

PIGEON HERPES INFECTION: NATURAL TRANSMISSION OF THE DISEASE

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INTRODUCTION

Vindevogel, Pastoret and Burtonboy (1980) showed that pigeons become asymptomatic carriers of pigeon herpesvirus (*Pigeon Herpesvirus 1*, PHV) after experimental infection. The results of studies on the persistence and transmission of the virus under natural conditions are presented in the present paper.

MATERIALS AND METHODS

Pigeons. All birds and samples originated from a dovecot where the disease was diagnosed 13 months previously. At the beginning of our observations pigeons had been clinically asymptomatic for 10 months and were rearing normal broods.

Study on the horizontal transmission of the virus. Titration of neutralizing antibodies and attempted isolation of the virus after pharyngeal swabbing were done on 10 mature pigeons, 5 squabs artificially incubated and hatched, and examined at day-old, 5 squabs 2, 7 and 14-days-old, and 10 weaned squabs 3 to 4-weeks-old. The last 10 birds were then injected with cyclophosphamide (Cy) on 4 successive days as described previously (Coignoul and Vindevogel, 1980), and submitted to pharyngeal swabbing 4 and 7 days after the beginning of this treatment. Techniques used to titrate and characterize infectious particles and to titrate specific neutralizing antibodies have been described previously (Vindevogel *et al.*, 1980).

The PHV-neutralizing capacity of egg yolk and milk of 5 pairs of pigeons as also evaluated by the 50 per cent plaque-reduction technique. Milk was extracted from the crops of pigeons, and was suspended (1/1 W/V) in phosphate-buffered saline solution (PBS) at pH 7.3. These suspensions were inactivated by heating for 30 min at 56 °C. Yolk was not inactivated.

Study on egg transmission of the virus. Cultures of cells from 25 pigeon embryos of 10 to 14 days incubation were made to detect the possible presence of PHV in the embryo.

Cultures of pigeon embryo fibroblasts (PEF) and hepatic cells (PEHC) were made on coverslips and in Falcon plastic bottles (25 cm²) according to the techniques described for the cultures of chicken embryo cells (CEF and CEHC) (Vindevogel, Pastoret, Burtonboy, Gouffaux and Duchatel, 1975; Vindevogel, Duchatel, Gouffaux and Pastoret, 1977).

Primary PEF cells were trypsinized at weekly intervals to obtain secondary and tertiary cultures and examined for 3 weeks. Dexamethasone (25 µg per ml; Opticortenol Ciba) was added to a portion of the primary PEF cultures. PEH cells were observed for 8 days.

After the observation period, cells of all cultures were suspended in the maintenance medium, 5 per cent dimethyl sulphoxide added, frozen and thawed, and assayed for PHV-infectivity by inoculation on secondary CEF. Indirect immunofluorescence (Vindevogel, Duchatel and Gouffaux, 1977) was used to detect PHV-antigen on coverslips of primary PEHC and tertiary PEF cells.

Primary and secondary PEF and CEF cells, as well as primary PEHC and CEF cells, were cultured together with 5×10^5 PEF cells, 7.5×10^5 PEH cells and 3.5×10^5 CEF cells per ml; they were screened for 10 days for cytopathic effect and viral antigen.

RESULTS

The results are summarized in Tables 1 and 2.

TABLE 1

TRANSMISSION OF PHV AND ANTIBODIES

<i>Birds sampled</i>	<i>Number of positive swabs Number of swabs taken</i>	<i>Antibodies*</i>
Mature pigeons	3/10	77
Egg yolk	—	33
Milk	—	<4
Squabs reared in incubator	0/5	49
Squabs 2-day-old	1/5	22
7-day-old	0/5	11
14-day-old	1/5	5
Weaned squabs	2/10	<4

* Geometric means.

Horizontal Transmission of the Virus (Table 1).

Infectious and immune status of the parents. All the mature pigeons possessed neutralizing antibodies (48 to 102). PHV was isolated from pharyngeal swabs of 3 of 10 of them (5×10^1 to 1.1×10^4 pfu per swab).

Neutralizing capacity of egg yolk and milk. Yolk neutralized PHV when diluted 27 to 48, whereas milk did not.

Infectious and immune status of the neonate squabs (1 to 14-day-old). PHV was not isolated from the pharyngeal swabs of the squabs reared in the incubator, but from two squabs reared by their parents, one 2-day-old (1×10^2 pfu per swab) and the other 14-day-olds (5×10^1 pfu per swab).

