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Host susceptibility of different *Avipoxvirus* isolates

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Abstract
A *Fowlpox virus* (FWPV) and another *Pigeonpox virus* (PGPV) isolate of domestic fowl and pigeon origin were reciprocally infected in both chicks and pigeon squabs to evaluate their host pathogenicity. The FWPV isolate could infect chicks but failed to produce infection in squabs, whereas the PGPV could infect squabs but failed to infect chicks. FWPV developed characteristic pox lesions in chicks within 7-10 days post infection with a latency of 4-6 days. FWPV induced lesions progressed through a mild subcutaneous oedema, Popular to pustule-like swellings, transformed to scabs, spread to nearby areas and later shed and healed by a month's duration post-inoculation. Similarly, pigeon squabs inoculated with PGPV developed characteristic pox lesions (pustules) within 5-10 days post infection with a latency of 5-6 days until visible development of lesions characterized progressively from macular lesions to raised popular, pustule-like swellings that were larger and numerous than those in chicks infected with FWPV inoculum. These lesions spread to nearby areas, formed scabs and healed after a month's time. No diphtheritic form was observed in any birds with either FWPV or PGPV isolates. Scabs lesions from both chicks and squabs were confirmed by amplification of the partial *P4b* gene of *Avipoxvirus*. It was concluded that FWPV and PGPV are host-specific as cross infection under controlled conditions did not occur. It is also speculated that natural transmission of avian pox between chicken and pigeons are therefore unlikely.

Keywords: *Avipoxvirus*, *Fowlpox virus* (FWPV). Lesions; *Pigeonpox virus* (PGPV), *P4b* gene

Introduction
Avipoxvirus genus currently comprises of ten recognized species [1], of which *Fowlpox virus* (FWPV) is the type species. Although there is evidence of considerable heterogeneity among species of avian poxviruses [2], antigenic relationship exists among them, and some avian poxviruses are more closely related to some than others. A great range of pathogenicity is reported among various bird species exposed to different types of avian poxvirus, particularly in wild birds, which have high host specificity [2].
Some workers have suggested means of *Avipoxvirus* differentiation based on their pathogenicity for chickens, turkeys, pigeons and canaries [3, 4]. These differences in their pathobiology have been documented when isolates fail to establish infection in heterologous hosts [5-7].
In our laboratory, domesticated fowls, pigeons and turkey birds were screened for avipoxvirus infection from different areas in Jammu region, India, and on the basis of the phylogenetic analysis of the partial *P4b* gene sequences, whereby three different isolates- *Fowlpox virus* (FWPV), *Pigeonpox virus* (PGPV) and *Turkeypox virus* (TKPV) were identified. The FWPV and TKPV were phylogenetic ally 99% related and clustered within clade 1, while PGPV clustered within clade 2 [8]. In this context it was proposed to attempt cross infection studies of the isolated viruses with their heterologous hosts to see if differences in pathogenicity and productivity with other virus strain exist. Whereas the TKPV samples comprised of formalin preserved and archival specimens, infection studies with only FWPV and PGPV isolates were possible. The present communication describes the pathogeneicity and pathobiology of FWPV and PGPV isolates in a cross infection model using young pigeons and chicks.

Materials and Methods
Virus isolates: Viruses obtained from infected tissue or scabs of clinically affected domestic fowls and pigeons designated as *Fowlpox virus* (FWPV) and *Pigeonpox virus* (PGPV) were used. The virus from processed clinical samples were adapted on chorioallantoic membranes

(CAM) of chicken embryonated eggs (CEE), confirmed by PCR amplification of the *P4b* gene of *Avipoxvirus* and by multiple nucleotide alignment with reported sequences in the public database [8].

Chicken embryonated eggs (CEE): Approximately 10-12 day old embryonated chicken eggs from Government Poultry Hatchery Unit, Belicharana, under Animal Husbandry Department, Jammu were procured for virus inoculation. The eggs were acquired after ensuring that there was no history of Fowlpox infection in the parent flock.

