VACCINATION TRIALS AGAINST PIGEON HERPESVIRUS INFECTION (*PIGEON HERPESVIRUS 1*)

By

H. Vindevogel, P. P. Pastoret and P. Leroy

Faculty of Veterinary Medicine, University of Liège, 45 rue des Vétérinaires, 1070 Bruxelles, Belgium

INTRODUCTION

Pigeon herpesvirus (*Pigeon herpesvirus 1*, PHV_1) infection is fairly common (Vindevogel and Duchatel, 1978; Vindevogel, Kaeckenbeeck and Pastoret, 1981).

The virus is not transmitted by the egg but from parents to their offspring mainly during the first days of life (Vindevogel and Pastoret, 1980). After primary infection, the infected pigeons become asymptomatic carriers and the virus is excreted intermittently (Vindevogel, Pastoret and Burtonboy, 1980a). Prevention of infection is therefore very difficult, more especially as chemotherapy has so far failed (Schwers, Pastoret, Vindevogel, Leroy, Aguilar-Setién and Godart, 1980; Schwers, Vindevogel, Leroy and Pastoret, 1981; Vindevogel, Pastoret and Aguilar-Setién, 1981).

We therefore tried to vaccinate pigeons and to compare the capacity of killed or attenuated vaccines to prevent infection or the carrier state or to control re-excretion.

MATERIALS AND METHODS

Pigeons

Five-week-old pigeons from parents free of infection were divided into 6 groups of 5 animals.

Virus Strains

The $PHV_1/B/Cu_1$ strain (Vindevogel, Pastoret, Burtonboy, Gouffaux and Duchatel, 1975) was used to prepare the vaccines and the $PHV_1/Cz/236$–69 strain isolated in Czechoslovakia (Krupička, Šmid, Valiček and Pleva, 1970) for the challenges.

Preparation of the Attenuated Vaccine

$PHV_1/B/Cu_1$ strain was serially passaged one hundred times on chicken embryo fibroblast cultures (CEF) according to methods previously described (Vindevogel *et al.*, 1975; Vindevogel, Duchatel and Gouffaux, 1977). Attenuation was monitored by inoculation of budgerigars (Vindevogel and Duchatel, 1977; Vindevogel, Pastoret, Leroy and Coignoul, 1980b) and the mortality rate compared, using the χ^2 test, with that of control birds inoculated with the original virulent strain (Vindevogel *et al.*, 1975).

Preparation of Inactivated Vaccine

Virus was cultivated on CEF grown in Falcon plastic bottles (75 cm^3). Twelve

0021–9975/82/040483+12 $03.00/0

hours after inoculation, the amount of medium was reduced to 10 ml per bottle. As soon as the cytopathogenic effect was generalized, cells were suspended in the medium, and frozen and thawed 3 times. The suspensions were centrifuged at 200 *g* for 10 min. The supernate was concentrated 5 times on polyethylene glycol 6000 according to methods previously described (Dagenais, Lansival, Pastoret and Kaeckenbeeck, 1980), 0·02 per cent beta-propiolactone was added and it was incubated for 30 min at 56 °C. The resulting preparation was thoroughly mixed with an equal volume of incomplete Freund's adjuvant. Inactivation of virus was tested on CEF cultures and bacterial sterility on blood agar.

Experimental Schedule

Details of the experimental schedule are given in Table 1 (day 1 was considered as the first day of experiment).

TABLE 1

PLAN OF TRIALS OF VACCINE AGAINST PIGEON HERPESVIRUS

Days	*Group 1*	*Group 2*	*Group 3*	*Group 4*	*Group 5*	*Group 6*	
1		Cy†			Cy		
2		Cy			Cy		
3		Cy			Cy		
4		Cy			Cy		
7							
8	va. inac.*	va. inac.		va. atten.**	va. atten.		
14		+Cy					
15	va. inac.	va. inac.					
		+Cy					
21		Cy					*Period a*
22	va. inac.	va. inac.					
		+Cy					
42							
43	chall.***	chall.	chall.	chall.	chall.	chall.	*Period b*
57							
58							
64			va. inac.				
71			va. inac.				
78			va. inac.				*Period c*
98							
99	Cy	Cy	Cy	Cy	Cy	Cy	
100	Cy	Cy	Cy	Cy	Cy	Cy	
101	Cy	Cy	Cy	Cy	Cy	Cy	
102	Cy	Cy	Cy	Cy	Cy	Cy	*Period d*
108							

* inactivated vaccine.
** attenuated vaccine.
*** challenge.
† cyclophosphamide treatment.

In group 1, pigeons were intramuscularly injected 3 times with 0·5 ml of the inactivated vaccine before challenge. Group 2 was first treated for 4 days with cyclophosphamide (Cy) as previously described (Coignoul and Vindevogel, 1980; Vindevogel *et al.*, 1980a), then vaccinated with inactivated vaccine, together with Cy-injections. Group 3 was vaccinated 3 times with inactivated vaccine after challenge.

