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DETERMINATION OF HOST SPECIFICITY OF PIGEON POX AND FOWL POX VIRUSES ISOLATED FROM A FIELD OUTBREAK

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Summary

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The aim of the study was to find out the host specificity of pigeon pox virus-1 (PPV-1) and fowl pox virus-2 (FPV-2) following cross infection with field strains from Bangladesh. The cross infection was investigated in five groups (n=5) of pigeons and seven groups of chickens (n=5) four weeks of age. Irrespective of route of inoculation each bird received either 0.1 mL of PPV-1 or FPV-2 having a virus concentration of $10^{6.2}$ EID_{50} and $10^{6.25}$ EID_{50} per 0.1 mL chorioallantoic membrane (CAM) suspension, respectively. A group of uninfected control birds were maintained in both cases. All pigeons infected either orally or intravenously did not respond to infection with FPV-2 and did not manifest any pox lesion. Experimentally infected chickens with PPV-1 by oral, intravenous (i.v.) or wing-web puncture (WWP) routes did not show any pox lesions except for a "take reaction" at the inoculation site. On the other hand, dispersed pox lesions were noticed in chickens exposed to FPV-2 by all three routes. The severity of infection in experimental birds was manifested with a descending magnitude after i.v., oral and WWP inoculation. It could be concluded that the field isolates of PPV-1 and FPV-2 manifested considerable host specificity to pigeons and chickens, respectively.

Key words: fowl pox virus, host specificity, pigeon pox virus

INTRODUCTION

The avian pox viruses form a subgroup of viruses of varying cross-relationship degrees. They affect a wide range of sixty bird species (Tripathy, 1991) of various families (Bolte *et al.*, 1999). The infection could be natural or artificial (Tripathy & Cunningham, 1984) in cutaneous or diphtheric/pharyngeal forms, or both (Fenner *et al.*, 1993). Sometimes viruses produce a protein similar to an epidermal growth factor (Moss, 1996). They multiply easily in cell cultures and on the chorioallantoic membrane of embryonated eggs and form type A cytoplasmic inclusions in the cells of these culture systems (Murphy *et al.*, 1995). The genome of the fowl pox virus is more than 100 kb larger than the orthopoxvirus vaccinia, indicating that the avipox viruses have the potential to code for more proteins than other groups of poxviruses (Coupar *et al.*, 1990).

Host specificity is considered to be one of the important criteria for differentiation of avian poxviruses. Vaccines of fowl pox or pigeon pox virus origin have been routinely used for more than half a century to prevent fowl pox in commercial poultry in endemic areas. However, in

recent years, outbreaks of fowl pox have occurred in previously vaccinated flocks due to the emergence of variant strains of fowlpox virus (FPV) and to the fact that the novel FPV exhibited enhanced virulence after integration of avian reticuloendotheliosis virus (REV) into their genomes (Singh *et al.*, 2000).

Mortality and morbidity related to pigeon poxvirus infection may be very high in pigeons (Tripathy, 1991). During the course of this study, a reference strain could not be procured in spite of repeated attempts. In order to overcome the problem, the identity of the field pigeon pox virus (PPV) isolates was compared with field fowl pox virus (FPV) isolates through *in vivo* cross infections of natural hosts such as pigeons and chickens, respectively. Interpretation of host specificity of agents was based on the formation of atypical pox lesions, generalized, dispersed or local (focal) infection, or death of experimental hosts.

The study was planned to unveil the host specificity of fowl pox and pigeon pox virus isolates prevailing in Bangladesh.

MATERIALS AND METHODS

Under a project of BARC (Bangladesh Agricultural Research Council) this study was conducted in the laboratory of the Department of Microbiology and Hygiene after the approval of Advance Studies Board of the University.

Viral isolates

One of the field isolates of PPV, identified as PPV-1, was selected for this investigation. This virus isolate was earlier adapted on chorioallantoic membranes (CAM) of growing CEE (chicken egg embryo) (Cornwell & Weir, 1970) through six serial passages and stored at –25 ^{o}C for future use.

