

THE OUTBREAK AND CONTROL OF DUCK VIRAL DISEASE IN TAIWAN, 1989-90

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ABSTRACT

An investigation was made into a disease causing up to 90% mortality in 3- to 4-day old ducklings, and up to 50-80% mortality in ducks from one to three weeks of age. Duck Viral Hepatitis virus (DVHV) and Parvovirus were isolated and identified. As an emergency treatment, sera from tolerant ducks were collected, and a dose of 0.5 ml was intramuscularly injected into 332,000 day-old ducklings. To give further protection to the ducklings, breeder ducks were vaccinated with bivalent inactivated vaccine. This emergency measure gave a protective effect of nearly 100%, and successfully prevented further outbreaks of the disease.

INTRODUCTION

The duck industry of Taiwan has grown rapidly over the past two decades. The annual production of meat ducks reached 36.2 million in 1988, which was about 3.6 times the number produced twenty years earlier in 1968 (Chen, 1990). The many rivers and streams of Taiwan, together with the high temperatures and humidity, provide an ideal environment for duck production. Muscovy ducks, Pekin ducks and mule ducks (a sterile, intergeneric cross of Muscovy, Pekin and the Chinese Kaiya breeds) are the major breeds used for meat. Two other breeds, the native Tsaiya and the Kaiya (a cross between Pekin and Tsaiya) are also reared for their eggs, and because of their adaptability to a wide range of environments and their low-fat meat.

During the October of 1989, an acute disease of ducklings with a very high mortality rate was seen for the first time in Taiwan. It was initially observed in Hualien county, on the east coast of the island, after which it was reported from several more counties where acute infections with the disease soon became common. The infected ducklings died rapidly, often showing acute opisthotonos*. Mortality ranged from 50 to 80% among ducks from one to three weeks of age. Surviving ducks showed symptoms of stunted growth and fragile bones. Characteristically, most of the surviving ducks also had an abnormality of the head, with a shortened

upper bill and a protruding tongue (Fig. 1).

There were no typical histopathological lesions in those ducks which had been infected. For this reason, etiological isolation was required for diagnosis. Two virus strains were isolated, and identified as Duck Viral Hepatitis virus (DVHV) and Parvovirus, respectively. Similar clinical symptoms could be reproduced in ducklings experimentally inoculated with the isolates.

In view of the rapid spread of the disease throughout Taiwan, and the tremendous economic losses caused by it, an emergency control program was organized by the Provincial Research Institute for Animal Health (PRIAH) and 15 Livestock Disease Control Centers. Veterinarians who were appointed to take part in the control program in different areas kept in close contact with PRIAH and reported any disease outbreak. Meanwhile, the tolerant sera of surviving ducks and inactivated vaccine were prepared as an emergency control measure. Epizootiological data, and the effectiveness of these control methods against the disease, are discussed below.

EPIZOOTIOLOGICAL DATA

In the first outbreak of duck viral disease, in Hualien, only young ducklings were affected. The younger they were, the more severe were the symptoms. During this first outbreak, adult breeders did



Fig. 1. Duck which has survived duck viral disease, showing the characteristic shortened bill and protruding tongue (normal duck below)

not show any symptoms. Field observations indicated that chickens and turkeys were resistant. The onset and subsequent spread of the disease were very rapid, with death generally occurring within 3-4 days of the first appearance of the symptoms.

As the disease progressed in a flock, the symptoms became more pronounced. Ducks showed droopiness associated with inappetence*, ataxia* and ruffled feathers, and excreted a watery diarrhea. Affected ducklings were unable to stand or stretch their wings, and some would have tremors of the head, neck and body. Birds fell onto their sides, kicked spasmodically with both legs, then died with their heads drawn back. Morbidity was generally almost 100%, although the mortality rate varied according to the location and the age of the birds. Mortality reached 95% in one-week-old ducklings, but fell to 40-80% in birds from one to three weeks of age. There was no difference in symptoms or mortality between different breeds of duck. Ducks which survived the disease were stunted, with shortened bills from which their tongues protruded in a very visible manner. The economic losses in Taiwan from the disease were estimated at close to US\$12 million.

