL HEPATITIS

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SUMMARY

This study succeeded in finding serologic evidence of duck hepatitis virus (DHV) in many kinds of domestic fowl. The antibody investigation revealed that 55% of duck flocks in Taiwan had been exposed to DHV. The antibody response of five survivors were evaluated for 120 days.

Twenty-three strains of DHV were isolated from intestines of Common carp and Tilapia taken from ponds where affected ducklings were raised. The titer was $10^{2.7-4.2}$ PFU/ml. Virus could be isolated from fish of the same pond even six years after the last DVH outbreak. Viruses isolated from fish were pathogenic to 5 days old duckling and produced typical lesion of DVH.

DHV ($10^{2.0-3.6}$ PFU/ml) was recovered from Tilapia 3-5 days after oral inoculation with 0.1 ml of Hunei-6 strain ($10^{5.2}$ PFU/ml). DHV ($10^{1.9-5.9}$ PFU/ml) was also recovered from Tilapia 2-7 days after feeding with intestines of ducklings died by intravenous injection with I-Lan strain ($10^{7.0}$ EID50/ml).

The data strongly suggested that Common carp, Tilapia and domestic fowls acted as a reservoir or carrier for DHV. They provided constant sources of infection and made it difficult to prevent DVH outbreak.

Duckling either naturally or artificially exposed to duck hepatitis virus (DHV) developed neutralizing antibody[2,3,8,10,13]. Therefore serum neutralization test was widely used for epidemiological study. Asplin[5] failed to find evidence in serums from 520 wild aquatic fowls of 6 species. Ulbrich[15] failed to find neutralizing antibody in 36 wild ducks (4 species) taken from ponds where the disease had occurred in domestic ducks. The author tried to find serologic evidence from ducks of several breeds, goose, turkey and chicken of Kaoshiung county where the first outbreak of DVH was noted in this island. Many kinds of fish taken from ponds where sick birds raised were studied to prove it's role as a reservoir or carrier of duck hepatitis virus.

MATERIALS AND METHODS

Serologic studies:

Sera: From January to June of 1972, samples from 15 counties were collected. One sample from every flock of Chinese common duck, mule duck, muscovy duck, goose, chicken and turkey of Kaoshiung county were taken. In addition, 281 samples from 66 duck flocks of the other fourteen

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counties were collected.

Antigen: K418 strain of DHV was used.

Neutralization test: Sera were inactivated at 56 C for 30 minutes and mixed with equal volume of viral fluid which was 10x diluted. The mixtures were incubated at 4 C or 37 C for 90 minutes before injecting into 7 days old chicken embryos, 0.1 ml for each embryo, using 4 chicken embryo per dilution[1]. The neutralization index (NI) was calculated by Behren-Karber method[9]. NI of 1.7 was judged as positive while 1.0 was considered to be negative[13].

Antibody response of survived ducks: Sera of five survivors which became sick at 3 days old were evaluated at 7, 14, 30, 60 and 120 days old, respectively.

Isolation of DHV from fish:

Common carp, Tilapia, silver carp, mullet and grass carp taken from ponds where DVH occurred were submitted for virus isolation. Common carp and Tilapia from clean pond serve as control. Common carp and Tilapia were brought for virus isolation one year after the last outbreak. Six years later when the second outbreak occurred at the same flock, common carp and Tilapia from the same pond were taken for virus isolation. Liver, intestine and brain of each fish were aseptically removed and made into emulsion with YLE solution before inoculating into chicken embryos and DK cells. The isolated virus was identified by conducting neutralization test with known DHV antiserum.

Duckling and gosling inoculation test with DHV isolated from fish:

Virus: Hunei-3 strain, isolated from intestine of common carp through 5 passages in chicken embryos. It's titer in DK cells was $10^{5.3}$ PFU/ml. Hunei-6 strain, isolated from intestine of Tilapia with chicken embryo. It's titer in DK cells was $10^{5.0}$ PFU/ml.

Animals: Five days old male ducklings and four days old gosling were used.

Method: Twenty ducklings and two goslings were injected via leg muscle with 0.1 ml of Hunei-3 virus, respectively. Twenty ducklings were injected with Hunei-6 virus by the same way described above. These birds were observed for 14 days and the dead birds were necropsied. Five healthy ducklings served as control.