Fowl pox virus (FPV) isolates were obtained from 5 clinical cases of naturally infected chickens showing characteristic pox lesions. The clinical signs of infection included manifestation of pox nodule (pox lesions) on the comb, wattles, commissure of beak, eyelids and in some cases – legs of birds (Fenner *et al.*, 1993). The isolation of FPV from clinical specimens was similar to the procedure described by Siddique (1998). The obtained FPV isolates were numbered as FPV-1-5 and stored at –25 ^{o}C. One of these isolates, namely FPV-2 was adapted through six serial passages on CAM of developing CEE (Cornwell & Weir, 1970) and was used for this experiment.

Experimental birds

A total of 25 healthy pigeons, four weeks of age were procured from the local market and were reared in the poultry house of the Department of Microbiology and Hygiene, Bangladesh Agricultural University.

Thirty-five White Leghorn healthy chicks aged four weeks were transferred to the departmental poultry shed. The chicks and pigeons were reared and maintained in separate houses and after one week of adaptation were used for experimental infection. The birds were provided with feed and other condition as recommended by management requirements except vaccination and medication through the period of study.

Titration of virus

This was performed with the respective CAM suspensions of the two virus isolates (PPV-1 and FPV-2) following the procedure described by Tripathy & Cunnigham (1984). The EID_{50} titre per

0.1 mL of suspension was found to be $10^{6.2}$ and $10^{6.5}$ for PPV-1 and FPV-2, respectively.

Route and dose of infection

The route of experimental infection for pigeons was either oral or intravenous (i.v.) through the wing vein, while for chickens an additional route of inoculation via wing web puncture (WWP) was used. Irrespective of host and route of infection, each bird was inoculated with 0.1 mL of virus suspension where the virus concentration was $10^{6.2}$ and $10^{6.5}$ EID_{50} of PPV and FPV, respectively.

Experimental design

Determination of host spectrum was carried out according to the layout shown in Table 1. Pigeons were divided into five equal groups. Birds of groups I and II were inoculated with PPV-1 via oral and i.v. routes respectively. The other two groups, III and IV, were inoculated with FPV-2 through oral and i.v. routes respectively while group V served as uninoculated control.

The chickens were divided into seven equal groups (I to VII). Birds of groups I, II and III were inoculated with PPV-1 using oral, i.v. and WWP routes respectively. Groups IV, V and VI were administered with FPV-2 via oral, i.v. and WWP routes, while group VII was maintained as uninfected control.

The birds were maintained for seven weeks following the experimental infection until they attained the age of 12 weeks.

RESULTS

Host specificity of PPV was determined on the basis of susceptibility and pathogenicity of PPV-1 and FPV-2 following exposure to virulent field isolates of the viruses by different routes of inoculation in pigeons and chickens (Table 2).

It was observed that pigeons inoculated with PPV-1 through oral route (Group I) manifested mild dispersed infection in three out of five birds while the remaining two exhibited local lesions (Table 2). On the other hand, four out of five birds of group II infected intravenously exhibited dispersed pox lesions on different parts of the body and the remaining one manifested local pox lesions. The

Table 1. Experimental design for host specificity of PPV and FPV

Host	Group of birds	Number of birds	Virus isolates	Virus titre (log10 ID_{50} /0.1 mL)	Route of infection
Pigeons	I	5	PPV-1	6.2	oral
	II	5	PPV-1	6.2	i/v
	III	5	FPV-2	6.5	oral
	IV	5	FPV-2	6.5	i/v
	V	5	Uninfected control	–	–
Chicks	I	5	PPV-1	6.2	oral
	II	5	PPV-1	6.2	i/v
	III	5	PPV-1	6.2	WWP
	IV	5	FPV-2	6.5	oral
	V	5	FPV-2	6.5	i/v
	VI	5	FPV-2	6.5	WWP
	VII	5	Uninfected control	–	–