In groups 4 and 5, animals were vaccinated before challenge by pharyngeal painting with 10^5 pfu of the attenuated strain with (group 5) or without (group 4) prior Cy-treatment.

Group 6 served as a control unvaccinated group.

All the animals were challenged on day 43 by pharyngeal painting with 10^5 pfu of the virulent PHV_1/Cz/236-69 strain and treated with Cy on days 99 to 102 in order to provoke viral re-excretion.

Monitoring of Viral Excretion and Development of Neutralizing Antibodies

To evaluate viral excretion, the pharyngeal mucous membrane of pigeons of groups 4 and 5 was swabbed from day 8 to 17, then on days 22, 29 and 36. From day 43, all the animals were swabbed daily until day 52, then on days 57, 64, 71, 78, 85 and 92, and from day 99 to 108 daily again. The number of infectious viral particles was titrated in each swab on CEF as previously described (Vindevogel *et al.*, 1980a).

Blood was taken for antibody titration from day 8 for groups 1, 2, 4, 5 and from day 43 for groups 3 and 6 every 7 days until day 99. Neutralizing antibodies were individually titrated as previously described (Vindevogel and Duchatel, 1978) in Falcon multiwells (16 mm) under 0·6 per cent carboxymethylcellulose, each well receiving 25 pfu in 0·1 ml of mixtures (1:1) of virus and serum.

Statistical Analysis of the Results

To simplify the statistical analysis, the results were grouped in 4 different periods: (a) from day 8 to 42 corresponding to the immunization period, (b) from day 43 to 57 corresponding to the challenge, (c) from day 58 to day 98 corresponding to the period of spontaneous viral re-excretion and (d) from day 99 to 108 corresponding to experimentally induced viral re-excretion.

For each pigeon the mean numbers of viral particles excreted and the antibody titres were calculated for the 4 different periods. Treated groups were compared with the control and with each other by the Mann and Whitney test (Snedecor and Cochran, 1968).

Clinical Score

Signs were scored according to methods previously described (Vindevogel and Pastoret, 1981).

RESULTS

Attenuation of $PHV_1/B/Cu_1$ Strain

The mortality rate of control budgerigars infected with the original virulent strain (8/10) was significantly higher than that of birds inoculated with the attenuated one (2/10) ($P<0·01$).

Comparison of Viral Excretion and Re-excretion Between Groups

Results for all pigeons are given in Figs 1 to 6. One pigeon each of groups 2 (Number 10) and 5 (Number 25) died after the initial Cy-treatment.

Comparison between groups 1 *and* 6 (*Table* 1; *Figs* 1 *and* 6). Period b: Vaccinated birds excreted significantly fewer viral particles than the control group ($P<0·01$). Moreover, 2 birds of group 1 did not excrete in this period.

Period c: Significant differences were observed between the groups ($P<0·01$). Indeed, no pigeon of group 1 spontaneously re-excreted virus, whereas spontaneous re-excretion was observed in the control group.

