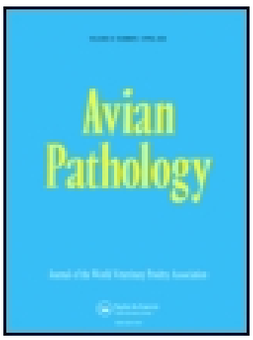

Avian Pathology

ISSN: 0307-9457 (Print) 1465-3338 (Online) Journal homepage: https://www.tandfonline.com/loi/cavp20

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To cite this article: Anne Schwers , H. Vindevogel , P. Leroy & P.P. Pastoret (1981) Susceptibility of different strains of pigeon herpesvirus to trisodium phosphonoformate, Avian Pathology, 10:1, 23-29, DOI: 10.1080/03079458108418455

To link to this article: https://doi.org/10.1080/03079458108418455

Published online: 12 Nov 2007.

Avian Pathology, 10: 23–29, 1981

SUSCEPTIBILITY OF DIFFERENT STRAINS OF PIGEON HERPESVIRUS TO TRISODIUM PHOSPHONOFORMATE

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SUMMARY

The susceptibility to trisodium phosphonoformate of five strains of pigeon herpesvirus (*Pigeon Herpesvirus 1*, PHV) was compared by measuring the mean plaque size variations in the presence of different concentrations of the compound.

Significant differences in their susceptibility to phosphonoformate were observed, but none of them was found to be naturally resistant. This finding may be of significance for a possible clinical application in the treatment of pigeon herpesvirus infection.

INTRODUCTION

Disodium phosphonoacetate and trisodium phosphonoformate are recently discovered antiherpetic compounds which are slightly cytotoxic and exhibit an important antiviral effect due to a specific inhibition of the herpesvirus-induced DNA-polymerase (Overby *et al.*, 1974; Mao and Robishaw, 1975; Helgstrand *et al.*, 1978). A possible mechanism of inhibition is an interference with a pyrophosphate binding site on the polymerase (Öberg and Helgstrand, 1978; Helgstrand and Öberg, 1978).

Susceptibility to these two compounds has been reported for all the herpesviruses so far studied, for instance turkey herpesvirus (*Turkey Herpesvirus 1*, HVT), Marek's disease virus (*Phasianid Herpesvirus 2*, MDV) (Lee *et al.*, 1978; Reno *et al.*, 1978) and pigeon herpesvirus (*Pigeon Herpesvirus 1*, PHV) (Schwers *et al.*, 1980). Some strains of several herpesviruses were found to be resistant (Jofre *et al.*, 1977; Duff *et al.* 1978; Reno *et al.*, 1978).

For therapeutic purpose, the use of phosphonoacetate is limited because of its topical irritative potential (Kern *et al.*, 1978), its toxicity for laboratory animals and its accumulation in bones (Bopp *et al.*, 1977). Phosphonoformate does not irritate the skin or the mucous membranes, and has been successfully used for the treatment of experimental cutaneous and ocular herpes simplex type 1 (*Human Herpesvirus 1*, HSV1) infection (Alenius *et al.*, 1978; Öberg *et al.*, 1978) or genital infection of guinea pigs

Received 21 April 1980
Accepted 31 July 1980

with herpes simplex type 2 (*Human Herpesvirus 2*, HSV2) (Alenius and Nordlinder, 1979). Furthermore, phosphonoformate has only minor effects on normal cellular metabolism (Stenberg and Larsson, 1978).

The present report deals with the comparison between the susceptibility of several strains of pigeon herpesvirus to phosphonoformate by measuring the effect of this compound on the mean size of plaques produced under agarose overlay. This investigation was performed with a view to a possible clinical application for the treatment of local lesions produced by PHV.

MATERIALS AND METHODS

Viruses

Two strains of PHV (PHV/B/Cu_2 and PHV/B/Cu_3) were isolated in our laboratory at Brussels (Cureghem) from young pigeons (Vindevogel and Duchatel, 1978), and a third one from psittacine birds (PHV/Psi/B/Cu) (Vindevogel *et al.*, 1978; Vindevogel, *et al.*, 1980).

A strain of PHV isolated in Czechoslovakia (PHV/Cz/V 236/69) (Krupička *et al.*, 1970) was kindly provided by Dr. Šmíd (Veterinary Research Institute, Hudcova), and another one isolated in Australia (PHV/A/B 50) (Boyle and Binnington, 1973) was obtained from Dr. Simmons (Animal Research Institute, Queensland).

Cell cultures

Chicken embryo fibroblasts (CEF) were cultured as previously described (Vindevogel *et al.*, 1975).

Production of plaques

Confluent cell monolayers, grown in Petri dishes (plastic Falcon; 30 mm diam.) were inoculated with 0.2 ml of 10-fold viral dilutions. After 1 hour of adsorption, the infected cells were covered with Minimum Essential Medium (MEM) containing 1% agarose (indubiose) and 2% foetal bovine serum. This overlay medium was supplemented with trisodium phosphonoformate at the following final concentrations: 10 and 100 μM/ml. As controls, the same procedure was followed on uninfected cells and inoculated cells were incubated with maintenance medium devoid of phosphonoformate.