Virus recovery from fish orally administered with DHV:

Virus: Hunei-6 strain ($10^{5.2}$PFU/ml)
I-Lan strain ($10^{7.0}$PFU/ml)

Fish: Tilapia from pond without ducks were fed with lyophilized earthworm for 1 week before test.

Exp. I: Sixteen fish were orally administered (forcefully) with 0.1 ml of Hunei-6 virus, respectively. Two healthy fish raised in separate tank were served as control. Virus recovery were conducted before and 1,2,3,4,5,6,7,14 days after infection.

Exp. 2: Five grams of intestine from ducklings died of intravenous injection with I-Lan strain was put into tank contained 100 liter water and 10 fish which were allowed to eat the intestine freely. Two healthy fish kept separately served as control. The feed was deprived from two days before the experiment and during the whole period of experiment. Virus recovery were conducted before and 2,4,7,14,28 days after feeding the DHV infected intestine materials. Intestines of the fish were removed and made into emulsion before inoculating onto DK cells in which

plaque formation unit (PFU) were calculated.

RESULTS

The results of serologic studies were summarized in Tables 1, 2 and Figure 1.

Table 1. Serologic Evidence of DVH in Domestic Fowls of Kaohsiung County

Poults	No. flock test	No. flock positive	Positive rate
Layer duck	14	14	100%
Mule duck	28	26	93%
Muscovy duck	27	17	63%
Goose	15	11	73%
Chicken	30	20	67%
Turkey	20	17	85%

The level of DVH antibodies (NI) in five surviving ducks were $10^{0.5-2.5}$ at 7-day-old, rised to $10^{1.3-4.8}$ at 14-day-old, $10^{3.0-5.25}$ at 60-day-old, and dropped to $10^{2.5-4.0}$ at 120-day-old.

The results of the isolation of DHV from fish were indicated in Tables 3, 4 and 5. Twenty-three strains of virus were isolated from intestines of the common carp and Tilapia. The virus titer ranged from $10^{2.7}$ to $10^{4.2}$ PFU/ml.

The results of duckling and gosling inoculation test with virus isolated from fish- were listed in Tables 6 and 7.
Virus isolated from fish were pathogenic to duckling and gosling, and produced typical lesion of DVH on them.

The results of virus recovery from fish orally administered with DHV were revealed in Table 8.

Table 2. Serologic Survey of DVH on Ducks in Taiwan

County	No. flock tested	No. duck tested	No. positive flock	No. positive duck
I-Lan	4	16	3	11
Taipei	5	23	3	12
Taoyuan	2	10	2	10
Hsinchu	4	15	1	4
Miaoli	4	15	1	4
Taichung	7	27	4	13
Changhwa	6	26	4	18
Yunlin	6	27	3	12
Chia-I	4	17	3	12
Tainan	8	38	3	12
Pingtung	6	27	5	18
Taitung	4	15	1	4
Hwalien	4	15	1	4
Penghu	2	10	2	10
Total	66	281	36 (55%)	144 (51.2%)

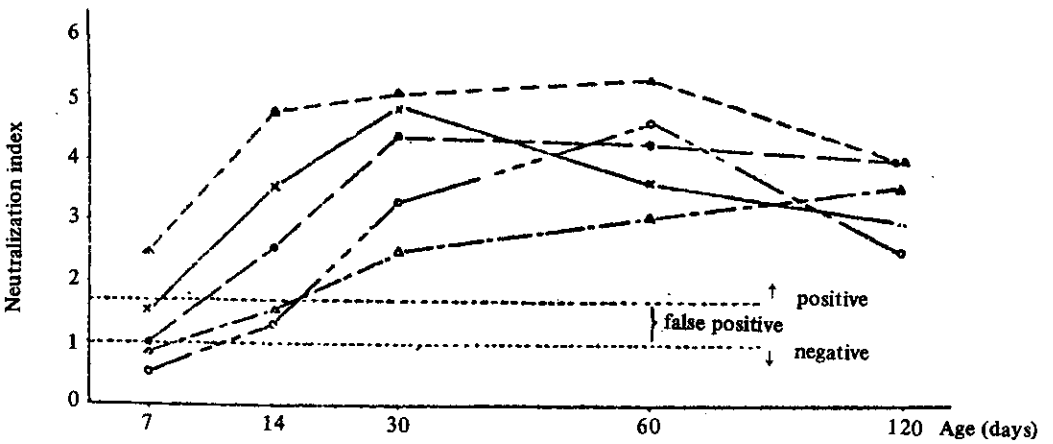

Fig. 1. Antibody response of ducks which survived the DVH.

