Case Report—

An Outbreak of Encephalitis in Pigeons (Columba livia) in the Canary Islands (Spain)

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SUMMARY. An encephalitic disease in pigeons, characterized by paralysis of extremities, torticollis, head tremor, and diarrhea, is described.

The negative titers in the hemagglutination tests, the presence of Feulgen-positive intranuclear inclusions in the cells of the granular layer of the cerebellum and the fibroblast culture, and the presence of viral particles with an icosahedral-herpetic morphology and a diameter of 100–170 nm all indicate that the etiology of this outbreak may be related to the encephalitis caused by herpesvirus, at present diagnosed only in Iraq and some African countries.

RESUMEN. *Reporte de Caso*—Brote de encefalitis en palomas (Columba livia) en las Islas Canarias (España).

Se investigó la presencia de un brote encefalítico en palomas, caracterizado por parálisis de las extremidades, tortícolis, temblores de la cabeza y diarrea.

Los títulos negativos de las pruebas de hemaglutinación, la presencia de cuerpos de inclusión intranucleares Feulgen positivos en células de la capa granulosa del cerebelo y en los cultivos de fibroblastos, sumados a la presencia de partículas víricas de morfología herpética icosahédrica de unos 100–170 nm; relacionan la etiología de este brote con la encefalitis causada por un virus Herpes, diagnosticada actualmente sólo en Iraq y algunos países Africanos.

In 1979 (1), a pigeon disease was described that presented encephalitic symptoms with no signs of respiratory distress; the causative agent was named pigeon herpes encephalomyelitis virus (PHEV). The outbreak, which occurred in Baghdad, Iraq, in 1977, affected a large number of pigeons, and mortality was high. This disease has spread to the neighboring countries of Syria (unconfirmed) and Egypt (8), as well as to more distant countries like South Africa (5).

A pigeon herpesvirus (PHV) had been isolated previously (3) that causes mainly respiratory distress and catarrhal manifestations.

Research carried out by various authors (3,7) shows that PHEV is not related to PHV or other animal or even human herpesviruses.

This paper reports an outbreak of non-suppurative encephalitis that appeared in the Canary Islands (Spain) in early 1985, at an altitude similar to that described for the outbreak of PHEV (1,3,7).

CASE REPORT

In 1985, a disease appeared in the Canary Islands that was characterized by acute nervous signs and a high mortality rate among sick pigeons, especially young birds and those subjected to stress by long-distance flying.

Nervous signs consisted mainly of paralysis of the extremities, torticollis, head tremor, and diarrhea. In no case did pigeons suffer from respiratory distress. The outbreak started on the island of Lanzarote and now affects other islands in the archipelago.

Pieces from brain, spinal cord, liver, spleen, kidney, lung, trachea, and intestines were collected from necropsied birds and fixed in 10% formalin. Following the usual paraffin-embedding technique, sections were cut at 5 μm and stained with hematoxylin and eosin (H&E). A histopathological study was performed, together with inoculation (0.2 ml of antibiotic-treated 10%

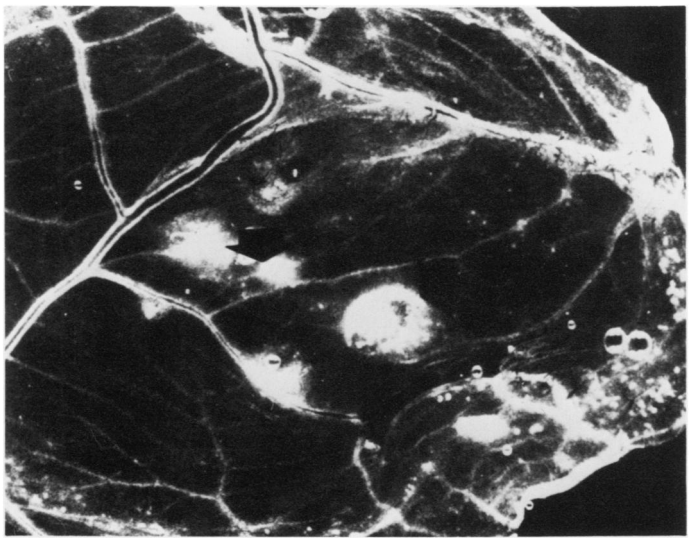

Fig. 1. Chorioallantoic membranes showing creamy, minute pocklike lesions (arrows).

brain suspension) onto the chorioallantoic membranes of 10-day-old chicken embryos and fibroblast cultures.

