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RESPONSE OF PEKIN AND MALLARD DUCKS AND CANADA GEESE TO EXPERIMENTAL INFECTION WITH DUCK PLAGUE VIRUS*

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Abstract

Studies show that duck plague virus elicits comparable clinical and pathologic responses in domestic White Pekin ducks *(Anas platyrhynchos domesticus)*, wild mallard ducks *(Anas platyrhynchos)*, and Canada geese *(Branta canadensis)*. All these birds were infected by oral inoculation or contact exposure. The antibody response was similar in both duck species. Clinical signs were ruffled feathers, increased lachrymation, nasal secretion, diarrhea, and reddening of the cloacal mucosae. The mortality rate was high in Pekin ducks and Canada geese, while mallard ducks were more resistant. Serums from birds that survived infection had a relatively high antibody level. The pathologic changes were qualitatively comparable in all three waterfowl species and were characterized by generalized congestion, hemorrhages in the serous and mucous membranes and necrosis of the gastrointestinal mucosae as well as the liver. Intranuclear inclusion bodies having staining characteristics of DNA viruses were found in the hepatic cells and epithelium of the esophageal and cloacal mucosae. These inclusions and other pathologic alterations might be valuable in diagnosing duck plague in wild waterfowl.

Duck plague was identified by Jansen [5,6,7,8,9,10] as a specific viral infection in Holland. This disease was suspected to exist in France[14] and China,[11] and its presence was confirmed in Belgium[4] and India.[15] An outbreak of duck plague occurred in 1967 on Long Island, New York, in an area of heavily concentrated, duck-producing farms.[12] The causative agent was isolated and identified by reciprocal serologic neutralization tests, using embryonating duck eggs as well as ducklings. The agent was a virus bearing relationship to the isolate from the Netherlands.[1,2,3]

This report describes the clinical, serologic, and pathologic responses of 3-week-old domestic White Pekin and mallard ducks and mature Canada geese to experimental infection with duck plague virus.

*The Long Island duck disease was designated duck virus enteritis by New York State and ARS, USDA, regulatory officials [9-Code of Federal Regulations—Part 83—duck virus enteritis (duck plague)].

Materials and Methods

Viruses

Virulent duck plague virus (LIDPV), isolated from ducks that died during the 1967 outbreak on a commercial duck farm on Long Island, New York, U.S.A., was used for challenge inoculation of Pekin ducklings and other waterfowl employed in these studies.[2]

A duck-plague chicken-embryo-adapted virus (DPAV), which originated in the Netherlands, was used as antigen in virus-serum neutralization tests.[7]

Waterfowl

Three - week - old White Pekin *(Anas platyrhynchos domesticus)* and 3-week-old mallard ducklings *(Anas platyrhynchos)* were used. Ten of each species were infected and 4 were maintained in contact as non-infected controls. Of 10 adult Canada geese *(Branta canadensis)*, 7 were infected. The ducks were housed together in one room and the geese in another.[2] All were fed commercial pellets and observed several times daily for clinical signs and disease course.

Virus inoculation

Each bird was given oral inoculation of 10^4 duck embryo 50% lethal dose ($DELD_{50}$) of LIDPV. The ducks were observed for 38 days. On the 5th day postinoculation (DPI), the degree of redness or congestion of the anal lips was scored from 0 to 5. A score of 5 signified a deep cherry-red color and appearance of inflammatory spots covered with white or yellow fibrinous exudate on the surfaces.

Sampling for virus recovery

At 7 DPI, one cotton swab was inserted into the esophagus and another into the cloaca; each was rubbed vigorously 10 times against the respective mucous membranes. Then, each swab was immersed and shaken well in 3 ml of a diluent consisting of PBS containing the following concentrations of antibiotics per ml: penicillin (1,000 units), streptomycin (1,000 mcg.), mycostatin (100 units), polymyxin (100 units), and kanamycin (50 units). The swab suspensions were incubated for 20 minutes at 37°C and centrifuged at 800 X g for 10 minutes. The supernatant fluid was removed and stored at —20°C until inoculation of duck or chicken embryos. Blood samples were collected from the birds by intracardial puncture 7, 24, and 38 DPI. Specimens from livers were obtained from the birds at necropsy. The blood obtained 7 DPI was mixed in 500 units of heparin/ml and then diluted in equal amounts of diluent. A 20% w/v suspension was made from the liver specimens and, following centrifugation at 800 X g for 10 minutes, the supernatant fluid was collected and stored at —20°C until used for testing.

