Infectious Bill Atrophy Syndrome Caused by Parvovirus in a Co-outbreak with Duck Viral Hepatitis in Ducklings in Taiwan

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SUMMARY. In October 1989, an epizootic duckling disease with high mortality occurred in Taiwan. The disease was characterized by droopiness, inappetence, ataxia, ruffled feathers, and watery diarrhea. Affected ducklings were lame, were unable to stand, showed opisthotonos, and often died 3 or 4 days after the onset of the disease. Tolerant maturing ducklings displayed atrophic upper bills with a protruding tongue and became stunted as they reached maturity. No diagnostic histopathologic lesions were found in these ducklings. Fourteen parvovirus isolates, 33 duck viral hepatitis virus (DVHV) isolates, two adenovirus isolates, and two reovirus isolates were obtained and identified from more than 500 sick ducklings in the epizootic. The epizootic was diagnosed as a co-outbreak of duck parvovirus infections and duck viral hepatitis. The high mortality in ducklings and the bill atrophy syndrome were reproduced in ducklings by inoculating the parvovirus isolates alone. The epizootic was controlled by an emergency immunization program of ducklings with sera collected from recovered ducks or a bivalent inactivated vaccine composed of local DVHV and parvovirus isolates.

RESUMEN. *Reporte de Caso*-Sindrome de la atrofia infecciosa del pico causado por parvovirus durante un brote simultameo con el virus de la hepatitis de los patos en patios de Taiwan.

En Octubre de 1989 ocurrio una epizootia con alta mortalidad en patitos en Taiwan. La enfermedad fue caracterizada por letargia, inapetencia, ataxia, plumas erizadas y diarrea acuosa. Los patitos afectados estaban cojos, eran ibcapaces de pararse, mostraban opistotonos y morian 3 o 4 dias despues de la iniciacion del brote. patos adultos sobrevivientes mostraban atrofia del pico superior, con la lengua salida y con enanismo. No se observaron lesiones histopatologicas diagnosticas en estos patis. A partir de mas de 500 muestras obtenidas de patitos enfermos, se aislaron 14 cepas de parvovirus, 33 cepas del virus de la hepatitis de los patos, dos cepas de adenovirus y dos cepas de reovirus. La epizootia fue diagnosticada como un brote simultaneo de infecciones por parvovirus y hepatitis viral de los patos. La alta mortaalidad de los patitos y el sindrome de la atrofia del pico fueron reproducidos en patitos mediante la inoculacion de los parvovirus solos. La epizootia fue controlada mediante un programa de emergencia inmunizando los patitos con suero obtenido de patos recuperados o mediante la administracion de una vacuna inactivada bivalante que contenia cepas locales del virus de la hepatitis de los patos y vatias cepas de parvovirus.

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Parvovirus has been foud to cause only silent infection in chickens [2,4,5] and turkeys [10]; in geese and Muscovy ducks [3], it causes Derzsy's disease, or goose parvovirus infection. Parvovirus disease only rarely causes other disease problems in poultry.

In October 1989, a new acute duckling disease causing great losses occurred in Taiwan. The disease had a very high morbidity and mortality, and infected ducklings died rapidly, often showing acute opisthotonos. Surviving ducklings had short upper bills and became stunted as they reached maturity. Parvovirus, duck viral hepatitis virus (DVHV), adenovirus, and reovirus were simultaneously isolated and identified in this outbreak. The purpose of the present report is to describe the epizootiological, pathological, and etiological studies; the experimental animal inoculation; and the emergency treatment of the disease during this epizootic.

CASE REPORT

Case history.

In October 1989, an acute disease with high morbidity and mortality was first found in ducklings in Hualien County, northeast Taiwan. The disease soon spread over the whole island. A total of 177 duck farms, mainly concentrated in nine counties, were found to be affected with the epizootic by an island-wide investigation in March 1990. Only young ducklings were affected. The resistance in ducklings against the disease increased with age. Adult breeders became fully resistant and showed no signs during the epizootic. No chickens, turkeys, geese, or other poultry were found affected with the disease. The onset and subsequent spread of the disease was very rapid, with death generally occurring within 3 or 4 days after the first appearance of the symptoms.