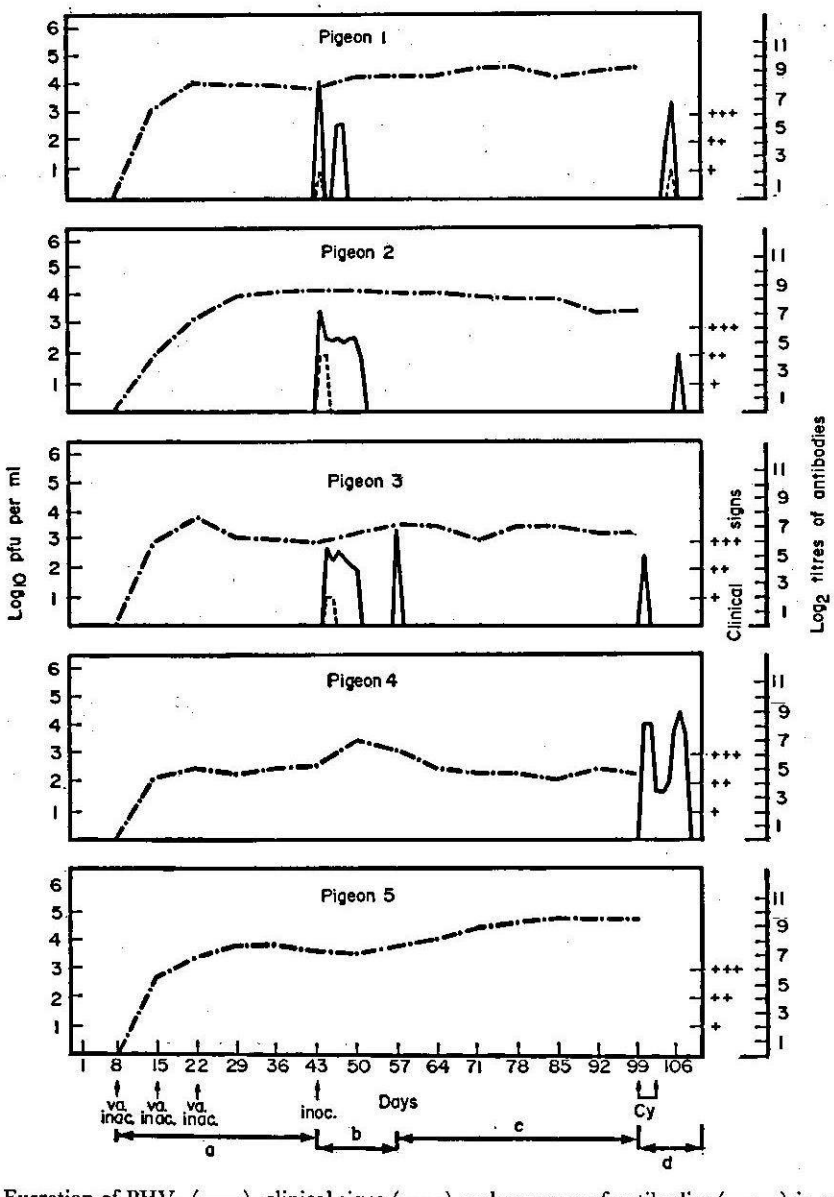

Fig. 1. Excretion of PHV_1 (——), clinical signs (– – –) and presence of antibodies (—·—) in group 1 pigeons. Cy = Cyclophosphamide treatment; va. inac. = inactivated vaccine; va. atten. = attenuated vaccine; inoc. = virulent challenge; a, b, c and d = periods of experiment.

Period d: After Cy-injections, vaccinated birds re-excreted significantly fewer particles than the control group ($P<0{\cdot}05$). One vaccinated pigeon did not re-excrete at all. It should be noted that the same bird (Number 5) neither excreted after primary infection nor re-excreted spontaneously. On the contrary, re-excretion was provoked in one bird (Number 4) where no primary excretion and no spontaneous re-excretion had been previously observed.

Comparison between groups 2 *and* 6 (*Table* 1; *Figs* 2 *and* 6). No significant differences were observed during periods b, c and d.

Comparison between groups 1 *and* 2 (*Table* 1; *Figs* 1 *and* 2). Periods b and c: Cy-treated animals excreted more particles than the untreated ones ($P<0{\cdot}01$).

Period d: No significant differences were observed.

Comparison between groups 4 *and* 6 (*Table* 1; *Figs* 4 *and* 6). Vaccinated pigeons excreted fewer particles during the three periods b, c and d ($P<0{\cdot}01$). More-