Pathological Findings

No lesions could be distinguished in most of the postmortem examinations. However, some infected ducks in two districts (Taoyuan on the west coast and Ilan on the east) showed haemorrhagic lesions in the liver, pancreas and kidney, while dead or dying ducks in a third district (Changhua, on the west coast) had haemorrhages in the duodenum and round casts in the rectum. Histopathological findings varied from one location to another. In Ilan, hepatocyte degeneration, bile duct hyperplasia and necrotic foci in the spleen were observed. Especially in the spleen, inflammatory cells and reticular endothelial cells were hyperplastic, while there was a decline in the number of matured lymphocytes.

In the Taoyuan cases, the renal tubular epithelium showed vascular degeneration. A considerable amount of crystal columnar renal casts were deposited in the tubules of the kidney, where eosinophiles infiltrated interstitially. The infiltration of eosinophiles was also found in necrotic foci of the limb muscles in which striation was not apparent.

However, in Changhua, the infiltration of inflammatory cells, epithelium necrosis and erosion were typically found in haemorrhagic mucosa of the

small intestine. There were associated *Cryptosporidium* on the cloaca bursa of some birds.

Etiological Examination

Specimens were collected from dead or dying ducks by veterinarians. Bacterial isolates were routinely cultured on Trypticase Soy agar, on Blood agar, or on DHL agar (Difco, U.S.A.). Although several strains of *Staphylococcus aureus* were isolated, those bacterial agents were probably not the major factors causing this infection, as experimental inoculation did not produce disease symptoms. The liver, spleen, intestine, kidney, lung and brain were used for virus isolation. The organs had to be delivered to the laboratory as rapidly as possible, either frozen or in a virus transport medium. Ten per cent (W/V) emulsions of selected organs were prepared in Eagle MEM which contained antibiotics and Fungizone. The emulsions were held at room temperature for approximately 30 to 60 minutes before clarification by centrifugation.

Eight- to ten-day-old embryos of Muscovy and Mule ducks, and SPF chicken eggs, were inoculated with the supernatant by the Amnioallantoic cavity route. The amnioallantoic fluid and dead embryos were harvested aseptically from three to five days after inoculation, and viruses present were identified by means of specific antisera as Duck Viral Hepatitis or Parvovirus, as described by Lu (1981) and Liao (1990).

In addition, primary cultures of chicken and duck embryo fibroblast cells were also used for virus isolation. The fluids from infected cultures were harvested and tested as described above. The isolated viruses were also morphologically confirmed by electron microscope.

A total of 51 virus isolates from 500 individual ducks were obtained in 1989-90 by the inoculation of embryonating eggs and by tissue culture. Of these, 33 isolates were identified as DVHV, 14 as Parvovirus, 2 as Adenovirus (one of the isolates associated with Parvovirus in the same isolation) and 2 as Reovirus (one of the isolates also associated with Parvovirus). The isolated viruses are shown in Fig. 2.

EXPERIMENTAL INOCULATION

Ducklings of mule ducks, Muscovy ducks and Tsaiya ducks aged from one to 16 days were inoculated with either the isolated Parvovirus or

DVHV. Inoculation was intramuscular, or by means of drops in the eyes, nose or mouth. After four to seven days, the ducks inoculated with Parvovirus had weak legs, droopiness and inappetence and were unable to stand. Mortality varied from 0 to 71% (average of 24%). Surviving ducklings were stunted in growth, and 14-71% of them had shortened bills (Table 1). There were no pathological findings correlated with such symptoms when postmortems were carried out on survivors.