All the squabs possessed antibodies when hatched. The antibody titres decreased rapidly and disappeared by day 14.

Infectious and immune status of squabs after weaning (Table 2). All squabs examined after weaning were devoid of neutralizing antibodies, and 2 were spontaneously shedding PHV. After Cy-treatment, 5 squabs developed typical lesions and 7 shed virus.

TABLE 2

COMPARISON BETWEEN LESIONS AND TITRES OF PHV EXCRETED BY WEANED SQUABS

Number	Before Cy-treatment		After beginning of Cy-treatment		
	lesions	pfu per swab	day 4 pfu per swab	day 7 lesions	day 7 pfu per swab
1	—	0	0	—	0
2	—	0	0	—	0
3	—	0	0	—	0
4	—	0	3.3×10^3	+	2.5×10^3
5	—	0	5.0×10^1	—	0
6	—	0	2.5×10^2	+	1.3×10^3
7	—	1.4×10^2	3.5×10^3	+	5.0×10^1
8	—	5.4×10^3	0	+	1.4×10^3
9	—	0	0	+	1.0×10^3
10	—	0	0	—	3.0×10^3

Absence of Egg Transmission of the Virus

No cytopathic effect was observed in pigeon embryo cell cultures even when cocultured with CEF, and no virus was isolated on secondary CEF from the pigeon embryo cell cultures. PHV-antigen was never detected.

DISCUSSION

Three out of 10 mature pigeons swabbed at random in a dovecot where disease associated with PHV had occurred more than one year before were still shedding virus. There had been no clinical signs over the previous 10 months. Pigeons may remain asymptomatic carriers of PHV for a long time after recovery from natural as well as experimental infection (Vindevogel *et al.*, 1980). Transmission of infection to their offspring is likely and the virus was isolated from the pharynx of a 2-day-old squab, and from several others during their first weeks of life.

Egg transmission of the virus seems unlikely since we were unable to demonstrate the virus or its antigen in cell cultures derived from embryos of infected pigeons. Similar observations have been made with avian infectious laryngo-tracheitis virus (*Phasianid Herpesvirus 1*, ILTV) (Brandly, 1934; Brandly and Bushnell, 1934). The egg does not thus seem to play a role in the transmission of pigeon herpes infection, at least after the acute phase of the disease. It would be interesting to study the pathogenesis of the disease and the distribution of the viral agent during the acute phase.

The squabs are protected in their early life by antibodies of parental origin and thus perhaps escape from lethal infection. None of the squabs presented clinical signs of the disease which is in contrast to those observed in the primary infection of susceptible squabs.

The passive protection of the squabs seems to be conferred through the egg and not by the pigeon milk. The neutralizing capacity of the pigeon egg yolk

is transferred to the serums of the squabs as soon as they are hatched; then the titres decline rapidly and disappear when the squabs are 2-weeks-old.

After weaning, most of the squabs, collected at random, were asymptomatic carriers of the virus, as has been shown after Cy-treatment, but were devoid of detectable neutralizing antibodies. Similar observations have been carried out with infectious bovine rhinotracheitis virus (*Bovid Herpesvirus 1*, IBRV) (Aguilar-Sétien, Pastoret, Jetteur, Burtonboy and Schoenaers, 1979).

We may conclude that in a flock of pigeons infected with PHV, some mature birds are asymptomatic carriers of the infectious agent and transmit it to their offspring. The squabs become infected but are protected from the more severe effects by the passive immunity of parental origin and most of them become themselves asymptomatic carriers after this initial infection. Clinical disease is principally observed in the primary infection of young pigeons derived from parents free of the infection, or in carriers of the virus with the help of debilitating factors.

SUMMARY

Studies on the persistence and transmission of pigeon herpesvirus (*Pigeon Herpesvirus 1*, PHV) in a dovecot containing pigeons which were naturally infected more than a year previously are reported.

Three out of 10 mature pigeons were still shedding virus. They transmitted it to their offspring during the first days of life. Egg transmission of the disease seems unlikely since PHV could not be unmasked in cell cultures of embryos of contaminated pigeons.

All mature pigeons possessed neutralizing antibodies. Parental passive immunity seems to be conferred on the squabs through the egg yolk and protects from the worst effects of infection. Seven of 10 squabs were asymptomatic carriers of the infectious agent after weaning although they were all devoid of detectable antibodies.

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