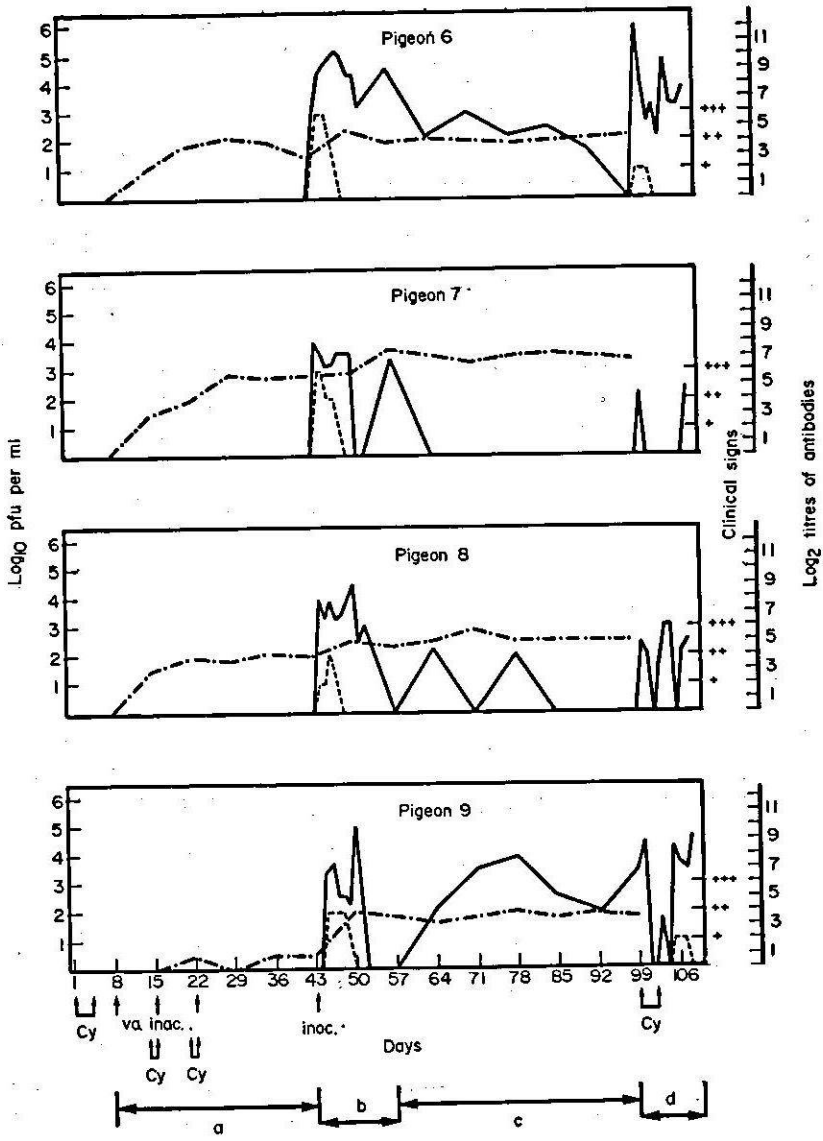

Fig. 2. Excretion of PHV_1 (——), clinical signs (– – –) and presence of antibodies (—·—) in group 2 pigeons. Cy = Cyclophosphamide treatment; va. inac. = inactivated vaccine; va. atten. = attenuated vaccine; inoc. = virulent challenge; a, b, c and d = periods of experiment.

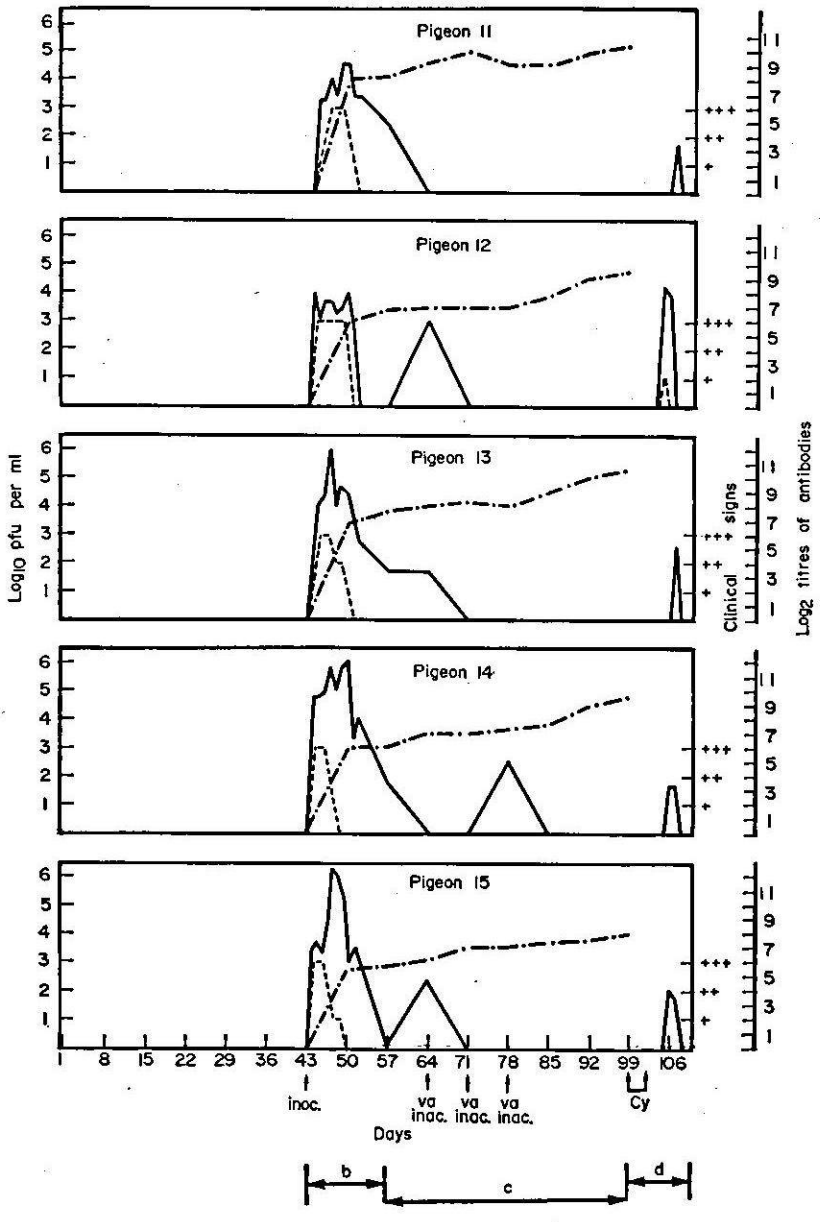

Fig. 3. Excretion of PHV_1 (——), clinical signs (– – –) and presence of antibodies (—·—) in group 3 pigeons. Cy = Cyclophosphamide treatment; va. inac. = inactivated vaccine; va. atten. = attenuated vaccine; inoc. = virulent challenge; a, b, c and d = periods of experiment.

over, no spontaneous re-excretion occurred in the vaccinated group and post-challenge Cy-treatment of these animals failed to provoke re-excretion in two of them.