Table 2. Determination of host specificity in pigeons and chickens

Host	Virus isolates	Route of inoculation (group)	Day of lesion appearance	Number of birds showing characteristic pox lesions			Dead birds	Number (%) of birds with infection
				Dispersed	Local	Site of inoculation		
Pigeon	PPV-1	oral (I)	8–11	3	2	–	1	5 (100%)
	PPV-1	i.v. (II)	7–10	4	1	+	3	5 (100%)
	FPV-2	oral (III)	–	–	–	–	–	0 (0%)
	FPV-2	i.v. (IV)	6–8	–	–	+*	–	0 (0%)
	Control	– (V)	–	–	–	–	–	0 (0%)
Chicks	PPV-1	oral (I)	–	–	–	–	–	–
	PPV-1	i.v. (II)	6–7	–	–	+* (5)	–	–
	PPV-1	WWP (III)	5–7	–	–	+** (5)	–	–
	FPV-2	oral (IV)	8–11	2	3	–	1	5 (100%)
	FPV-2	i.v. (V)	6–9	4	1	♦ (5)	2	5 (100%)
	FPV-2	WWP (VI)	5–7	1	–	*** (5)	–	5 (100%)
	Control	– (VII)	–	–	–	–	–	–

+: positive; –: negative; * mild take reaction (2–4 papules) ** prominent take reaction (>4 papules); *** severe inflammatory take reaction; ♦: papules; (5) number of birds showing lesions

intensity of infection (pox lesions) was found to be severe in birds inoculated via the i.v. route (Group II) compared to those infected orally (Group I). On the other hand, none of the pigeons of groups III and IV, inoculated with FPV-2 either orally or i.v., where noticed to develop any lesions except mild take reactions (2–4 papules) at the site of i.v. inoculation in group IV (Table 2).

In case of experimental infection of chickens with PPV-1 none of the birds of groups I-III exhibited any pox lesion (nodules) irrespective of inoculation route. However, the birds from group III inoculated via WWP route exhibited a characteristic take reaction while birds of group II infected i.v. showed a mild reaction (2–4 papules).

In contrast, chickens inoculated with FPV-2 through the oral route (Group IV) exhibited dispersed as well as local pox lesions. Two birds died which either might be due to toxaemia produced by secondary bacterial infections or due to lesions in mouth that hampered feed intake and cause nutritional deficiency. When inoculated intravenously, all chickens from group V manifested dispersed pox lesions similar to the natural infection as described by Cunningham (1974). The chickens of group VI (infected with FPV-2 via WWP route) exhibited characteristic 'take reaction'. These lesions were mild and limited at the site of inoculation in all cases except one with dispersed pox lesions.

DISCUSSION

In general, the infection following i.v. inoculation was manifested more severely compared to these using either oral or WWP route. With this regard Cunningham (1972), Tripathy & Cunningham (1984) recorded that the severity of infection depended on the multiplication of the virus in the internal organs which nor-

mally followed the intravenous inoculation.

The authors further stated that the propagation of the virus at the site of inoculation resulted in local lesions which were signs of virus multiplication. Generalized pox lesions were observed when the birds were inoculated intravenously. Similarly, focal and generalized lesions appeared but in case of intradermally and orally inoculated birds, a mild intensity of infection was exhibited. These findings were very similar to those of Heuschele (1986) and Fenner *et al.* (1993).

The most important finding was that both the PPV and FPV were host specific. The results of the investigation were in agreement with the findings of Cunningham (1966; 1972) and Tripathy & Cunningham (1984) who described that pigeon pox and fowl pox viruses are host specific and produced disease in their respective hosts only. The finding also partially agreed with Afonso *et al.* (2000) who reported that the occurrence of the cyclobutane pyrimidine dimer photolyase homologue in FPV suggests the presence of a photoreactivation DNA repair pathway and this diverse complement of genes with likely host range functions in FPV demonstrates the significant viral adaptation to the avian host. However, Khodir & Mikhail (2006) reported that FPV is not extremely host specific and that PPV also produces the characteristic pox lesions in quails. So, with the above discussions, it can be concluded that the tested field isolates of pigeon pox virus-1 and fowl pox virus-2 were extremely host specific.

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