Sixty-three inoculated embryos were sacrificed 48, 72, and 96 hours after inoculation, and inoculation was continued in blind passages until the fifth passage. These samples were used for both microscopic and ultramicroscopic pathological studies. The hemagglutinating ability of the chorioallantoic fluid was tested, and 15 young, healthy pigeons were experimentally inoculated with material obtained from chicken embryos in the third passage.

The effects of bromodeoxyuridine (BUdR; 100 μg/ml of the culture medium) and 20% diethyl ether on virus replication were studied.

RESULTS

Macroscopic lesions. Most pigeons showed marked congestion and hemorrhages in different organs, particularly in the brain, liver, pancreas, and intestine.

Microscopic lesions. The most significant findings were perivascular cuffs of mononuclear cells, neuronal degeneration, destruction of Purkinje neurons in the cerebellum, and spongiosis phenomenon.

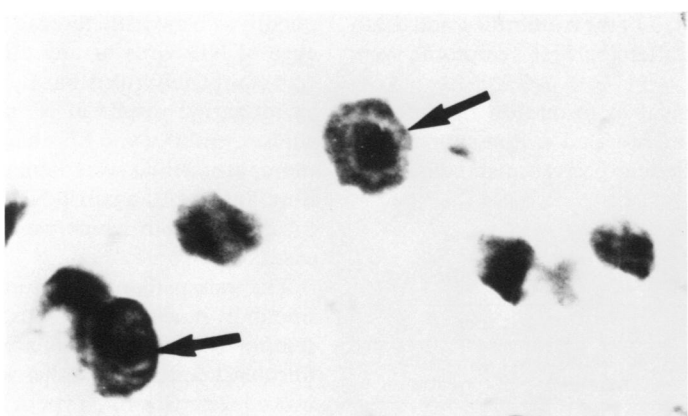

Fig. 2. Cowdry-A Feulgen-positive basophilic inclusions (arrows). H&E. 1000×.

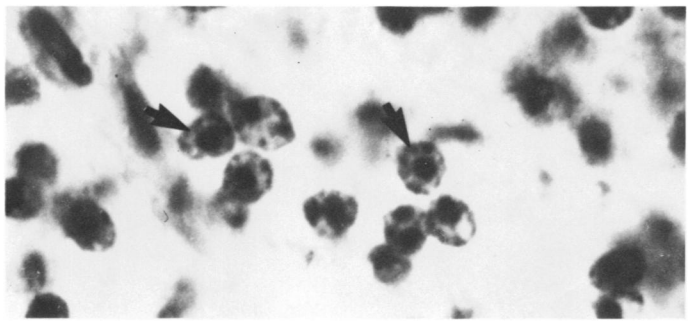

Fig. 3. Feulgen-positive inclusions in the granular layer of the cerebellum (arrows). Feulgen technique. 1000×.

Virus isolation. Six inoculated embryos had died 48 hr postinoculation, and six died 72 hr postinoculation. Chorioallantoic membranes showed creamy, minute pocklike lesions (Fig. 1). The embryos were curled and showed signs of dwarfism, with subcutaneous hemorrhages over the whole body. The visceral organs showed hemorrhages and a decrease in coloring of the liver.

The embryos had necrosis in the ectodermal cell layer of the chorioallantoic membrane and Cowdry-A Feulgen-positive basophilic inclusions (Fig. 2). Similarly, a strong reaction was provoked in immature eosinophilic cells in these membranes.

Cytopathic effects appeared in the fibroblast culture 72 hr postinoculation, with monolayer destruction, syncytial formation, and the presence of Feulgen-positive inclusions.

No substrates exhibited hemagglutinating ability.

Experimental inoculation of young pigeons. After 17 and 19 days, inoculated pigeons exhibited signs of paralysis of the extremities, and death occurred 7 days after the first symptoms were observed.

Histopathological examination of different parts of the brain revealed a non-suppurative encephalitis, with few perivascular cuffs, neuronal degeneration with satellitosis, and the presence of Feulgen-positive inclusions in the cells of the granular layer of the cerebellum (Fig. 3).

Inoculation of chicken embryos with material from experimentally inoculated pigeons caused reproduction of pocklike lesions of the membrane and intranuclear inclusions in ectodermal chorioallantoic membrane cells, together with the other lesions described earlier for inoculation using field material.

Ultrastructural examination of the chorioallantoic membranes and cerebellum of affected pigeons revealed intranuclear viral particles (Fig. 4) with a diameter of about 100–170 nm, depending on the presence or absence of an outer covering.