Affected ducklings showed listlessness, inappetence, ataxia, ruffled feathers, and watery diarrhea. Most of the ducklings were lame or unable to stand. Some ducklings had tremors of the head, neck, and body. Birds often fell on the floor on one side, kicked spasmodically with both legs, and finally died with their heads drawn back.

Morbidity and mortality rates in the islandwide epizootic were 24.7 % (446,300 /1,803,266) and 67.7 % (302,350/446,300), respectively. Morbidity and mortality varied with the age of the birds. Mortality was about 90 % in ducklings of 3 or 4 days of age and 50-80 % in ducklings of 1 to 3 weeks of age. All breeds of ducklings (e.g., Muscovy ducks, pekin ducks, mule ducks [a sterile, intergenetic cross of Muscovy, pekin, and the Chinese kaiya breeds], native tsaiya ducks, and the Kaiya ducks [a cross between pekin and tsaiya ducks]) were affected and showed no differences in signs and severity. Ducklings that survived the acute disease displayed atrophic bills with protruding tongues and stunted growth (Fig. 1).

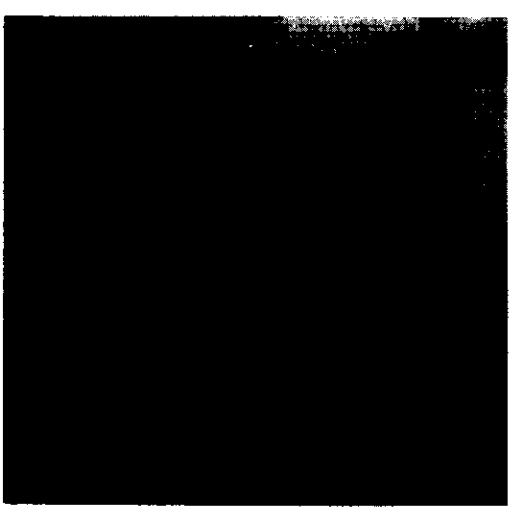

Fig. 1. The upper duckling survived the parvovirus infection with the characteristic atrophic bill and protruding tongue. The lower duckling is normal.

Gross findings.

Gross lesions were found to vary by different locations. Some infected ducks in two districts (Taoyuan and Ilan counties) had hemorrhagic lesions in the liver, pancreas, and kidney. Dead or dying ducklings in Changhua

County had hemorrhagic foci in the duodenum and round casts in the rectum. According to Dr. M. Y. Lin of National Pintung Polytechnic Institute, the cases in Taiwan, Kaohsiung, and Pintung counties had necrotic foci on the bill surface and moderate encephalitis.

Histopathological findings.

The lesions varied from one location to another. In the cases from Ilan County, hepatocyte degeneration, bile duct proliferation, and necrotic foci in the spleens were observed. In the spleen, the number of inflammatory cells and reticular endothelial cells was highly increased, while mature lymphocytes were decreased. In the cases from Taoyuan County, the epithelial degeneration was seen in the renal tubule. A considerable number of columnar crystal casts were found deposited in the renal tubules. Granular leukocytes were found infiltrating the renal interstitial space and the necrotic foci of the limb muscles. Striations of the skeletal muscles were not apparent. In the cases from Changhua County, infiltration of inflammatory cells, epithelial necrosis, and erosion were mainly found in hemorrhagic foci of the small intestine. *Cryptosporidia* were sometimes found on the mucosal surface of the bursa of Fabricius.

Microbiological findings.

More than 500 sick ducklings from different areas were submitted to this laboratory for etiologic study. Tryptic soy agar, blood agar, and desoxycholate hydrogen-sulfide lactose (DHL) agar were used for bacterial isolation. In some cases, *Staphylococcus aureus* was isolated. Duck embryos and primary duck kidney cell cultures were employed for virus isolation.