Calculation of EID_{50}: The EID_{50} was estimated as per the 50% endpoint calculation [9]. Briefly, 20% suspensions from highly positive CAM infected with FWPV and PGPV was prepared and serial diluted in phosphate buffer saline (PBS, pH 7.4) up to 10^{-6} dilution. About 0.1 ml of each diluted inoculum (10^{-1} to 10^{-6}) was infected on the CAM of three 10-12 day old developing chicken embryos. A total of eighteen chicken embryonated eggs, three from each serial dilution were inoculated with FWPV isolate. Similarly, another eighteen eggs were inoculated with PGPV isolate. After 5 days of incubation, all the 36 eggs were opened for observing the presence or absence of Avipox induced CAM lesions. The calculated EID_{50} for FWPV isolate was 3.25 and for PGPV isolate were 3.50.

Birds for experimentation: To study the pathobiology of FWPV and PGPV field isolates in their respective hosts and between heterologous hosts, ten-day old fowl chicks and pigeon squabs were used. The birds were kept in separate groups with provision of *ad libitum* feed and water throughout the acclimatization and experimentation period.

Experimental Avipox induction in birds: Before inoculation, the birds were immunosuppressed with parental steroid administration using 0.2 ml dexamethasone (Dexasone, Cadila Pharmaceuticals Ltd., India) injected on two occasions on alternate days intramuscularly. On the next day of second injection, the birds were given three linear scratches about 2 cm long and 0.5-1 cm apart with a sterile needle below the wings. About 0.1 ml processed virus suspension after appropriate virus titre dilution was swabbed over the scarified skin on the under wings in chicks (Fig. 1A) and pigeon squabs (Fig. 1B). FWPV isolate was inoculated in five chicks and two squabs, while PGPV isolate was inoculated in another five chicks and two squabs as shown in Table 1. The scars were monitored for a period of 28 days for development and progression of Avipoxvirus induced lesions.

PCR confirmation of reinfection: For diagnosis and confirmation of reinfection, scabs were collected from the birds, processed for DNA isolation [8], and PCR was done targeting the *P4b* gene of *Avipoxvirus* using reported primers by Lee and Lee [10].

Results

Experimental infection with different *Avipoxvirus*: The pathobiology of different *Avipoxvirus* inoculation in chicks and pigeon hosts with development of cutaneous lesions is given in Table 2. It was observed that all five chicks inoculated with FWPV samples could be infected, whereas the same inoculum failed to establish infection in two squabs. Likewise, PGPV inoculum could infect the two squabs but failed to develop lesions in any of the five chicks.

Progression of lesions with different *Avipoxvirus*: The progressive lesions developed in fowl chicks and pigeon squabs at different post infection days with FWPV and PGPV are shown in Figure 2.

Fowl chicks inoculated with FWPV developed characteristic pox lesions within 7-10 days post infection. A latency of 4-6 days was observed for visible development of characteristic lesions. Initially a mild subcutaneous oedema developed around the scarified area and formation of a scar over the streaks. Papular lesions appeared as tiny raised areas of skin-approximately 3-5 mm across, and also around the feather follicles (Fig. 3A-C). The papules became darker and developed to soft, pustule-like swellings probably after secondary infection and persisted approximately till 10 days post inoculation (d.p.i.) or transformed to scabs. The spread of lesions was simultaneously observed after 14 d.p.i. in nearby areas of the skin, including spread to eyelids, head and face of the chicks. These scabs were sometimes naturally shed by abrasions and healed by a month's duration post-inoculation. No diphtheritic form was observed in any of the five chicks.

Similarly, pigeon squabs inoculated with PGPV developed characteristic pox lesions (pustules) within 5-10 days post infection. The progression of cutaneous lesions was similar to those in chicks infected with FWPV inoculum. A latency of 5-6 days was observed for visible development of lesions. A mild subcutaneous oedema developed around the scarified area and formed a scar over the streaks. Macular lesions appeared by 5 d.p.i. as discoloured areas (white to yellowish) on skin- approximately 3-8 mm across, and also around the feather follicles which developed to soft, raised papular lesions (Fig. 3D-F). The papules became darker and developed to soft, enlarged pustule-like swellings till 10 d.p.i. or transformed to scabs. The pustules were larger and numerous than those in chicks infected with FWPV inoculum. The spread of lesions was simultaneously observed after 14 d.p.i. in nearby areas of the skin, including spread to eyelids, head and face of the chicks. These scabs were sometimes naturally shed by abrasions and healed by a month's duration post-inoculation. No diphtheritic form was observed in either of the two squabs.

