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Virulence and Charecteristics of Attenuated Field Fowl Pox Virus in Embryonated Chick Embryo (Ece)

KEYWORDS: Fowl pox virus, Chick, Embryo, Passage, Virulence,

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ABSTRACT *Avian pox virus represents a large group of virus which belongs to the pox viridae family. This virus mainly infect and cause diseases in the poultry which causes economic losses to the poultry industry. The present work is based on the analysis of field fowl pox virus with attenuation of different passages in ECE's. For this, the sample was collected from the infected birds and the fowl pox virus was cultured in embryonated chick egg and its virulence was seen at different passages like 3, 6 & 9. As a result the attenuation of virus in ECE reduces its virulence by increasing the number of passages. The same was observed in the PCR product with decreasing the length of DNA and the effective vaccination process was observed with 9 times passaging in ECE's.*

INTRODUCTION

Avian pox virus represent a large group of virus present in bird population (Joshi & Shakya, 1997). APV's belongs to the chordopoxvirinae subfamily of the poxviridae family which infect and causes diseases in poultry and causes economic losses to the poultry industry (Fauquet et al., 2005). This disease was characterized by proliferative lesions on skin and diptheric membranes of respiratory tract, mouth and oesophagus. APV vector vaccines are already in use in veterinary medicine (Binns & Avery, 1986 and Beard et al., 1991). APV allows many of its genes to suppress the host immune responses. These include genes that encode proteins which act on early innate pathways such as pathways involving interferon (Mayr et al., 1962), pattern recognition receptors as Toll-like receptor (TLR) (Bowie & Unterholzner, 2008), chemokines and cytokines (Alcami & Smith, 1992), as well as pathways that act on subsequent adaptive responses (Dasgupta et al., 2007 and Antoniou & Powis, 2008). Host protein interaction network and biochemical pathways are altered in most cases by the viral proteins that deliver virus from normal cellular controls and induce nucleotide metabolism in cells results in shut down DNA synthesis (Boehmer & Lehman, 1997). Therefore the present work analyses the field fowl pox virus with attenuation at different passage in ECE's on the molecular characteristics and pathogenicity.

MATERIALS AND METHODS

Scab material samples from dry pox cases and pieces of trachea and esophagus from wet pox cases were collected in sterile containers from fowl pox infected farms in and around Namakkal. Samples of fowl pox virus were inoculated by following Tripathy (1993). Chorioallantoic membranes with characteristic pock lesions were triturated in a mortar and pestle using sterile sand and PBS (pH 7.2), then centrifuged at 3000rpm for 15 minutes and used as CAM antigen for AGPT (Singh *et al.*, 2007). PCR of DNA, extracted from fowl pox virus. Extraction of DNA was carried out as per method prescribed by Handberg *et al.*, (2001) with slight modification. The template DNA was amplified in PCR utilizing ATI gene specific primer, as described by Singh *et al.*, (2007). 1ml attenuated viral samples collected from 3, 6 and 9 time's passage through ECE were treated to the 11 weeks chicks in 10 birds batch in triplicate along with control triplicate birds batch without treatment. Chicks were observed daily and readings on their disease development were taken on a weekly basis.

RESULTS

AGPT test showed an intense precipitins line of the samples collected from the fowlpox virus infected birds as against the control reference antigen. Samples inoculated into the chorioallantoic membrane (CAM) of the embryonated chick embryo (ECE) which, showed characteristic pock lesions and thickening of the membrane at the site of inoculation in all the passaged embryonated eggs whereas in negative control not such lesions were observed.