Virus isolation and identification

Attempts were made to isolate virus from the collected blood samples. Amounts of 0.4 ml from each sample were inoculated in each of five 9- to 10-day-old embryonated duck eggs *via* the chorioallantoic membrane (CAM). A 0.2-ml amount of each sample was also inoculated in each of five 8- to 9-day-old embryonated chicken eggs *via* the chorioallantoic sac (CAS). The duck and chicken embryos were observed for 14 and 10 days, respectively. Upon death, the duck embryos were examined and a 20% suspension was prepared from the CAM membranes as described for the liver suspension. The supernatant fluid was used as an antigen in neutralization tests with duck plague hyperimmune serum. For identification of the isolates from the CAM, dilutions of supernatant fluids were mixed with equal amounts of 1:5 dilution of duck plague hyperimmune serum. Each of the serum-virus mixtures was inoculated in embryonated duck eggs and treated as described above for virus isolation. A virus neutralization index (VNI) of 2 $\log_{10}$ $DELD_{50}$ or more was considered positive.[2]

Virus neutralization tests

The serums collected from the coagulated blood specimens were heated for 30 minutes at 56°C before use. The "constant serum-variable virus" method was used to determine the VNI. An

initial dilution of 1:5 (1 part serum and 4 parts diluent) was made. Serial 5-fold dilutions of viral antigens were prepared in diluent, mixed with equal amounts of the diluted serums and incubated at 37°C for 30 minutes. Positive and negative control serums were diluted, mixed, and incubated in the same manner. The serum-virus mixtures were inoculated in 8- to 9-day-old chicken-embryonated eggs *via* the CAS, using 0.2-ml amounts of serum-virus mixtures. They were examined daily for 10 days and the ELD_{50} VNI determined.

Control serums

Normal serums were obtained from mature White Pekin ducks which had not been exposed to duck virus hepatitis. Duck plague hyperimmune serum was collected from similar ducks which had recovered from experimental infection and withstood repeated challenge inoculations with virulent virus. The VNI of the undiluted immune serum was 10^5 50% chicken-embryo lethal doses ($CELD_{50}$)/ml. Endpoints were calculated according to the method of Reed and Muench.[10]

Histologic techniques

Birds were necropsied approximately 2 to 8 hours after death, and specimens were collected from the esophagus, proventriculus, gizzard, small and large intestines, ceca, cloaca, heart, lungs, pancreas, spleen, liver, and kidneys. They were fixed in 10% neutral formalin, embedded according to the conventional technique, sectioned at 6 μ, and routinely stained with hematoxylin and eosin. The Giemsa, Feulgen, methyl green-pyronin and other stains, when required, were used according the methods of Lillie.[13]

Results

White Pekin Ducks

The clinical course: Of the ten 3-week-old ducklings inoculated with duck plague virus, 2, 1, 1, and 1 died 5, 7, 11, and 13 DPI, respectively. Of the 4 contact, noninoculated ducklings, 1 died 13, another 17 DPI. Two to 5 days before death, the ducklings had congested, watery eyes. As lachrymation increased, a circle of wet feathers formed around the eyes.

The ducks assumed a sitting position and had difficulty in moving. They crept with extended wings to reach the water and feed troughs. When the cloacae of the infected ducks were examined 5 DPI, 7 of the ducks that had been given virus orally had various degrees of reddening but the cloacal mucosae of the contact ducks appeared normal.

Virus isolation using duck embryonated eggs: Virus was recovered from the esophageal swab samples taken from 8 of the orally-infected birds. Similarly, virus was recovered from the cloacae of 8 birds, but it was isolated from the blood of 5 birds only. Virus was not recovered from any samples collected from one bird. The virus was present in the esophageal, cloacal, and blood samples of the 2 birds that died 5 DPI, and also in those that died 7 and 13 DPI. Blood taken 7 DPI from the bird that died 11 DPI contained no virus, but virus was found in the samples from the esophagus and cloaca. No virus could be found in samples taken from contact-control ducklings (Table 1). The respective specimens produced no response upon inoculation into chicken-embryonated eggs.

Serological response: The antibody level in the preinoculation blood samples ranged from negative to 0.5 (Table 1). In the ducklings that survived inoculation, there was a slight rise in the antibody level 7 DPI. However, there was a marked rise in the antibody level 24 DPI, and a further increase was noticed 38 DPI. Seven, 24, and 38 DPI the VNI range was negative to 1.25, 2.0 to 3.75, and 3.25 to 4.0, respectively. This pattern of antibody response was common to both the orally-infected and contact-control fowl.

Gross lesions: The esophageal mucosa was covered with yellowish, elongated pseudomembranes. Upon their removal, the mucosa had multiple, linearly arranged, petechial hemorrhages extending from the upper portion of the esophagus down through its transition into the proventriculus that was filled with thick

TABLE 1. *Results of Experimental Infection of 3-Week-Old White Pekin Ducks with Duck Plague Virus*

Duck	Mortality	Intensity* of cloacal congestion	Virus isolation			Virus neutralization index** DPI			
No.	(DPI)	5 DPI	B	E	C	0	7	24	38
Infected orally:									
212	5	3	+	+	+	NA			
213	5	4	+	+	+	NA			
214		0	—	+	+	0.25	0.25	2.0	3.25
215		0	—	—	+	0.5	1.25	2.75	3.75
216	11	2	—	+	+	0.25	0.25		
228		0	+	+	—	0.5	0.75	3.0	4.0
229	7	2	+	+	+	NA			
230		0	—	—	—	NA	NA	3.75	4.0
232		2	—	+	+	0.25	1.0	3.0	4.0
233	13	2	+	+	+	NA	0.5		
Contact:									
214		0	—	—	—	NA	0.25	2.0	3.25
217		0	—	—	—	0.5	0.75	2.5	4.0
231	17	0	—	—	—	0.25	0.25		
234	13	0	—	—	—	0.5	0.75		