A total of 51 viruses were isolated, Transmissible electron microscopic (Fig. 2), biological, and immunological examinations identified 14 as parvoviruses, 33 as picornaviruses, two as adenoviruses (one associated with a parvovirus isolation), and two as reoviruses (one also associated with a parvovirus isolation). The duck parvovirus isolates multiplied in duck or goose embryos but not in chicken embryos. They also grew on duck or goose embryo fibroblasts or kidney cell cultures and caused cytopathic effects. The nucleic acid type of the parvovirus was determined to be DNA by the reduction in virus titer of log 6.1-6.8 in the presence of 5'-iodo-2-deoxyuridine (IUdR; Sigma Chemical Co., St. Louis, Missouri) in cell culture. Cell cultures infected with duck parvovirus had an intranuclear immunofluorescence reaction by indirect immunofluorescent staining using a hyperimmune serum against Derzsy's prepared as described [8]. The hyperimmune serum had a neutralization titer of $10^{3.8}$ against Derzsy's disease virus (isolate 90483) and a titer of $10^{3.5}$ against duck parvovirus isolated in this epizootic (isolate 902193).

Experimental inoculation.

One picornavirus isolate (Ilan isolate) was inoculated into eight groups of ducklings by intramuscular or oral routes. The inoculated dose for each duckling was 0.2 ml or 0.5 ml virus suspension containing $10^{6.5}$ plque-forming units (PFU)/ml. Each experimental group contained five to 20 ducklings of different ages, ranging fron 2 to 16 days (Table 1). Four groups of ducklings of the same ages served as controls. Each control group contained 20 to 80 ducklings. Two or 3 days later, the ducklings inoculated with picornavirus had the following symptoms: weakness, opisthotonos, torticollis, prostration, and disseminated hemorrhagic foci. They died rapidly from duck viral hepatitis (DVH); Table 1 shows mortality rates. Surviving ducklings did not become stunted or display atrophic bills. None of the controls died, were stunted, or displayed atrophic bills.

Ten groups of ducklings were individually inoculated with one of three parvovirus isolates (isolates 41, 902193, and 902153) via intramuscular, intraocular, or oral routes (Table 2). Each duckling was inoculated with 0.2 ml of virus suspension containing $10^{6.5}$ mean embryo infective dose (EID_{50})/ml. Six groups of ducklings ranging from 1 to 16 days of age served as controls. Each control group contianed 10 to 30 ducklings. Ducklings inoculated with parvovorus showed weakness, anorexia, inability to stand, and high mortality. Most of the surviving ducklings became stunted, and 14 % to 75 % had atrophic bills similar to those observed in the field cases (Table 2). None of the controls died, were stunted, or displayed atrophic bills.

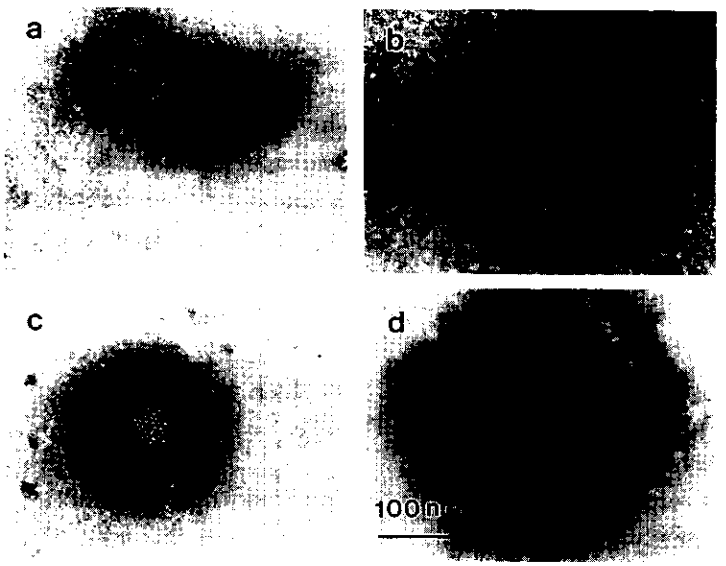

Fig. 2. The isolated viral particles:a) Parvovirus,b) Picornavirus (DVHV),c) Adenovirus,d)Reovirus. Bar = 100 nm.