Comparison between groups 5 *and* 6 (*Table* 1*; Figs* 5 *and* 6). Period b: Mean excretion did not differ between the groups.

Period c: Spontaneous re-excretion was observed in 3 pigeons of group 5; nevertheless, the overall re-excretion was significantly less in that group than in the control one ($P<0{\cdot}05$).

Period d: No significant differences were observed.

Comparison between groups 4 *and* 5 (*Table* 1; *Figs* 4 *and* 5). Periods a and b: No significant differences were observed.

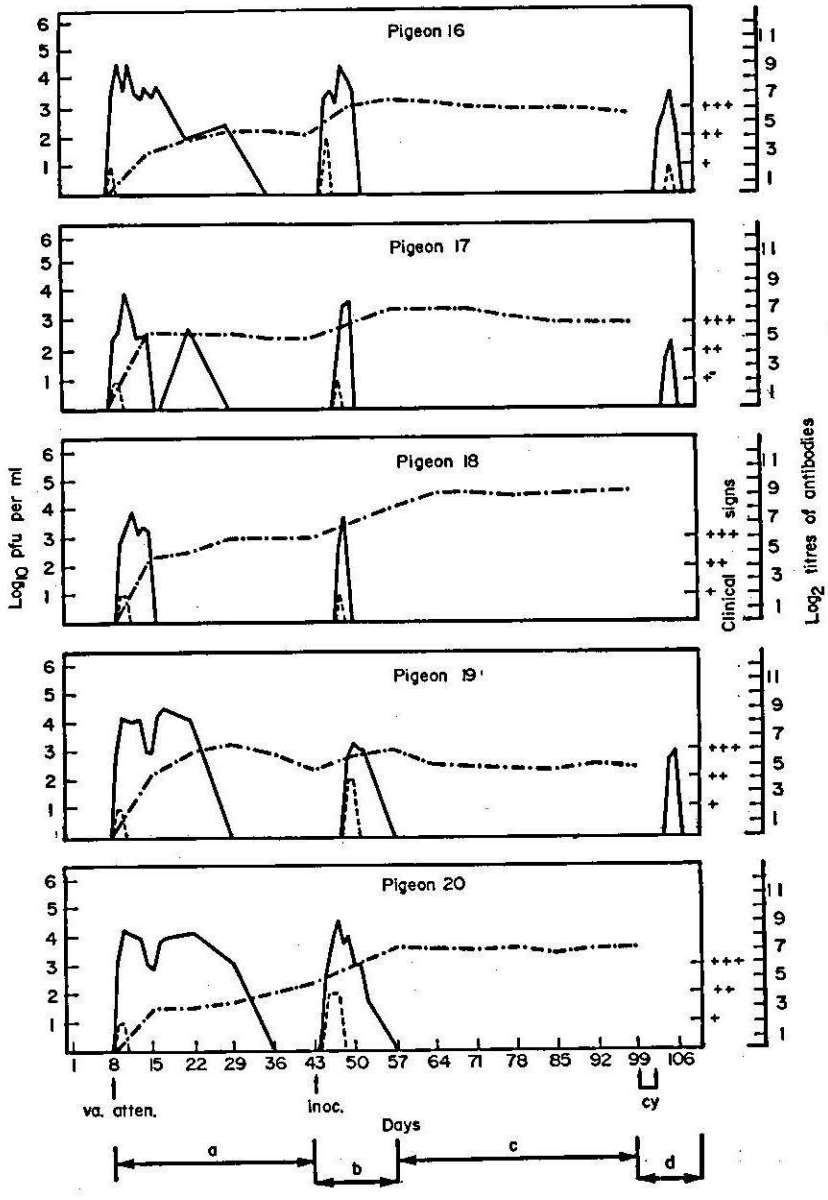

Fig. 4. Excretion of PHV_1 (——), clinical signs (– – –) and presence of antibodies (—·—) in group 4 pigeons. Cy = Cyclophosphamide treatment; va. inac. = inactivated vaccine; va. atten. = attenuated vaccine; inoc. = virulent challenge; a, b, c and d = periods of experiment.