All the experiments were done on the same day, in the same CEF culture and with the same medium.

The cells were fixed with formaldehyde after 4 days of incubation, then stained with May-Grünwald Giemsa (Vindevogel *et al.*, 1975).

The experiments were twice repeated.

Measurement of mean plaque size

Cell cultures inoculated with the optimal viral dilution, causing a sufficient number of isolated plaques, were examined according to a method previously described (Jetteur *et al.*, 1979; Pastoret *et al.*, 1979; Schwers *et al.*, 1980).

Briefly, plaques were printed with an offset camera to obtain a final magnification of x 20. For each virus at each concentration of phosphonoformate, the areas of 50 plaques taken at random were measured with an OH Kempton planimeter.

Statistical tests

Normality test. The normality of the plaque size distribution was studied by skewness and kurtosis tests.

Analysis of variance. The effect of the addition of trisodium phosphonoformate at increasing concentrations in the medium was studied by a one-way analysis of variance.

Student's t-test was used to determine which concentrations of phosphonoformate were producing a significant reduction of the plaque size.

Interaction test. To see if the susceptibility to phosphonoformate was the same for each strain, a two-way analysis of variance was performed and the hypothesis of absence of interaction was tested by the F-test.

RESULTS

The mean of the plaque size for PHV is given in Table 1.

Table 1. Mean plaque size of PHV strains.

Strains	Mean plaque size at different concentrations (μM/ml) of phosphonoformate		
	0	10	100
PHV/B/Cu_2	0.7627[a]	0.5432	0.1316
PHV/B/Cu_3	0.4438	0.2331	0.1910
PHV/Psi/B/Cu	0.5644	0.4769	0.2135
PHV/Cz/V 236/69	0.5160	0.2910	0.1157
PHV/A/B 50	0.4800	0.3452	0.1621

a Values are given in mm^2 and calculated from 50 plaques.

Normality test

The sizes of the plaque population produced by PHV strains were not quite normally distributed in the presence of phosphonoformate. The square root of the plaque size always followed a normal distribution, thus allowing variance analysis.