Virus replication was inhibited by BUdR and by ether treatment (Table 1).

Table 1. Effect of chemical agents on the virus.

Chemical agent	Virus titer	
	Before treatment	After treatment
BUdR	10^4	10^2
Ether	10^4	10^0

DISCUSSION

In processes of non-suppurative avian encephalitis, one of the diseases present most frequently is Newcastle disease (ND) (4). The presence of ND virus or any other paramyxovirus was completely ruled out by the negative hemagglutination results in chorioallantoic fluid of chicken embryo and fibroblast culture. Furthermore, at no time were hemagglutination-inhibiting antibodies against ND virus observed in those pigeons in which we reproduced the disease.

The lesions found in naturally sick and experimentally inoculated pigeons, in the chorioallantoic membranes of inoculated embryos, and in fibroblast cultures, together with the presence of intranuclear Cowdry Type A inclusions revealed by electron microscopy and the presence of viral particles with an icosahedral-herpetic morphol-

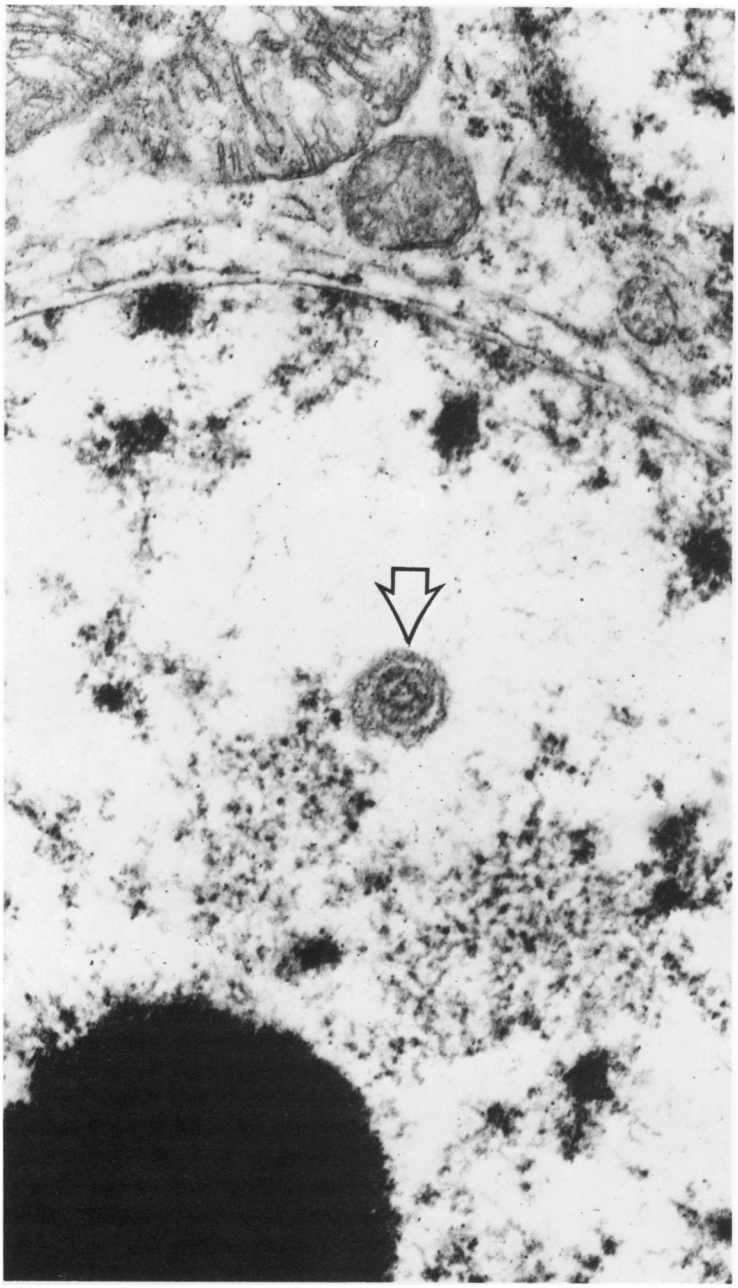

Fig. 4. Intranuclear viral particles in the granular layer of the cerebellum (arrow). 73,000×.

ogy (herpesvirus morphology) and a diameter of 100–170 nm, imply a close relationship to the herpesvirus described earlier (1,2,6).

The presence of a DNA virus is demonstrated by the inhibition of virus replication by BUdR and diethyl ether.

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