Confirmation of experimental *Avipoxvirus* infection in birds: Confirmation of successful establishment of *Avipoxvirus* infection in chicks infected with FWPV and squabs infected with PGPV inoculums was done by PCR. A predicted amplified product of 578 bp could be demonstrated in positive cases (Fig. 4).

Discussion

Experimental Study

In the present experimental study, it was observed that the Embryo Inoculation Dose (EID_{50}) of both FWPV and PGPV were similar. The reciprocal infection with FWPV and PGPV in heterologous hosts could not establish infection. Host specificity of FWPV and PGPV is in agreement with the findings of earlier workers [7, 11, 12]. Subtle differences in infection is otherwise described by Kabir *et al.* [13] in terms of the host specificity of PGPV to pigeons alone, but FWPV was found to infect both chicken and pigeons. Contrarily, Mohammed [14] reported that fowls could be infected with both FWPV and PGPV isolates, but reciprocally, pigeons could be infected with FWPV isolate only. With the same FWPV and PGPV isolates, earlier attempts to grow them on chicken embryonated eggs showed that PGPV produced

earlier and aggravated lesions on the chorioallantoic membrane than FWPV [15]. The reason for such variation is unclear, and is only speculative. But the general consensus is that most Avipoxviruses are host-specific [2].

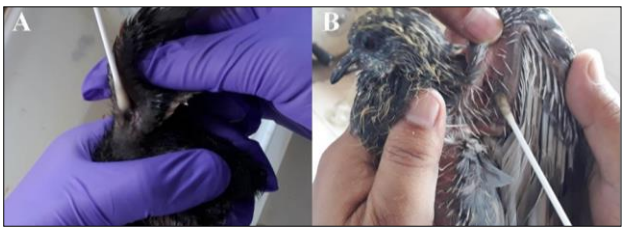

Fig 1: Experimental infection of birds with *Avipoxvirus* inoculum. (A) Application of *Fowlpox virus* (FWPV) inoculum by smearing on scarified skin on under-wings of 5 day old fowl chicks; (B) Application of *Pigeonpox virus* (PGPV) inoculum by smearing on scarified skin on under-wings of pigeon squabs

Table 1: Experimental design to determine host specificity of Fowlpox and Pigeo pox isolates

Avipoxvirus isolate	Species of birds infected	Number of birds	Virus titre (log_{10} ID_{50}/ 0.1 ml)
Fowlpox virus (FWPV)	Fowl chick	5	3.25
	Pigeon squab	2	3.50
Pigeonpox virus (PGPV)	Fowl chick	5	3.25
	Pigeon squab	2	3.50

Table 2: Pathobiology of different Avipoxvirus in homologous and heterologous hosts and development of cutaneous lesions

Avipoxvirus	Host species infected (number of birds)	Number of birds with cutaneous lesions (inoculated/ affected)
Fowlpox virus (FWPV)	Fowl chicks (n=5)	5/5
	Pigeon squabs (n=2)	0/2
Pigeonpox virus (PGPV)	Fowl chicks (n=5)	0/5
	Pigeon squabs (n=2)	2/2