Ducklings were experimentally inoculated with the isolated DVHV (Ilan strain) in the same way as with Parvovirus. Symptoms of weak legs, so that the ducklings were unable to stand, and of opisthotonos, appeared 2-3 days after inoculation. Two groups of ducks aged between 13 and 16 days, which had been inoculated orally, were not infected. The other ducks had a mortality rate of 20-80%, with an average of 24% (Table 2). This was a higher rate than found in ducks inoculated with Parvovirus. The postmortem examinations of dead birds showed the typical lesions from hepatic haemorrhages of DVH. The control group had no clinical signs or pathological findings.

Preparation of Tolerated Sera

Surviving ducks were collected from affected farms, and duck sera were prepared as an emergency treatment. Before use, the sera were tested for neutralization titer with DVHV and Parvovirus, respectively. A minimum SN titer of $10^{2.5}$ was established. The method of preparing the antisera followed that described by Lu (1972 and 1985). A total of 332,000 ducklings on 42 breeder farms were inoculated with antisera (0.5 ml each). The ducks treated with antisera resisted infection in the field. This emergency treatment with antisera thus brought about an abrupt halt in the spread of infection.

Development of Mixed Inactivated Vaccine

The Ilan isolate of DVHV and the isolated Parvovirus were used as a seed strain for the preparation of inactivated vaccine. DVHV was multiplied in chicken embryos, and Parvovirus in duck eggs. Viral fluid was prepared from a mixture of amnioallantoic fluid and an emulsion of homogenized dead embryos clarified by centrifugation at 7,000 rpm. The virus fluid of both DVHV ($10^{7.5}$ EID₅₀/ml) and Parvovirus ($10^{7.0}$ TCID₅₀/ml) was inactivated by adding 0.2% (V/V) formalin and

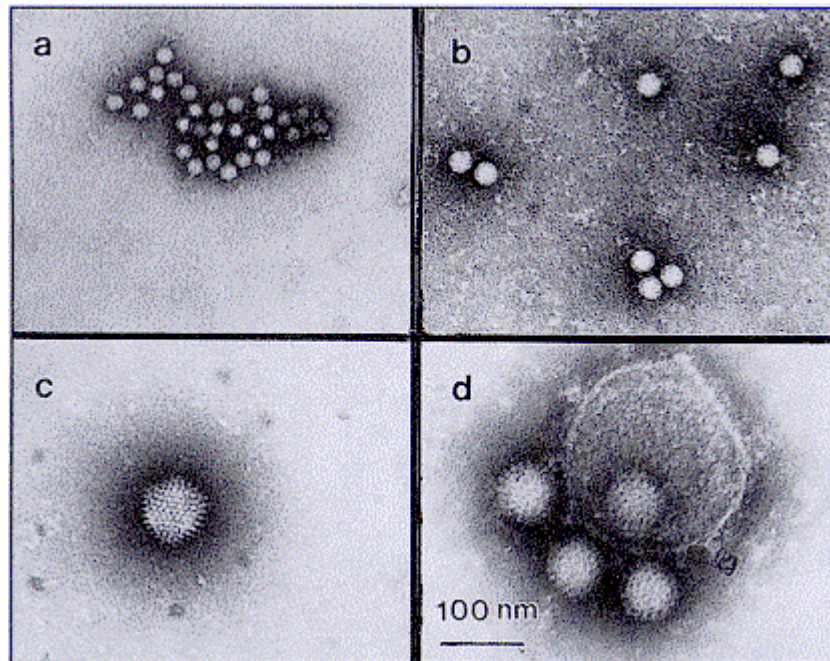


Fig. 2. The isolated viral particles:
 (a) Parvovirus (b) Picornavirus (DVHV)
 (c) Adenovirus (d) Reovirus
 Bar = 100 nm.

maintaining the mixture at 4°C for one week. After inactivation, an equal volume of both viral fluids was combined, mixed with an equal volume of mineral oil adjuvant, and homogenized.