Table 3. Virus Isolation from Fish when DVH Occurred

Time	Fish	Species	Organ		Titer LogPFU/ml	Designate*
			Intest.	Others		
197.9	A1	CC	+	–	3.6	Hunei-1
	A2	CC	+	–	2.8	Hunei-2
	A3	CC	+	–	2.7	Hunei-3
	A4	CC	+	–	3.5	Hunei-4
	A5	CC	+	–	4.0	Hunei-5
	A6	T	+	–	2.9	Hunei-6
	A7	T	+	–	3.2	Hunei-7
	A8	T	+	–	3.0	Hunei-8
	A9	T	+	–	3.6	Hunei-9
	A10	T	+	–	2.9	Hunei-10
	A11	SC	–	–	0	
	A12	SC	–	–	0	
	A13	M	–	–	0	
	A14	M	–	–	0	
	A15	GC	–	–	0	
	A16	GC	–	–	0	
1971.9	B17	CC	+	–	2.9	Hunei-11
	B18	CC	+	–	3.6	Hunei-12
	B19	T	+	–	4.2	Hunei-13
	B20	T	+	–	2.7	Hunei-14
	B21	T	+	–	3.1	Hunei-15
1971.9	C22	CC	–	–	0	
	C23	CC	–	–	0	
	C24	T	–	–	0	
	C25	T	–	–	0	
	C26	T	–	–	0	
	C27	T	–	–	0	

* Cultured by chicken embryo and DK cell. CC = common carp, T = tilapia, SC = silver carp, M = mullet, GC = grass carp.

Table 4. Virus Isolation from Fish One Year after the Last Outbreak

Time	Fish	Species	Organ		Titer LogPFU/ml	Designate*
			Intest.	Others		
1972.9	B28	CC	+	–	3.0	Hunei-16
	B29	CC	+	–	3.2	Hunei-17
	B30	T	+	–	3.5	Hunei-18
	B31	T	–	–	0	
	B32	T	+	–	3.4	Hunei-19
	B33	T	–	–	0	

* cultured by DK cell.

Table 5. Virus Isolation from Fish Six Years after the Last Outbreak

Time	Fish	Species	Organ		Titer LogPFU/ml	Designate*
			Intest.	Others		
1978.2	A34	CC	–	–	0	
	A35	CC	+	–	3.1	Hunei-20
	A36	CC	–	–	0	
	A37	T	+	–	2.9	Hunei-21
	A38	T	–	–	0	
	A39	T	–	–	0	
	A40	T	–	–	0	
	A41	T	+	–	3.0	Hunei-22
	A42	T	+	–	3.2	Hunei-23
	A43	T	–	–	0	

* cultured by DK cell.

DISCUSSION

Hawang[6] recovered DHV from liver of many kinds of poults, except pigeon, 7 days after experimental infection. The mortality rate was 100% in guinea fowl, 75% in goose, 60% in phesant, 33.3% in quail and 67% in turkey. No death was observed in chick and muscovy duck. The neutralizing index was 3.0-6.4 in chicken, pheasant, turkey and quail evaluated 21 days postinfection. Asplin and McLauchlan [3] reported that the level of neutralization antibody of experimentally infected chicken was 4.0. These results complied with the present serologic studies. The data recommended that most kinds of poults are susceptible to DHV so that the virus is widespread.

DHV is highly heat-resistant[4,7,11,14,16]. Therefore, the transmission by direct contact or contaminated materials are highly possible. Asplin[4] demonstrated that wild birds might act as mechanic carrier for DHV, but Ulbrich[15] failed to find neutralizing antibody from wild ducks lived in DVH outbreak area. Demakov[6] indicated that brown rat could act as a reservoir of DHV. The problem of reservoir and vector is so complicated that remained to be studied.