*Range of intensity of congestion: none (0) to severe (5).
**Log 10
B = blood; E = esophagus; C = cloaca
NA = no detectable antibody
+ = virus isolated
— = no virus isolated
DPI = days postinoculation

sanguinous mucous. The small intestines had dark red, circular bands that were translucent through the serosa. When examined from the mucosal surface, these bands were found as confluent, slightly elevated, circular hemorrhages. These bands occurred in several portions of the small intestines alternating with apparently normal sections of the intestinal tract. The contents of the ceca and large intestines were soft and bloody. The cloacal mucosa was dark red and overlayered with elongated, grey, cheesy pseudomembranes, sometimes 2.5 cm long. The pancreas and kidneys had minute petechial hemorrhages. Most livers appeared normal, except one that manifested bright, paint-brush-like hemorrhages. Petechiae in the heart were common. *Pasteurella* organisms were isolated from the livers and the cardiac blood. The incidence of gross lesions in the Pekin and mallard ducklings is summarized (Table 2).

Microscopic changes: Histologic examination of the formalin-fixed specimens showed that the lesions associated with duck plague virus infection were localized primarily in the digestive tract. While there were variations among the birds in the extent of the lesions, the main qualitative characteristics appeared to be generalized congestion, accompanied by the petechiae and extravasations; the degeneration and necrosis of the epithelial cells of the gastrointestinal mucosa; and the occurrence of the intranuclear inclusion bodies in the degenerating epithelial cells. The typical microscopic changes found in the Pekin ducks usually were most pronounced in the esophagus, cloaca, and liver.

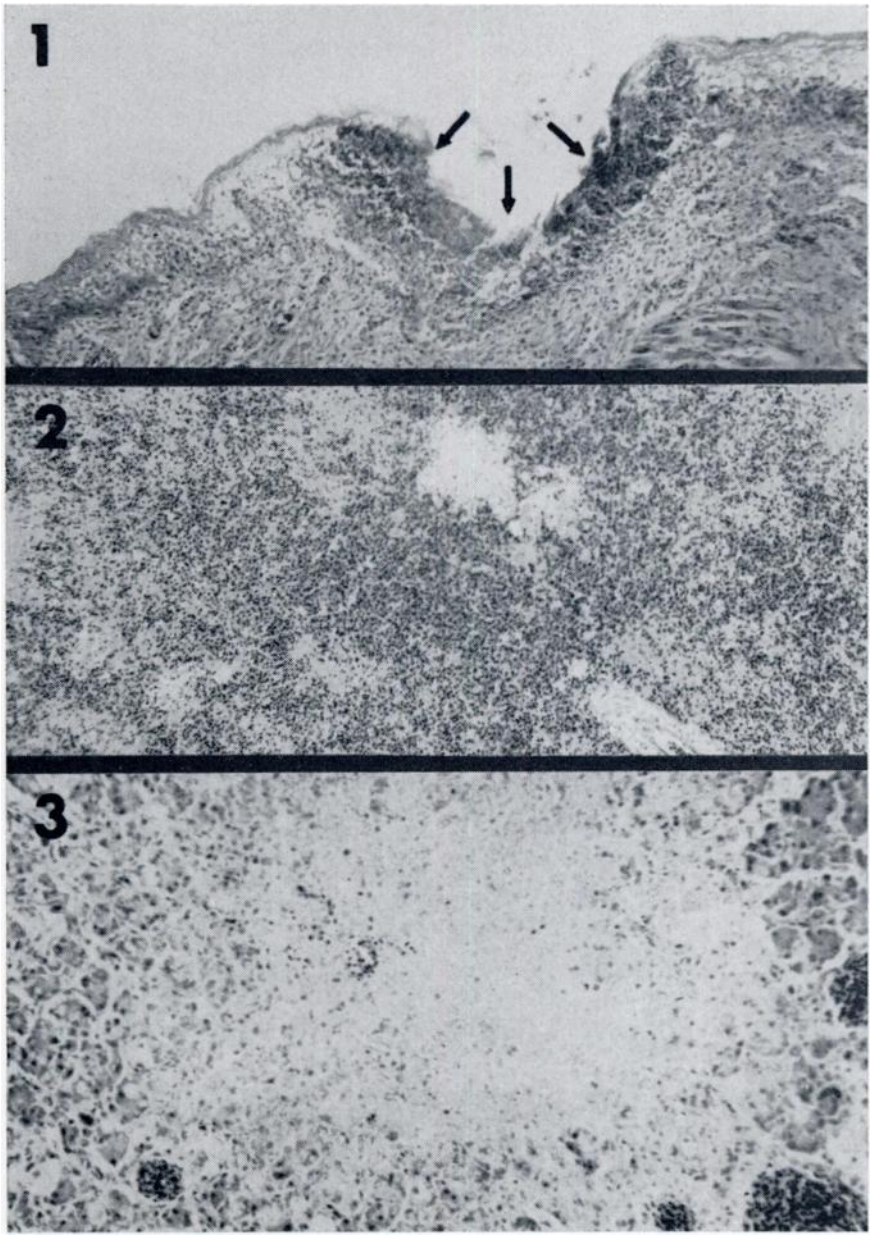

FIGURE 1. *Erosion (arrows) in the cloaca of a Pekin duck infested with duck plague virus. H & E stain. X 122.*