Table 1 Pathogenicity of duck viral hepatitis virus isolate in ducklings.[A]

Breed	Age at inoculation (days)	Inoculation		No. dead/ total inoculated (%)
		Route	Volume	
Mule	2	IM	0.2	2 / 5 (40)
Mule	2	IM	0.5	4 / 5 (80)
Tsaiya	5	IM	0.2	12 / 20 (60)
Tsaiya	5	Oral	0.2	12 / 19 (63)
Mule	13	IM	0.2	0 / 10 (0)
Mule	13	Oral	0.5	0 / 10 (0)
Tsaiya	16	IM	0.5	2 / 10 (20)
Tsaiya	16	Oral	0.5	0 / 10 (0)

[A] Ducklings were inoculated with Ilan isolate of picornavirus (DVHV). No atrophic bills were observed in surviving ducklings.
[B] IM = intramuscular. Each ml of virus suspension contained $10^{6.5}$ plaque-forming units of picornavirus.

Twenty-one specific-pathogen-free (SPF) Pekin ducklings (provided by the Chemo-Sero-Therapeutic Research Institute, Kumsmoto, Japan) were allotted into four groups; each group was raised in separate isolation units with filtered air under positive pressure. Ducklings in each group were then inoculated intramuscularly at 3 days of age with three parvovirus isolates (Table 3). Among them, isolate 861015 was isolated from Muscovy ducks and isolate 90483 was isolated from geese infected with Derzsy's disease. The fourth group served as a control group. Four weeks after inoculation, body weights and bill lengths were measured. Only isolate 902193 caused atrophic bills and stunting (Table 3).

Emergency treatment.

One-half ml of the convalescent duck sera collected from the affected farms with neutralization titers against DVHV over $10^{2.5}$ mean tissue-culture-infectious dose ($TCID_{50}$)/ml was injected subcutaneously into each of 400,000 ducklings. Later, an inactivated bivalent vaccine composed of local parvovirus and DVHV isolates was developed, and more than 330,000 ducklings were immunized. This emergency measure gave a protective effect of nearly 100 % and prevented further outbreaks.

DISCUSSION

The cmplicated etiologic nature of the outbreak made it difficult to give a complete diagnosis at the first stage of the epizoptic in

Table 2 Pathogenicity of parvovirus isolates in ducklings.

Breed	Age at inoculation (days)	Inoculatijon		No. dead/ total inoculated (%)	Syndrome in survivors	
		Route	Isolate		Stunting	Bill atrophy
Mule	1	IM	41	5 / 7 (71)	2 / 2 (100)	1 / 2 (50)
Muscovy	4	IM	902193	3 / 8 (38)	5 / 5 (100)	3 / 5 (60)
Muscovy	4	IM	902193	1 / 8 (13)	6 / 7 (71)	5 / 7 (71)
Mule	10	IM	902193	1 / 5 (20)	4 / 4 (100)	1 / 4 (25)
Mule	11	IM	41	3 / 10 (30)	7 / 7 (100)	1 / 7 (14)
Mule	11	IN & IO	41	0 / 10 (0)	8 / 10 (80)	2 / 10 (20)
Muscovy	13	IM	902153	1 / 5 (20)	4 / 4 (100)	3 / 4 (75)
Mule	13	IM	902193	4 / 10 (40)	6 / 6 (100)	2 / 6 (33)
Mule	13	Oral	902193	1 / 10 (10)	9 / 9 (100)	2 / 9 (22)
Tsaiya	16	IM	902153	1 / 10 (10)	9 / 9 (100)	2 / 9 (22)

[A] Each duckling was inoculated with 0.2 ml of virus suspension ($10^{6.5}$ EID_{60} /ml). IM = intramuscular, IN = intranasal, IO = intraocular.

[B] No. showing signs/total examined (%).

Table 3 Pathogenicity of three parvovirus isolates in SPF ducklings.[A]

Inoculated isolate	Bird No.	Body weight		Length of bill	
		kg	Mean ± SD	cm	Mean ± SD
902193	1	0.30*		3.1*	
	2	0.30*		3.2*	
	3	0.35		3.6*	
	4	0.35		3.6*	
	5	0.30*		3.5*	
	6	0.40	0.34 ± 0.04	4.0	3.6 ± 0.35
861015	1	0.40		4.8	
	2	0.50		4.8	
	3	0.50		4.8	
	4	0.45		4.7	
	5	1.40	0.44 ± 0.05	4.5	4.7 ± 0.13
90483	1	0.70		4.8	
	2	0.70		4.8	
	3	0.50		5.1	
	4	0.40		4.7	
	5	0.40	0.52 ± 0.15	4.8	4.8 ± 0.14
Control	1	0.50		4.5	
	2	0.45		4.8	
	3	0.50		4.5	
	4	0.40		4.2	
	5	0.40	0.44 ± 0.05	4.2	4.4 ± 0.24

[A] Values marked by asterisks indicate stunting and bill atrophy, as indicated by body weights and bill lengths smaller than the mean of the control group minus two standard deviations.