The author recovered DHV from intestine of ducks 8-11 days after the first sign noted, but Reuss[12] reported that

Table 6. Duckling and Gosling Inoculation Test with Virus Isolated from Fish

Poults	Age (day)	No. test	Virus	Days postinoculation											Death (%)
				0	1	2	3	4	5	6	7	8	9	10	11-14	
Gosling	4	2	Hunei-3	0	0	0	0	1	0	0	0	0	0	1	–	2 (100%)
Duckling	5	20	Hunei-3	0	0	0	3	4	2	1	0	0	0	0	0	10 (50%)
Duckling	5	20	Hunei-6	0	0	2	5	2	1	1	0	0	0	0	0	11 (55%)

Table 7. Incidence of Gross Lesion of Dead Birds Inoculated with Virs Isolated from Fish

Poults	Virus	Liver			SM	Kidney		Lung Edema
		Enl.	Decol.	HR.		Enl.	HR.	
Gosling	Hunei-3	1/2	2/2	2/2	1/2	0/2	2/2	2/2
Duckling	Hunei-3	7/10	5/10	7/10	9/10	4/10	9/10	6/10
Duckling	Hunei-6	3/11	7/11	7/11	7/11	4/11	7/11	6/11

En. = enlarged, Decol. = decolored, HR. = hemorrhage, SM = splenomegaly.

Table 8. Virus Recoery from Fish Orally Administered with DHV

Exp. No.	Fish No.	Virus	Method	Days passed	Virus titer Log PFU/ml
I	1,2	Hunei-6	Forcefully	0	0
	3,4	Hunei-6	Forcefully	1	0
	5,6	Hunei-6	Forcefully	2	0
	7,8	Hunei-6	Forcefully	3	2.0
	9,10	Hunei-6	Forcefully	4	3.6
	11,12	Hunei-6	Forcefully	5	2.9
	13,14	Hunei-6	Forcefully	6	0
	15,16	Hunei-6	Forcefully	7	0
	17,18	Hunei-6	Forcefully	14	0
II	19,20	I-Lan	Freely	0	0
	21,22	I-Lan	Freely	2	2.5
	23,24	I-Lan	Freely	4	5.9
	25,26	I-Lan	Freely	7	1.9
	27,28	I-Lan	Freely	14	0
	29,30	I-Lan	Freely	28	0

recovered duck may excrete virus in the feces up to 8 weeks after infection. These facts suggested that duck itself may act as a reservoir.

The author succeeded in isolating virus from intestines of common carp and Tilapia fed partly on duck excreta in pond water. Though the viral multiplication in fish body had not been proved, this success suggested the possibility of fish as a reservoir.

Virus could not be recovered from intestines of fish later than 7 days after experimental infection. The problems such as, the periods virus harbored in fish body, the transmission between fish, the transmission from fish to duck, remained to be studied.

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鴨病毒性肝炎之流行病學

呂榮修

1972 年1月至6月，對高雄縣內各種禽類以群爲單位作中和抗體調查，呈現陽性者蛋鴨（菜鴨）100%，土蓄鴨93%，正蓄鴨63%、鵝73%、雞67%、火雞85%。同一期間調查本省 14 縣之鴨群 66 群計 281隻，呈陽性者計 36 群（ 55%）144隻（ 51.2% ）。自然感染耐過鴨之抗體自 11日齡起開始上昇至120日齡仍居高不下。

由病鴨場之魚池之鯉魚及吳郭魚的腸管分離到 23株鴨肝炎病毒，力價爲 $10^{2.7-4.2}$ PFU/ml 。爆發六年後仍可由該魚池之魚分離到病毒。

將由魚群分離之病毒，肌肉注射0.1 ml於 4日～5日齡的小鴨和小鵝，於接種 5日內死亡 50% 以上，屍解呈現典型鴨肝炎病變。

用感染 Hunei-6株之雞胚尿液（ $10^{5.2}$ PFU/ml ）0.1 ml强制口投吳郭魚，投予後3～5日回收病毒力價達 $10^{2.0-3.6}$PFU/ml 投予後第 4日回收病毒力價量高。

用感染 I-Lan（ $10^{7.2}$ EID50/ml）死亡小鴨之腸管（ $10^{5.8}$ PFU/gm ）餵飼吳郭魚，投予後2～7日回收病毒力價達 $10^{1.9-5.9}$PFU/ml ，投予後第 4日回收病毒力價量高。

本研究證明鯉魚、吳郭魚及各種魚類擔當鴨肝炎病毒 Carrier 之角色，使本病之防疫更困難。

台灣省家畜衛生試驗所