FIGURE 2. *Focal disseminated necrosis in the spleen of a mallard duck. H & E stain. X 200.*

FIGURE 3. *Large focus of coagulative necrosis in the pancreas of a mallard duck. H & E stain. X 200.*

TABLE 2. *Incidence of Gross Duck Plague Lesions in 3-Week-Old Pekin and Mallard Ducklings*

Duck No.	Death (DPI)	Esophagus	Proventriculus	Liver	Intestines	Cloaca
White Pekins Inoculated:						
212	5	+	+	+	+	+
213	5	+	+	+	+	+
216*	11	+	+	+	+	+
229	7	+	+	+	+	+
233	13	+	0	0	+	+
Contact:						
231	17	+	+	+	+	+
234	13	+	+	+	+	+
Mallards Inoculated:						
219	7	+	+	+	+	+
221	7	+	+	+	+	+
Contact:						
236	14	+	+	+	+	+

DPI = days postinoculation
* Fowl-cholera-like lesions
+ = Characteristic macroscopic lesions
0 = No mascroscopic lesions

In most ducks, there was a severe epithelial necrosis in both esophagus and cloaca, resulting in deep erosions (Fig. 1.) Portions of the detached necrotic epithelium, sometimes invaded by rod-shaped microorganisms, were seen in the lumen. Most of the mucous glands in the propria of the esophagus were in a state of coagulative necrosis. There was hyperemia, edema, and heterophilic infiltration of the propria and muscularis. Subserous hemorrhages and edema were prominent.

In the liver, the extent of changes varied considerably between different specimens, but usually they consisted of irregular foci of necrosis, congestion of the venous vessels and extravasation. Diffuse vacuolization of the hepatic cells was not uncommon. The necrotic foci tended to localize in the periportal areas and ranged in size from several cells to areas approximating a liver lobule. They contained a moderate number of heterophils.

The changes in the proventriculus, gizzard, small intestine, ceca, and large intestine involved variable degrees of necrosis of tubular glands and villi. Hemorrhages and edema in the propria and muscularis occurred in most instances. Spleen and pancreas were hemorrhagic.

Intranuclear inclusions bodies were found in the hepatic and epithelial cells of the esophagus and cloaca. The sites of predilection for these inclusions seemed to be the edges of the necrotic foci in the liver and the sloughed-off mucosal cells in the esophagus and cloaca. They were relatively numerous in the hepatic parenchyma; as many as 4 or 5 could be observed in a high-dry vision field of liver sections (Fig. 4).

Morphologically, the inclusion bodies appeared as granular structures of variable shape and size, the majority tending to be circular or eliptical, with fairly smooth borders. A clear halo was ob-