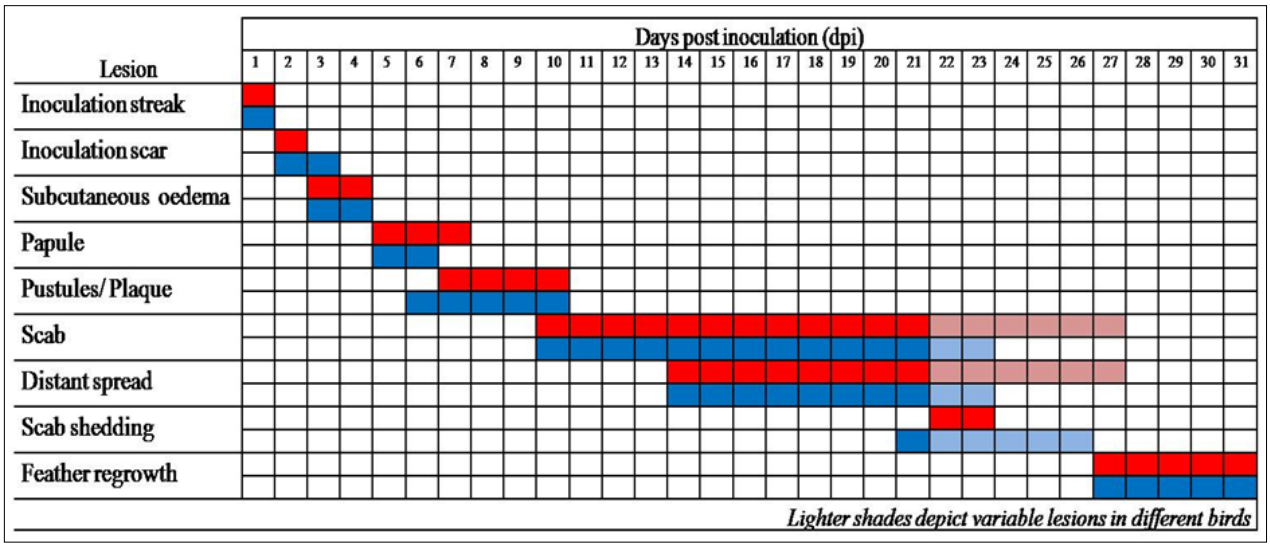

Fig 2: Progression of cutaneous lesions of *Fowlpox virus* (FWPV) inoculation in fowl chicks (red bar) and *Pigeonpox virus* (PGPV) inoculation in pigeon squabs (blue bar) in days post inoculation (dpi)

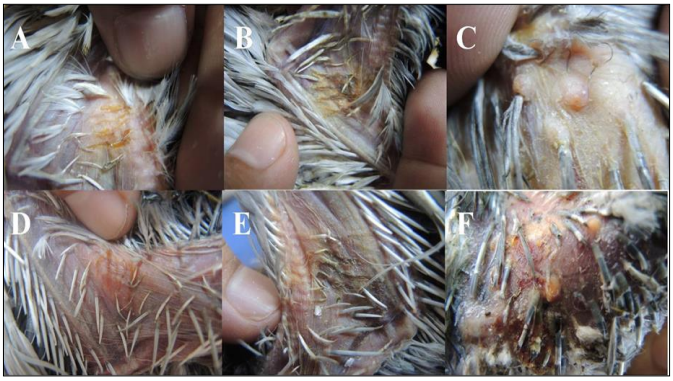

Fig 3: Experimental infection of fowl chicks with *Fowlpox virus* (FWPV) inoculum and pigeon squabs with *Pigeonpox virus* (PGPV) inoculum. (A) FWPV inoculated scarified tissue in chicks on 2 d.p.i.; (B) FWPV inoculated scarified tissue in chicks on 4 d.p.i. showing subcutaneous oedema; (C) FWPV induced lesions in chicks with development of papules on 7 d.p.i; (D) PGPV inoculated lesions in squabs on 2 d.p.i.; (E) PGPV inoculated scarified tissue in squabs on 4 d.p.i. showing oedema; (F) PGPV induced lesions in squabs depicting development of papules on 6 d.p.i.

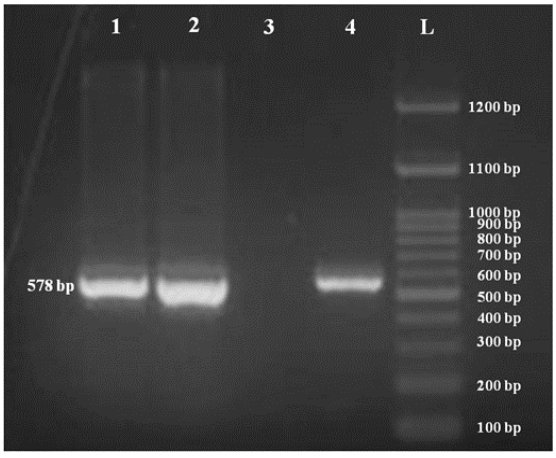

Fig 4: Confirmation of *Avipoxvirus* reinfection by PCR targeting the partial *P4b* gene (578 bp). Lane 1- Cutaneous scabs from fowl chicks experimentally infected with *Fowlpox virus* (FWPV); 2- Cutaneous scabs from pigeon squabs experimentally infected with *Pigeonpox virus* (PGPV); 3- Negative control; 4- Fowlpox commercial vaccine virus; L- 1,2 kbp DNA Ladder

Conclusion

The present investigation indicates that FWPV and PGPV are host-specific since cross infection under controlled conditions did not occur. In such event it can be concluded that natural transmission between chicken and pigeons are unlikely.

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