The prepared inactivated vaccine was intramuscularly inoculated into breeder ducks, with a second booster shot four weeks later. A total of 40,150 breeder ducks were vaccinated (Table 3). The ducklings born from vaccinated breeders in contaminated counties were closely observed to evaluate the effect of vaccination. During the experimental period, no such ducklings from infected farms showed any clinical signs of the disease. Breeders immunized with the inactivated vaccine had effectively transferred the maternal antibodies to their ducklings.

CONCLUSION

It seems clear that the disease described in Taiwan's ducks in 1989-90 was caused by DVHV and Parvovirus. The emergency control measures which were carried out effectively stopped the spread of infection, and restored the confidence of duck

farmers. Although live DVH vaccine has been used to control this disease for the past 20 years, some farmers had not continued to vaccinate breeder ducks on their farms because the disease had become rare in recent years. The level of maternal antibodies was therefore too low to protect ducklings from DVHV infection. An attenuated vaccine against Parvovirus (Derzy's disease) was used for geese and Muscovy ducks.

However, the reversion of attenuated DVH vaccine to a virulent strain with the passage of the virus from duck to duck had always been a problem in using live vaccine, as pointed out by Crighton (1978) and Woolcock (1979). Because of the reversion to virulence of live DVH vaccine, it was necessary to look at the preparation and effectiveness of oil emulsion inactivated DVH vaccines. The oil inactivated vaccine was developed by means of the formula described by Gough and Spackman (1981a). The results of this study indicated that vaccination with inactivated vaccine was satisfactory, as measured by serum-neutralization antibody production and the transfer of protection to progeny ducklings. Such a vaccine could be of particular value in areas

Table 1. Inoculation of duck with isolated parvovirus

Breed of duck	Age (days)	No. of ducklings	Inoculation route*	Volume of inoculation (ml)	No. of deaths (Mortality %)	No. of survivors (Survival %)	Syndrome in survivors	
							Stunted	Short bill
Mule	1	7	IM	0.2	5(71)	2(29)	2(100)	1(50)
Muscovy	4	8	IM	0.2	3(37.5)	5(62.5)	5(100)	3(60)]
Muscovy	4	8	IM	0.5	1(12.5)	7(87.5)	6(85.7)	5(71.4)
Mule	10	5	IM	0.5	1(20)	4(80)	4(100)	1(25)
Mule	11	10	IM	0.5	3(30)	7(70)	7(100)	1(14)
Mule	11	10	IN & Eye drops	0.5	0(0)	10(100)	8(80)	2(20)
Muscovy	13	5	IM	0.5	1(20)	4(80)	4(100)	3(75)
Mule	13	10	IM	0.5	4(40)	6(60)	6(100)	2(33.3)
Mule	13	10	Oral	0.5	1(10)	9(90)	9(100)	2(22.2)
Tsaiya	16	10	IM	0.5	1(10)	9(90)	9(100)	2(22.2)
Control								
Mule	1	20			0	20	0	0
Mule	11	15			0	15	0	0
Mule	13	30			0	30	0	0
Muscovy	4	20			0	20	0	0
Muscovy	13	20			0	20	0	0
Tsaiya	16	10			0	10	0	0

* IN: Intranosal
 IM: Intramuscular
 Virus titer: 10^{6.5} EID₅₀/ml

Table 2. Inoculation of ducklings with isolated DVHV

Breed of duck	Age (days)	No. of ducklings	Inoculation route*	Volume of inoculation (ml)	No. of deaths (Mortality %)	No. of survivors (Survival %)	Syndrome in survivors	
							Stunted	Short bill
Mule	2	5	IM	0.2	2 (40)	3 (60)	0	0
Mule	2	5	IM	0.5	4 (80)	1 (20)	0	0
Tsaiya	5	20	IM	0.2	12 (60)	8 (40)	0	0
Tsaiya	5	19	Oral	0.2	12 (63)	7 (37)	0	0
Mule	13	10	IM	0.2	0 (0)	10 (100)	0	0
Mule	13	10	Oral	0.5	0 (0)	10 (100)	0	0
Tsaiya	16	10	IM	0.5	2 (20)	8 (80)	0	0
Tsaiya	16	10	Oral	0.5	0 (0)	10 (100)	0	0
Control								
Mule	2	20			0	20 (100)	0	0
Tsaiya	5	50			0	50 (100)	0	0
Mule	13	30			0	30 (100)	0	0
Tsaiya	16	80			0	80 (100)	0	0