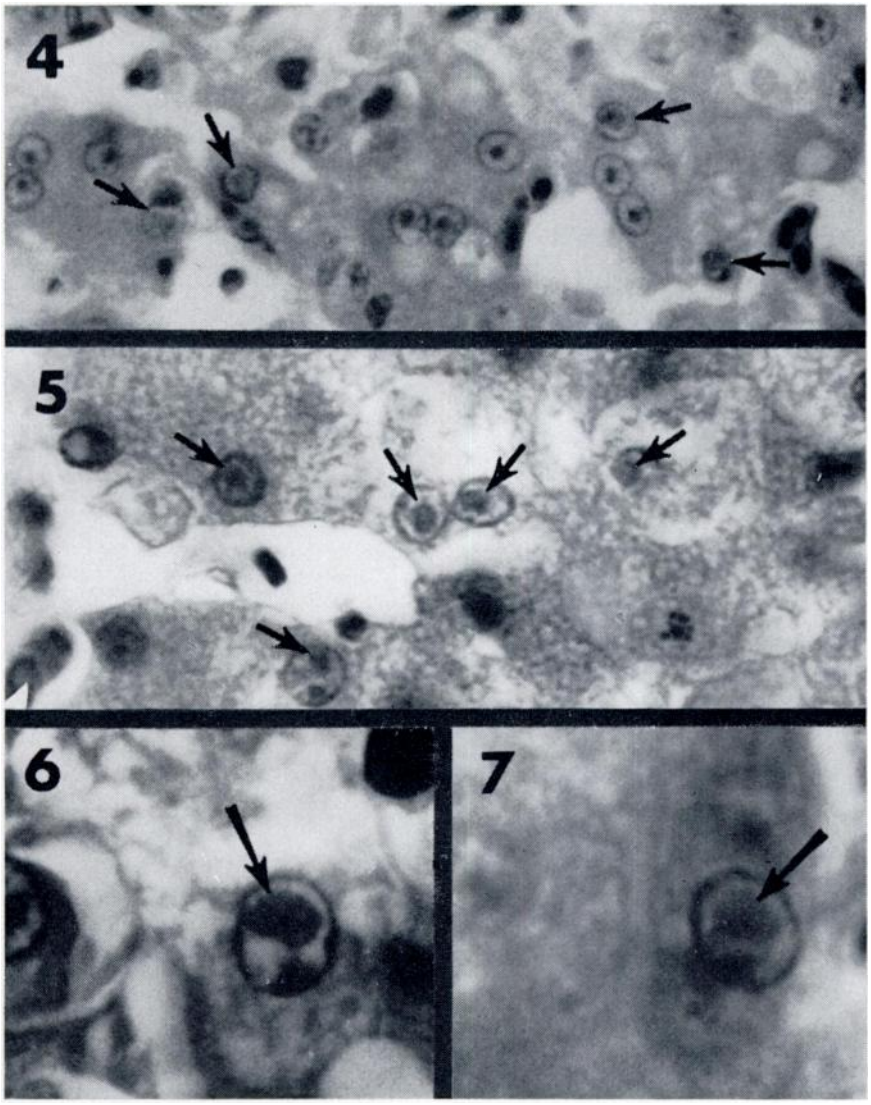

FIGURE 4. *Intranuclear inclusions (arrows) in the liver of a Pekin duck. H & E stain. X 1000.*

FIGURE 5. *Inclusions (arrows) in the liver of a mallard duck. H & E stain. X 1500.*

FIGURE 6. *High power magnification of a kidney-shaped intranuclear inclusion in the hepatic cell of a Pekin duck. H & E stain. X 2800.*

FIGURE 7. *Comparable inclusion to that in Fig. 6. It is in the liver of a Canada goose. H & E stain. X 2800.*

served frequently, and the margination of nuclear chromatin was usually discernable (Fig. 6). They stained purple with hematoxylin and eosin; pink with Giemsa; greyish-green with methyl green-pyronin; and gave positive reaction with the Feulgen stain, consistent with the presence of deoxyviruses.[1,2] No inclusions were found in the noninfected control ducks.

Mallard Ducks

Clinical course: As indicated in Table 2, of the ten 3-week-old mallard ducklings inoculated with duck plague virus, 2 died 7 DPI, and 1 of the 4 contact birds died on the 14th DPI. The ducks had signs identical to those seen in the White Pekin ducklings. Those infected were lethargic, had circles of wet feathers around congested eyes, and 1 had a sticky, closed eye. Clear serous discharge dripped from the nasal passages. The other external body signs of the disease were difficult to observe as they were masked by the dark feathers and pigmentation. The congestion on the beaks and unfeathered parts of the body were less pronounced than in the Pekin ducklings. Five ducklings had cloacal inflammation that varied in the degree of redness. Seven DPI, the 4 contact ducklings were active, moved about normally, and appeared healthy.

Virus isolation: Virus was recovered from the blood, esophageal and cloacal samples of 5, 7, and 4 inoculated ducklings, respectively. It was also isolated

TABLE 3. *Results of Experimental Infection of 3-Week-Old Mallard Ducks with Duck Plague Virus*

Duck	Mortality	Intensity* of cloacal congestion	Virus isolation			Virus neutralization index** DPI			
No.	(DPI)	5 DPI	B	E	C	0	7	24	38
Infected orally:									
218		4	—	+	+	0.25	0.75	1.5	3.75
219	7	2	+	+	+	0.25			
220		0	—	—	—	0.75	NA	2.25	3.5
221	7	4	+	+	+	NA			
222		0	—	+	—	0.25	0.5	3.25	4.25
223		2	—	+	+	NA	0.75	3.2	4.2
224		1	+	+	—	0.25	0.5	2.0	3.5
225		0	+	—	—	0.5	0.25	2.5	4.0
226		0	+	+	—	NA	0.5	2.5	2.5
227		0	—	—	—	0.75	0.75	3.5	4.0
Contact:									
235		0	+	—	+	0.25	0.75	2.0	3.75
236	14	0	—	—	+	0.25	0.25		
237		0	—	—	—	NA	0.5	1.75	4.0
238		0				NA	0.5	2.25	3.75

*Range of intensity of congestion: none (0) to severe (5).
**Log 10
B = blood; E = esophagus; C = cloaca
NA = no detactable antibody
+ = virus isolated
— = no virus isolated
DPI = days postinoculation

from the cloaca of 2 of the 4 contact ducklings and from the blood of one of them (Table 3). No virus could be isolated from 2 birds of either the inoculated or contact group. The virus was recovered from the blood, and esophageal and cloacal swabbings of the 2 inoculated ducklings that died 7 DPI. None of the chicken embryos that had been inoculated with the swab and blood samples from mallard ducklings died during a 10-day observation period.