1989. After through pathological and microbiological studies of more than 500 affected ducklings distributed throughout Taiwan, the epizootic was diagnosed as a mixed infection of parvovirus and DVHV. The diagnosis was based on the frequency of the virus isolation from field cases and the reproducibility of the clinical signs and lesions in experimentally inoculated ducklings. It was also proven that parvovirus alone could cause high mortality and bill atrophy in ducklings. To our knowledge, this is the first report on the clinical disease caused by parvovirus infection in ducks including breeds other than Muscovy duck.

In 1970-71, there was a major outbreak of DVH in Taiwan [6,7]. After an emergency treatment with immune sera and subsequent use of live DVH vaccine for breeders, the disease was well controlled [7]. The possibility of the reversion of the attenuated vaccine strain to a virulent strain in the field has been reported [1,11]. However, we speculated that relaxation of the vacccination program may be the main cause of the epizootic in 1989. Since field cases of DVH in Taiwan have been rarely reported in recent years, some farmers might have forgotten or were reluctant to vaccinate breedrs. This eventually results in insufficuently low maternal antibody to protect ducklings from virulent DVHV infection. It is not yet known whether the occurrence of DVHV and duck parvovirus infection at the same time was coincidental or whether there was some synergistic effect between these two viruses.

During an outbreak of Derzsy's disease in goslings and Muscovy ducks in Taiwan in 1982, no other breeds of ducks were affected [8]. Surviving birds showed no bill atrophy. In the 1989 epizootic, all breeds of ducks were affected, but geese were spared. The observation of the involvement of all breeds of ducks in the 1989 epizootic was confirmed by the results of our experimental inoculation (Tables 2,3). Thus, the host range and pathogenicity of the parvovirus isolated in the 1982 epizootic and in the 1989 epizootic are different. Still, the duck parvovirus had some similarity in antigenicity with goose parvovirus by showing cross-reaction in an indirect immunofluorescence test and in the neutralization test. Further investigations of the virus are needed to determine the relationships between the parvoviruses isolated in the two epizootics.

Although atrophic bills could be reproduced in ducklings experimentally inoculated with parvovirus, the pathogenesis of the bill atrophy is not quite clear. Shortened upper bill has been described in the Ammi majus-induced photosensitization of ducks [9]. However, we did not see the photosenitization lesions in ducklings with atrophic bills in the 1989 epizootic. Some field cases in the south of Taiwan had necrotic foci on the surface of the upper bill that could have caused degeneration and atrophy of the cells in the upper bill, leading to tissue shrinkage.

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台灣鴨小病毒性短啄症及鴨病毒性肝炎共同爆發流行病

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摘要 1989 年 10 月台灣發生幼齡鴨高死亡率之流行病，感染鴨精神萎靡，食慾不振，運動失調，羽毛膨鬆雜亂無章，並有水樣下痢，病鴨跛行，或無法站立，並呈角弓反張現象，常在發病後 3～4 天內死亡。耐過鴨發育遲滯，達性成熟時鴨隻常有上喙萎縮，呈舌頭突出狀。病死鴨未發現有具診斷性之組織病理學病變。在本次大流行期間共收集 500 隻以上病鴨進行病原分離,結果分離到 14 株小病毒，33 株鴨病毒性肝炎病毒，2 株腺病毒及 2 株里奧病毒。本次大流行經診斷爲由鴨小病毒及鴨病毒性肝炎所共同引起。分離之鴨小病毒經人工感染幼齡鴨可引起高死亡率及短喙症經以耐過鴨血清及由本所分離之鴨小病毒及鴨小病毒及鴨病毒性肝炎病毒所製成之雙價不活化疫苗，而能成功的控制住此次大流行。