* IM: Intramuscular
Virus titer: $10^{6.5}$ PFU/ml

Table 3. Field test of inactivated vaccine used on breeder ducks

County	No. farms	Breed	No. vaccinated ducks	Volume of vaccine	Safety test	Protection
Yunlin	13	Kaiya	15630	0.5 ml	100%	98%
Ilan	2	Kaiya	2820	0.5 ml	100%	100%
Chiayi	4	Muscovy	10000	0.5 ml	100%	95%
Tainan	1	Tsaiya	11700	0.5 ml	100%	98%
Total	20		40150			

or countries where the disease is epizootic.

When an outbreak of Derzy's disease occurred in goslings in 1983, the attenuated live vaccine was used for breeder geese and Muscovy ducks (Lu *et al.*, 1985). In this year, the infection did not seem to spread to Mule ducks, Tsaiya ducks or Kaiya ducks (Lu *et al.*, 1990). Why did the infection appear in all breeds of ducks and cause such severe losses in 1989-90? Did a different strain of virus cause the outbreak, or was it caused by genetic reversion of the vaccine virus? This question needs further investigation in the future.

Although it has been suggested that it is a nutritional deficiency of Ca, P, Se and Vitamin A which caused the shortened bills of surviving ducks, in fact the levels of Ca, P, Se and Vitamin A in the sera of sick birds were found to be at a normal level. The factor that caused the shortened bills could be replicated by experimental inoculation of ducks with Parvovirus. The small intestine of the infected ducks had abnormal absorption, which might have affected their growth. On the other hand, we did not find the "shortened bill syndrome" after the outbreak of Derzy's disease in 1983. The different pathogenesis of the two outbreaks should receive further study. The shortened bill syndrome should also be considered along with other symptoms, such as the stunting, femoral head necrosis, brittle bones, malabsorption and osteoporosis.

To conclude, etiological identification, histopathological findings and epizootic investigation were all essential factors in making a diagnosis. To retain control of the disease, continuous surveillance for disease outbreaks is necessary, and an effective vaccination program must be a basic part of duck production.

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DISCUSSION

- Q.** You mention that in the field tests, the vaccination gave 100% protection. Were the birds experimentally challenged?
- A.** Yes, this was done. We used the isolated strain to challenge experimental birds.
- Q.** Do you think there was any relation between the outbreak of duck viral disease, and the outbreak of parvovirus in geese ten years earlier?
- A.** We are working now to try and find out whether there is any relationship between the two parvoviruses, by trying to identify the antigen differentiation between goose and duck parvovirus. We used the Western blot method to differentiate the antigenicity between isolated duck and goose parvoviruses.
- Q.** Has any of the parvovirus isolated from ducks been used on geese?
- A.** We have tried using virus isolated from ducks on geese, but the antigens are very different.
- Comment: (Nadzri Bin Salim)
Parvovirus tends to be species specific. The only parvovirus we know that crosses from one species to another is the canine strain which crosses to the feline. It is likely that goose and duck parvovirus are very different.
- Q.** You mentioned that survivors of duck parvovirus have a shortened bill. What is the pathogenesis? Does goose parvovirus have a similar effect on geese?
- A.** Parvovirus in geese does not produce the same syndrome. We don't know the reason for the shortened bill in ducks, and are still working to understand the syndrome. It may be the result of a nutrient deficiency caused by the low absorption of the intestine.
- Q.** How long did the outbreak of duck viral disease last?
- A.** It was first observed in October 1989, and lasted until February 1990 when we began to use the tolerant serum.