Serologic response: Virus neutralizing indices of the serums collected from the ducklings pre- and postexposure are shown in Table 3. Traces of nonspecific antibodies were found in the preinoculation serum. The VNI gradually increased to markedly high levels by the 38th DPI. This pattern was similar to that described previously for the Pekin ducklings.

Gross lesions: Necropsy of the 3 mallard ducklings that succumbed to infection with duck plague virus revealed lesions qualitatively identical to those seen in the Pekin ducks but much more severe. There was generalized congestion, multiple petechiae and ecchymoses in the visceral organs as well as serous membranes and extensive necrosis, chiefly involving the esophageal and cloacal mucosae. The pericardial sac and peritoneal cavity contained a moderate amount of serosanguinous exudate. The liver was yellow and friable, having paint-brush-like subcapsular and punctiform hemorrhages on the cut surface. The spleen was enlarged and studded with punctiform white-grey areas of a dry, granular substance. The mucosal folds of the esophagus and cloaca were covered with a cheesy exudate that, spotwise, was hardened into elongated grey-yellow plaques. Upon removal of the exudate adhering to the necrotic mucosa, the propria was diffusely hemorrhagic, swollen, and watery. *Pasteurella* organisms, lethal for rabbits, were recovered from the blood and livers of the dead birds.

Microscopic changes: Besides the pathologic alterations seen in the visceral organs of the Pekin ducks, the spleen, pancreas, lungs, and heart of mallard ducks were severely affected. The spleen had lost most of its trabecular framework, and had many degenerated splenic corpuscles, resulting in multiple, small and large, partially confluent, irregular foci of necrosis (Fig. 2). They were characterized by a strongly acidophilic, vacuolated caseous mass, fragmented nuclear debris, numerous degenerating heterophils, and occasional rods of microorganisms.

The pancreas showed multiple, perivascular foci of necrosis dispersed throughout the parenchyma (Fig. 3). Degenerate heterophils were scattered in the necrotic areas. Periacinar spaces were enlarged, vacuolated, and extended by the edema fluid.

The prominent changes in the lungs were congestion, hemorrhage, and focal necrosis. The heart had subpericardial hemorrhages, and there were a few foci of hyaline degeneration in the myocardium. The intranuclear inclusions identical to those found in the Pekin ducks were present in the hepatic cells (Fig. 5) as well as in the mucosae of the esophagus and cloaca.

Canada Geese

Clinical course: Approximately 4 to 10 days elapsed before the appearance of signs. The geese were listless, depressed, and moved slowly with their necks retracted. Later in the progress of the disease, they stayed apart, were prone to assume a sitting position, with closed eyes, and did not respond to auditory stimuli. Green diarrhea and soiling of the feathers in the cloacal area were observed. All the Canada geese succumbed to infection between 7 and 15 DPI (Table 4).

Gross lesions: In the birds that died relatively early, the lesions were only slightly suggestive of duck plague and largely consisted of petechiation and ecchymoses of the serous membranes and the abdominal fat. Hemorrhages were extensive in the epicardium and the yellow-discolorated liver. The incidence of gross lesions in the organs of the Canada geese is shown (Table 4). Paint-brush-like hemorrhages of the liver surfaces were common in all the birds,

TABLE 4. *Incidence of Duck Plague Lesions in Canada Geese*

Goose No.	Death (DPI)	Lesions				
		Esophagus	Proventriculus	Liver	Intestines	Cloaca
Infected orally:						
609	7	±	0	+	+	+
610	7	±	0	+	+	+
611	8	±	0	+	+	+
612	9	±	+	+	+	+
613	9	+	+	+	+	NF
615	10	+	+	+	+	NF
616	14	+	+	+	+	NF
Contact:						
617	14	+	+	+	+	NF
619	15	+	+	+	+	NF
620	15	+	+	+	+	NF

DPI = Days postinoculation
± = Moderate petechiation and no diphtheritic membrane
NF = Necrotic foci
0 = No macroscopic lesions
+ = Characteristic lesions

especially those that died on or before 10 DPI. In contrast, characteristic duck plague lesions were more frequent in those geese that had a prolonged disease course. The mucosa of the esophageal folds had a linear petechiation. In 4 geese, the esophageal mucosa was covered with a continuous diphtheritic pseudomembrane extending from the oral cavity down to the proventriculus. In others, only the hemorrhagic petechiation of the esophagus was present. In 2 geese, the diphtheric lesions were limited to the areas of petechiation. The lesions in the cloaca were more prominent than those in the esophagus and were characterized by severe congestion, necrosis, and the formation of the diphtheric plaques. *Pasteurella* organisms lethal to rabbits were isolated from the hearts and livers of all the Canada geese. Occasionally, the lungs were moderately congested and, in some, congestion of the duodenum was prominent.

Microscopic changes: Microscopic changes in the esophagus and cloaca were characterized by the hemorrhages, hydropic degeneration, extensive necrosis of the epithelial cells and erosions of the mucosa. Portions of the pseudomembranes were calcified (Fig. 8). The congestion of the venous vessels was prominent in the liver, spleen, and pancreas. Retrograde changes in the liver were minimal and only occasional single foci of caseous necrosis could be found in the parenchyma. Intranuclear inclusions similar to those in the Pekin and mallard ducks, were constantly present in the hepatic cells (Fig. 7), and in the epithelium of the esophagus (Fig. 9) but rarely in the cloaca.