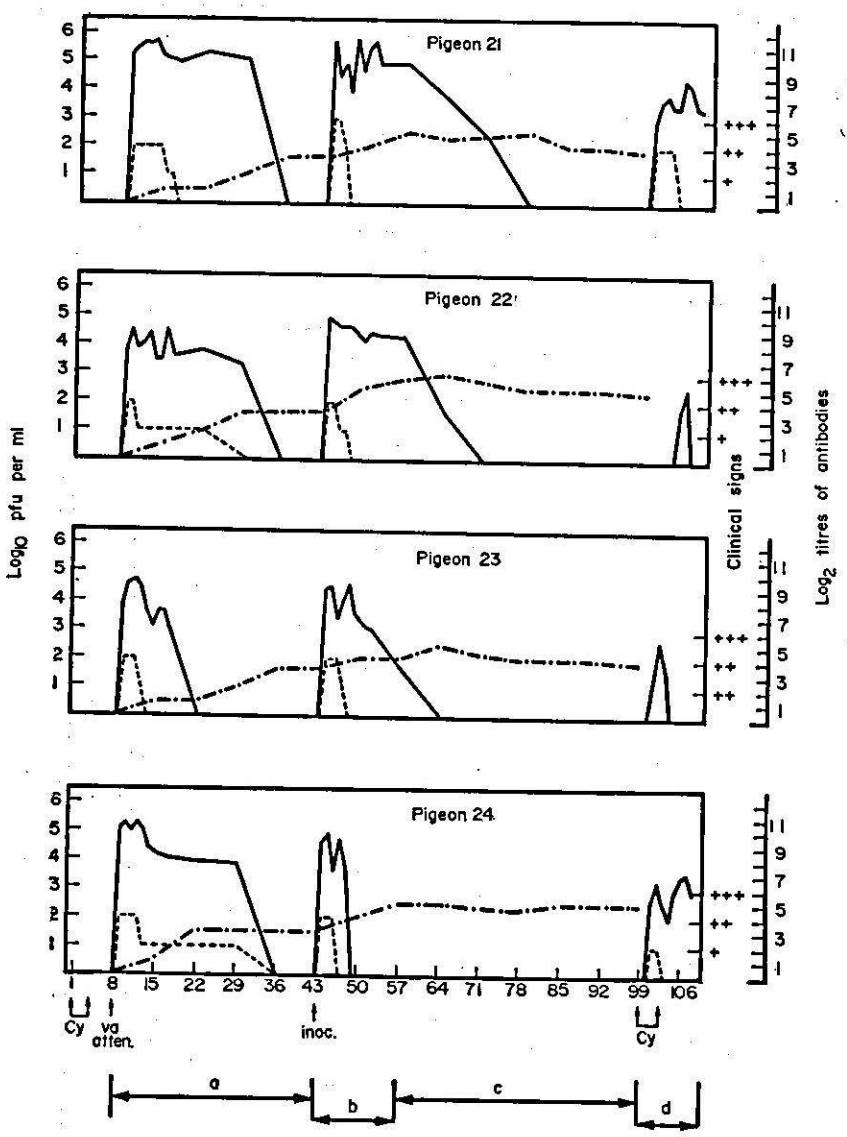

Fig. 5. Excretion of PHV_1 (——), clinical signs (– – –) and presence of antibodies (—·—) in group 5 pigeons. Cy = Cyclophosphamide treatment; va. inac. = inactivated vaccine; va. atten. = attenuated vaccine; inoc. = virulent challenge; a, b, c and d = periods of experiment.

Period c: Significant differences appeared between the groups ($P<0{\cdot}05$). Indeed, no spontaneous re-excretion occurred in group 4, whereas 3 pigeons of group 5 re-excreted virus.

Period d: Only 3 pigeons of group 4 re-excreted viral particles after Cy-treatment but the mean amount of virus was not significantly different between the groups.

Comparison between groups 3 *and* 6 (*Table* 1*; Figs* 3 *and* 6). During periods b

and c excretion was similar in both groups, but significant differences were observed during period d ($P<0{\cdot}01$): less viral re-excretion was observed in the group injected with inactivated vaccine.

Comparison between groups 1 *and* 4 (*Table* 1; *Figs* 1 *and* 4). No significant differences were observed during periods b, c and d.