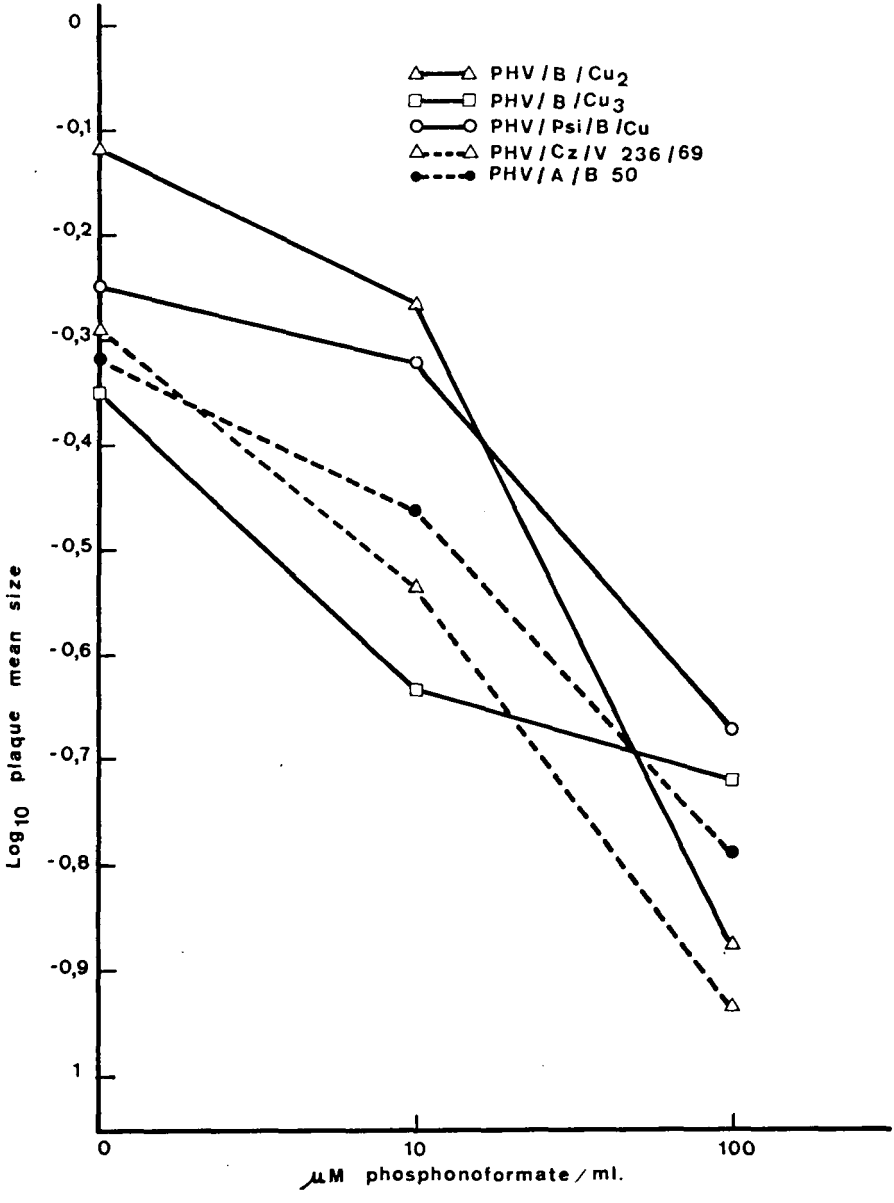
Analysis of variance

Addition of phosphonoformate induced a marked reduction of the mean plaque size ($P < 0.001$) for each of the five strains studied.

By use of the t-test, a significant reduction of the mean plaque size with each concentration of trisodium phosphonoformate (10, 100 μM/ml) ($P < 0.02$) was found, except for PHV/B/Cu_3 and PHV/Psi/B/Cu strains. Indeed, the addition of more than 10 μM/ml phosphonoformate in the presence of PHV/B/Cu_3 did not induce a further reduction in the mean size; for PHV/Psi/B/Cu we did not observe a significant reduction of the plaque size after addition of 10 μM/ml of phphonoformate, but we did so after the addition of 100 μM/ml (Text-fig.1).

Comparison between the strains by their mean plaque size reduction in the presence of phosphonoformate

Significant differences were observed between the strains from the point of view of mean plaque size reduction in the presence of phosphonoformate ($P < 0.001$). All the strains studied differed from each other on the basis of the determination of their reduction rate in the presence of 10 or 100 μM/ml of phosphonoformate (Text-fig.1). The most susceptible strain was PHV/Cz/V 236/69, followed by PHV/B/Cu_2, PHV/A/B 50 and PHV/B/Cu_3; PHV/B/Cu_3 was less susceptible than the other strains at high concentrations, whereas PHV/Psi/B/Cu was the least susceptible. A second experiment gave similar results.



Text-fig. 1. Comparison between the mean plaque size reduction of PHV strains with increasing concentrations of trisodium phosphonoformate.

DISCUSSION

All the PHV strains studied were susceptible to trisodium phosphonoformate. In fact, the mean plaque size of the different PHV strains was reduced in the presence of phosphonoformate and, by comparing their reduction rate, it can be shown that each strain differs from any other according to its susceptibility to the compound. This agrees with the observations of Reno *et al.* (1978) who described variations between several strains of HVT in their susceptibility of phosphonoformate, some of them being resistant.

The strain isolated from psittacines was less susceptible than the other strains although it was a typical PHV strain (Vindevogel *et al.*, 1980).

It remains to be studied whether these differences within the same viral species are as important as those between two different species (Schwers *et al.*, 1980).

The finding that there are differences between several strains of PHV in the presence of phosphonoformate may be important from the point of view of a possible clinical application in the treatment of local lesions produced by PHV infection, notwithstanding the fact that none of the PHV strains studied was found to be naturally resistant to the compound.

Acknowledgements

We wish to thank Dr. B. Öberg (Astra Häkemedel AB, Sweden) for providing us with trisodium phosphonoformate, Dr. Šmíd (Veterinary Research Institute, Hudcova, Czechoslovakia) and Dr. Simmons (Animal Research Institute, Queensland, Australia) for providing the pigeon herpesvirus strains V 236/69 and B 50.

We also wish to thank Dr. A. Aguilar-Setién, Annick Verheyden and Martine Godart for their help, and Lise Dagenais for revising the manuscript.

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RESUME

Etude la sensibilité de différentes souches de virus Herpès du pigeon au phosphonoformate de sodium

La sensibilité au phosphonoformate de sodium de cinq souches de virus herpès du pigeon (*Pigeon Herpesvirus 1*, PHV) a été étudiée en comparant les variations de la surface des plages liées aux différentes concentrations du produit.

Les cinq souches montrent des sensibilités différentes au phosphonoformate, mais aucune d'entre elles n'y est naturellement résistante. Ces résultats sont encourageants pour une utilisation clinique du phosphonoformate dans le traitement de l'infection herpétique du pigeon.

ZUSAMMENFASSUNG

Die Empfindlichkeit verschiedener Taubenherpesvirusstämme gegen Trinatriumphosphonoformat

Die Empfindlichkeit von fünf Stämmen des Taubenherpesvirus (Taubenherpesvirus 1, PHV) gegen Trinatriumphosphonoformat wurde durch Messungen der Größenvariationen der Plaques in Gegenwart verschiedener Konzentrationen dieser Substanz vergleichend untersucht.

Es wurden signifikante Unterschiede in der Empfindlichkeit gegenüber Phosphonoformat beobachtet, aber keinerlei natürliche Resistenz. Dieses Ergebnis könnte bei einer evtl. klinischen Anwendung zur Behandlung der Taubenherpesvirus-Infektion von Bedeutung sein.