Discussion

In January of 1967 — for the first time — duck plague was reported in the United States.[12] It appeared and spread among the highly concentrated White Pekin duck population raised commercially on Long Island, New York. The question of the source of infection and its origin is unresolved. During the outbreak, a few carcasses of mallard ducks and Canada geese were found in the

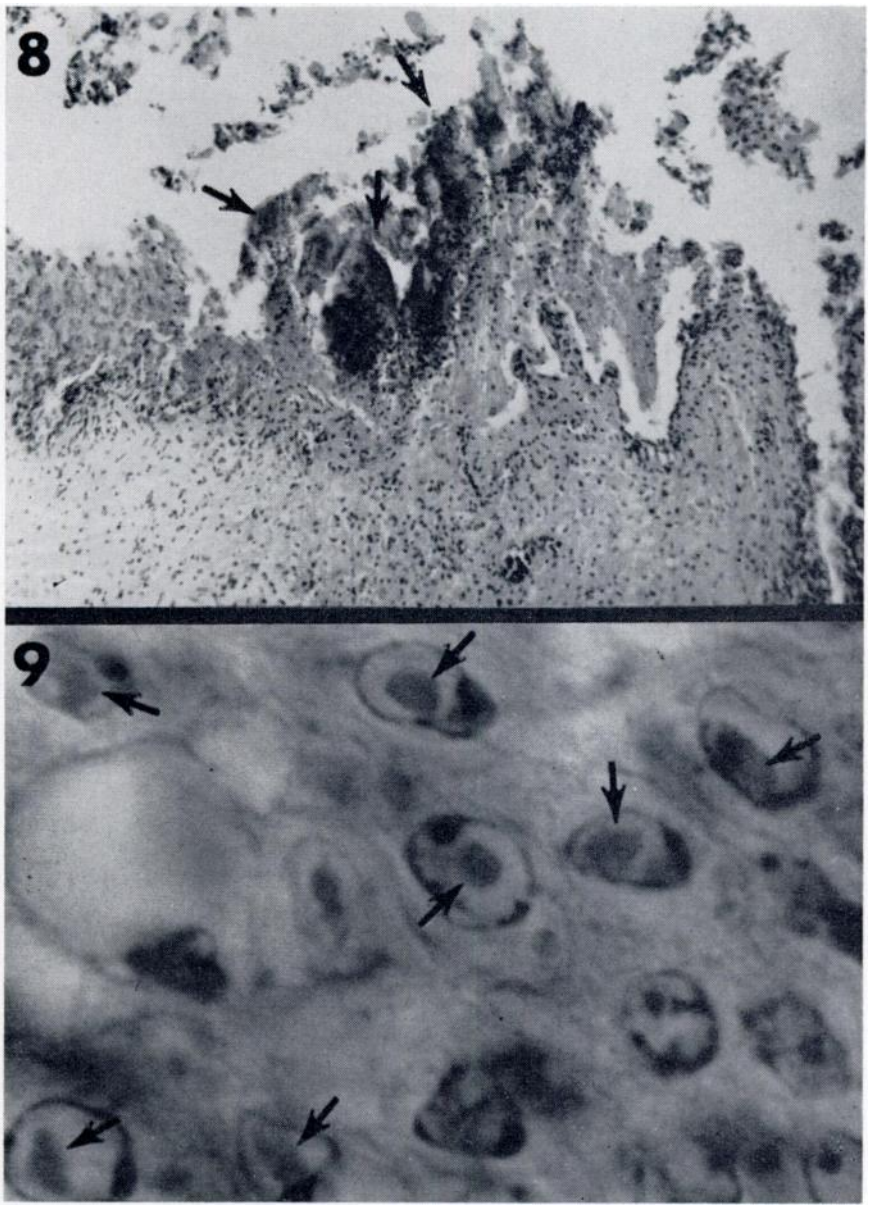

FIGURE 8. *Necrosis and calcification (arrows) of the cloacal mucosa of a Canada goose. H & E stain. X 170.*

FIGURE 9. *Numerous intranuclear inclusions (arrows) in the epithelial cells of the esophagus of a Canada goose. H & E strain. X 2800.*

TABLE 5. *Comparative Mortality: Rate of Three Species of Waterfowl*

Waterfowl species	No. died/used Exposure		Mortality (DPI) Exposure		Average Mortality	
	Oral	Contact	Oral	Contact	Oral	Contact
Ducks:						
White Pekin	5/10	2/4	5,5,7,11,13	13,17	50%	50%
Mallard	2/10	1/4	7,7	14	20%	25%
Geese:						
Canada	10/10	0	7,7,8,9,9,10 14,14,15,15	0	100%	