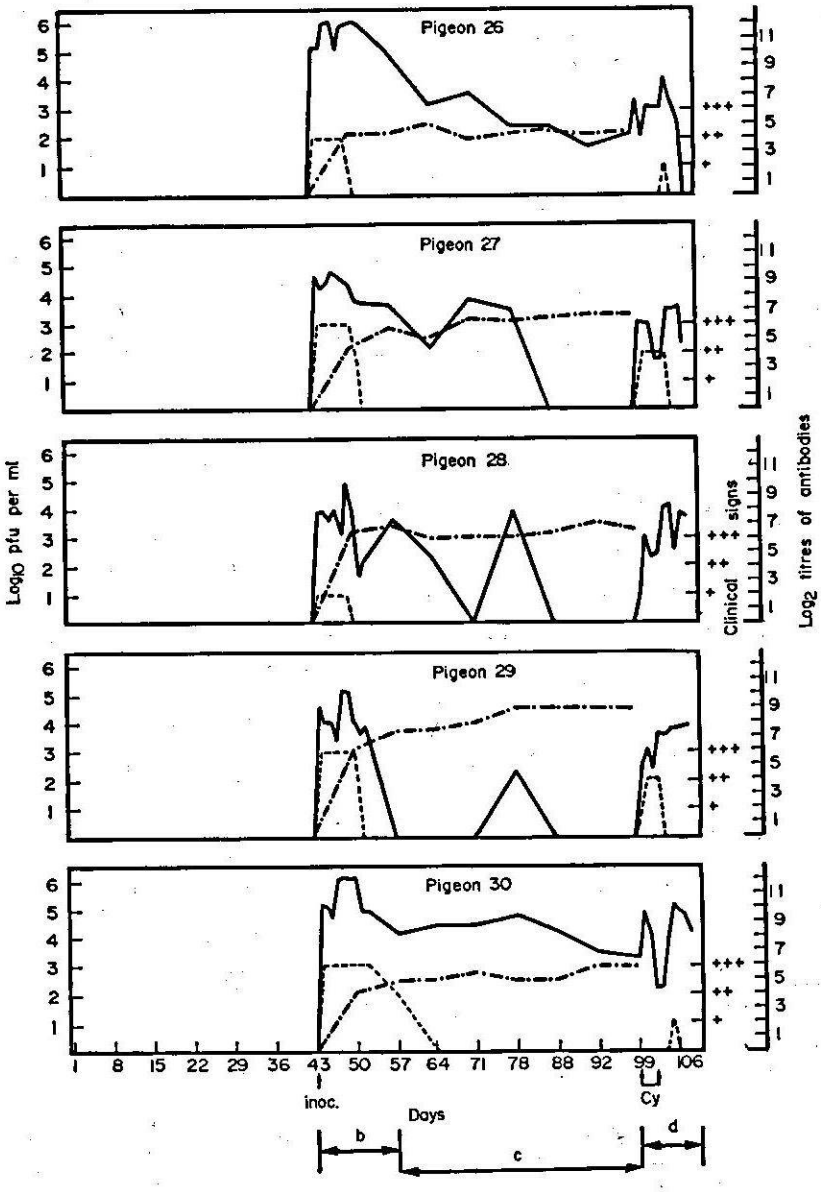

Fig. 6. Excretion of PHV_1 (——), clinical signs (– – –) and presence of antibodies (—·—) in group 6 pigeons. Cy = Cyclophosphamide treatment; va. inac. = inactivated vaccine; va. atten. = attenuated vaccine; inoc. = virulent challenge; a, b, c and d = periods of experiment.

Comparison of Sero-neutralization Titres Between Groups

The only significant differences were observed during period a between groups 1 and 2 ($P<0{\cdot}05$) (Figs 1 and 2), 4 and 5 ($P<0{\cdot}05$) (Figs 4 and 5) and 1 and 4 ($P<0{\cdot}01$) (Figs 1 and 4). Indeed, titres were less important in groups immunocompromised by Cy-treatments before vaccination, and titres were higher in the group vaccinated with inactivated vaccine than in the group vaccinated with the attenuated one.

Clinical Signs

These are given on Figs 1 to 6.

The attenuated vaccine strain was still mildly pathogenic for pigeons. Indeed, during 1 or 2 days after vaccination, oedematous congestion of the pharyngeal mucous membrane was observed and signs and lesions were exacerbated in animals previously Cy-treated. Vaccination decreased viral excretion after challenge as well as re-excretion after Cy-treatment, but mild and transient signs were observed in some pigeons, especially in groups where animals were immunocompromised.

DISCUSSION

Both vaccines, whether attenuated or inactivated, gave similar results in that they were able to control primary excretion after challenge and reduce clinical signs. However, sero-conversion was higher after injections of inactivated vaccine, which also prevented detectable viral excretion in 2 animals. Moreover, the attenuated vaccine was mildly pathogenic, especially when the birds were immunocompromised by prior Cy-treatment.

Neither attenuated nor inactivated vaccines were able to prevent the appearance of carriers since most of the pigeons re-excreted virus after Cy-treatment subsequent to challenge. It should be noted that it was not possible to distinguish between virulent and attenuated viral particles during episodes of re-excretion in pigeons which had been vaccinated with live vaccine.

Both vaccinations helped to prevent spontaneous viral re-excretion, except in previously immunocompromised animals, and therefore helped to control viral dissemination. Experimental re-excretion was also reduced if animals were vaccinated with inactivated vaccine after challenge.

For the control of subclinical infection vaccination is of little value, mainly because young pigeons are often infected shortly after hatching (Vindevogel and Pastoret, 1980) and therefore before vaccination is practicable. Furthermore, it does not prevent the carrier state. A solution might be to hyperimmunize parents with killed vaccine before the breeding period. In that instance, inactivated vaccine seems to be better than the attenuated one since it gives a higher serological response; but repeated intramuscular injections with oil adjuvant are not advisable in flying pigeons. It should also be mentioned that Cy-treatment impedes vaccination and failures may thus be expected in practice.