0 = None tested

vicinity and neighborhood of the infected flocks. Wild mallard ducks are in abundance on Long Island. They swim and mingle with the domestic ducks and fly freely in and out of the premises were ducks are raised commercially. Jansen[7] stated that most mallard ducks did not die from infection but produced antibodies. This fact was confirmed in our laboratory.[3] The information developed in the present studies demonstrated that the disease syndrome was identical in all respects, in all ducklings, regardless of whether they were infected orally or by contact exposure. The similarity of the disease signs and the pathologic alterations in individual ducks of the 2 species were striking. The severity of signs varied among individual birds, and some of those mildly affected recovered. The histopathologic findings revealed the consistent presence of intranuclear inclusion bodies in the esophageal and cloacal epithelial, as well as in the parenchymal hepatic cells. The inclusion bodies reported by Leibovitz and Hwang[12] in the lumen of blood vessels and Kupffer's cells were not detected in the specimens from the birds employed in the present study. In spite of these similarities, and within the limitation of these trials, more Pekin ducklings died than did mallard ducklings (Table 5). This may reflect an inherent species resistance.

The disease features in the Canada geese were characterized by 100% mortality, multiple hemorrhages, and other pathognomic signs and lesions found in Pekin and mallard ducks, and the presence of rabbit-lethal *Pasteurella* organisms in all geese used in this study. Moreover, inclusion bodies similar to those observed in both Pekin and mallard ducks were also found in the Canada geese at the same sites of predilection as in the ducks.

We noted that extensive hemorrhages were also found in the ducklings from which *Pasteurella* organisms were isolated. Whether there is symbiosis between duck plague virus and *Pasteurella* organisms in eliciting the expression of the duck plague disease signs and severity of mortality is not known. However, from the present observation, it may be conjectured that the hemorrhages described and emphasized in the literature as one of the pathognomic duck plague disease signs may result from mixed viral and bacterial infections. A study of the disease in gnotobiotic ducks is warranted and may provide worthwhile information.

The inclusion bodies observed in the esophageal, cloacal, and hepatic epithelial cells of the mallard ducks and Canada geese constitute a new finding. Since these inclusions are also asscciated with duck plague infection in domestic ducks, they may be important in diagnosing duck plague in waterfowl.

Acknowledgments

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Literature Cited

1. BREESE, S. S. and A. H. DARDIRI. 1968. Electron Microscopic Characterization of Duck Plague Virus. Virology 34(1): 160-169.
2. DARDIRI, A. H., HESS, W. R., BREESE, S. S., Jr. and H. R. SEIBOLD. 1967. Characterization of Duck Plague Virus from a Duck Disease Outbreak in the United States. Proc. 39th Northeastern Conf. on Avian Dis., State University of New York, June 19-21, 1967. Stony Brook, N.Y.
3. DARDIRI, A. H., and W. R. HESS. 1968. The Incidence of Neutralizing Antibodies to Duck Plague Virus in Serums from Domestic Ducks and Wild Waterfowl in the United States of America. Proc. 71st Ann. Mtg. USLSA, Phoenix, Arizona, 225-237.
4. DEVOS, A., VIAENE, N., and M. STAELENS. 1964. Eendenpest in België (Duck Plague in Belgium). Vlaams Diergeneesk. Tijdschr. 33(6): 260-266.
5. JANSEN, J., and H. KUNST. 1949. Is eendenpest verwant aan hoenderpest of pseudohoenderpest? (Is duck plague related to fowl plague or Newcastle disease?) Tijdschr. Diergeneesk. 74: 705-707.
6. JANSEN, J. 1951. On the Difference Between Fowl Plague, Newcastle Disease and Duck Plague. Proc. IX World Poult. Congr. (Paris) 3: 65-68.
7. JANSEN, J. 1961. Duck Plague. Brit. Vet. J., 117: 349-356.
8. JANSEN, J. 1961. Wederom gevallen van eendenpest. Tijdschr. v. Diergeneesk. 86(9): 631-632.
9. JANSEN, J. 1963. Over het voorkomen van eendenpest. Tijdschr. v. Diergeneesk. 88(21): 1341-1343.
10. JANSEN, J. 1964. Duck Plague (A Concise Survey). Indian Vet. J. 41(5): 309-316.
11. JANSEN, J., and H. KUNST. 1964. The Reported Incidence of Duck Plague in Europe and Asia. Tijdschr. v. Diergeneesk. 89(11): 765-769.
12. LEIBOVITZ, L., and J. HWANG. 1968. Duck Plague on the American Continent. Avian Diseases 12(2): 361-378.
13. LILLIE, R. D. 1965. Histopathologic Technic and Practical Histochemistry. McGraw-Hill Book Co., New York.
14. LUCAM, F. 1952. La Peste Avaire en France. Report of the XIVth Internat. Vet. Congr., London, II: 380-386.
15. MUKERJI, A., DAS, M. S., GHOSH, B. B., and J. L. GANGULY. Duck Plague in West Bengal., Pt. 1, Indian Vet. J. 40(8): 457-462.
16. REED, L. J., and H. A. MUENCH. 1938. A Simple Method of Estimating Fifty Per Cent Endpoint. Am. J. Hyg. 27: 493-497.