SUMMARY

Vaccination trials were made with live attenuated or inactivated vaccines to control pigeon herpesvirus infection (*Pigeon herpesvirus 1*, PHV_1).

Both kinds of vaccine gave similar results. Both of them were able to reduce primary viral excretion and clinical signs after challenge. Nevertheless, neither attenuated nor inactivated vaccines were able to prevent the appearance of carriers since most of the pigeons re-excreted virus after cyclophosphamide-treatment. However, vaccination would help to prevent spontaneous viral re-excretion and therefore to control viral dissemination. When animals were vaccinated with inactivated vaccine after challenge with a virulent strain, re-excretion was also reduced.

REFERENCES

Coignoul, F., and Vindevogel, H. (1980). Cellular changes in the bursa of Fabricius and thymus of cyclophosphamide-treated pigeons. *Journal of Comparative Pathology*, **90,** 395–400.

Dagenais, L., Lansival, B., Pastoret, P. P., and Kaeckenbeeck, A. (1980). Détection par contre-immuno-électro-osmophorèse (C.I.E.O.P.), des *rotavirus* dans les matières fécales de veau, après concentration au polyéthylène glycol 6000 (P.E.G. 6000). *Annales de Médecine Vétérinaire*, **124,** 223–227.

Krupička, V., Šmid, B., Valiček, L., and Pleva, V. (1970). Isolation of a herpesvirus from pigeon on the chorioallantoic membrane of embryonated eggs. *Veterinary Medicine (Praha)*, **15,** 609–612.

Schwers, A., Pastoret, P. P., Vindevogel, H., Leroy, P., Aguilar-Setién, A., and Godart, M. (1980). Comparison of the effect of trisodium phosphonoformate on the mean plaque size of pseudorabies virus, infectious bovine rhinotracheitis virus and pigeon herpesvirus. *Journal of Comparative Pathology*, **90,** 625–633.

Schwers, A., Vindevogel, H., Leroy, P., and Pastoret, P. P. (1981). Susceptibility of different strains of pigeon herpesvirus to trisodium phosphonoformate. *Avian Pathology*, **10,** 23–29.

Snedecor, G. W., and Cochran, W. G. (1968). *Statistical Methods*, 6th Edit. Iowa State University Press, Ames, Iowa, U.S.A., pp. 130–131.

Vindevogel, H., and Duchatel, J. P. (1977). Réceptivité de le perruche au virus herpès du pigeon. *Annales de Médecine Vétérinaire*, **121,** 193–195.

Vindevogel, H., and Duchatel, J. P. (1978). Contribution à l'étude de l'étiologie du coryza infectieux du pigeon. *Annales de Médecine Vétérinaire*, **122,** 507–513.

Vindevogel, H., Duchatel, J. P., and Gouffaux, M. (1977). Pigeon herpesvirus. Pathogenesis of pigeon herpesvirus in chicken embryo fibroblasts. *Journal of Comparative Pathology* **87,** 597–603.

Vindevogel, H., Kaeckenbeeck, A., and Pastoret, P. P. (1981). Fréquence de l'ornithose-psittacose et de l'infection herpétique chez le pigeon voyageur et les psittacidés en Belgique. *Revue Médicale de Liège*, **36,** 693–696.

Vindevogel, H., and Pastoret, P. P. (1980). Pigeon herpetic infection: Natural transmission of the disease. *Journal of Comparative Pathology*, **90,** 409–413.

Vindevogel, H., and Pastoret, P. P. (1981). Pathogenesis of pigeon herpesvirus infection. *Journal of Comparative Pathology*, **91,** 415–426.

Vindevogel, H., Pastoret, P. P., and Aguilar-Setién, A. (1981). Assessment of phosphonoformate-treatment of pigeon herpesvirus infection in pigeons and budgerigars, and Aujeszky's disease in rabbits. *Journal of Comparative Pathology*, **92,** 177 180.

Vindevogel, H., Pastoret, P. P., Burtonboy, G., Gouffaux, M., and Duchatel, J. P. (1975). Isolement d'un virus herpès dans un élevage de pigeons de chair. *Annales de Recherches Vétérinaires*, **6,** 431–436.

Vindevogel, H., Pastoret, P. P., and Burtonboy, G. (1980a). Pigeon herpetic infection: Excretion and re-excretion of virus after experimental infection. *Journal of Comparative Pathology*, **90,** 401–408.

Vindevogel, H., Pastoret, P. P., Leroy, P., and Coignoul, F. (1980b). Comparaison de trois souches de virus herpétique isolées de psittacidés avec le virus herpès du pigeon. *Avian Pathology*, **9,** 385–394.

[*Received for publication, July 4th* 1981]