The attenuated fowlpox virus passaged for three times through the chorioallantoic membrane (CAM) of the embryonated chick embryo (ECE) inoculated to 11 weeks chicks showed consistent primary lesions for 21 days and reduced to 4 birds in the 42nd day (Figure 1), 35 days and reduced to 4 birds on the 35th day (Figure 2) and 21 days and reduced to 2 birds on the 21st day (Figure 3). Where as in the control chicks the lesions started only from 21 days for six birds and increased to 6 birds and increased to 10 birds in 28 days and lesions continued to appear. Secondary lesions also occurred in the birds treated with 3 times passages fowl pox virus from the 7 days time, gradually reduced to one bird on 42nd day, birds treated with 6 times passaged fowl pox virus from the 7 days time, gradually reduced to two birds on the 21st day and 9 times passages fowl pox virus from the 14 days time, gradually reduced to one bird on the 21st day. In control birds secondary lesions also occurred on the 21st day for one bird

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and spread to nine bird on 42nd day. Viremia was found to be high initially with 3 times passaged virus treated chicks reduced to one on 35th day and nil on 42nd day, 4 birds initially with 6 times passaged virus treated chicks reduced to two on the 14th day and nil on 42nd day and Viremia was not found at 9 times passaged virus treated chicks during the study period where as control checks showed increasing viremia from the 28th day.

The PCR analysis of the attenuated fowl pox virus showed changes in the DNA fragment length, which ranged between 1.8 kb to 2.2 Kb the non attenuated DNA strand was much longer with 2.2 Kb followed by 3 times passaged with 2.1 Kb, 6 times passaged attenuated 1.9 Kb and 1.8 for 9 times passaged (Plate 1). However the results showed slight variations among the replicated samples.

DISCUSSION

The effect of APVs in the poultry industry is highly pronounced in their economic output, which also affects pet and wild birds. APVs with its large genome makes it complex in their expression in different host cells, which produce a variety of versions and complete knowledge of their behavior is lacking although it forms into multi species targeted vaccine. As FPV replicates only in avian cells, we chose to study FPV production on ECEs under different passage and used, non passaged virus and control with the commercial FPV virus as a control. FPV production had been shown to be affected by different passage in ECE. Passaging from 3 times to 6 times showed increased reduction in secondary lesions and viremia infected birds and mortality rate is also low. However, effective vaccination effect was seen in the 9 times passaged virus vaccination into chicks.

The characterization of the non-attenuated and ECE passaged showed differences in their PCR products the variations in their length as the number of time's passage increased the length of amplified DNA, reduced this may be due to deletion of mutation genes which is the host interaction factor. This response to passaging is the response of LTR sequence which has been reported in many studies on their sequence alignment (Fatunmbi and Reed, 1996: Boulanger, *et al.*, 1998; Afonso, *et al.*, 2000; Garcia, *et al.*, 2003). The present study also showed a sudden change in the PCR product of DNA length from 3 time passage to the 6 times passage and reached the length of 1.8Kbp length which is also similar to the commercially available fowl pox vaccine. This depicts that passaging more than times gives better deletion of virulent factor.

CONCLUSION

The present study showed decreased pathogenicity in passaging of field virus in the ECE's this due to deletion of the mutated gene in the fowlpox virus genome, which is the interaction factor with the host as determined by the LTR sequence, creating rapid change in the virus genomic sequence which imbalances the host immune system and makes the host susceptible. Reduction of the mutant gene through passaging in ECE's reduces and this rapid change and allows the host to face the virulence by producing a defense immune response.

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Figure 1. Comparitive analysis of 3 times passaged fowl pox virus innoculated chicks with control on their lesions and viremia

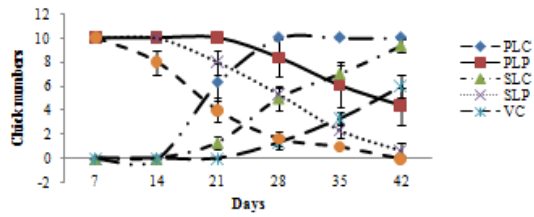


Figure 2. Comparitive analysis of 6 times passaged fowl pox virus innoculated chicks with control on their lesions and viremia

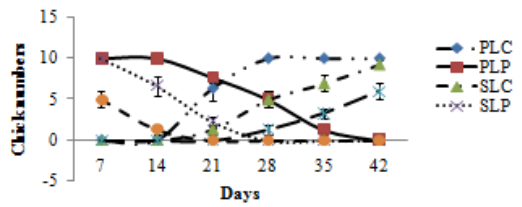
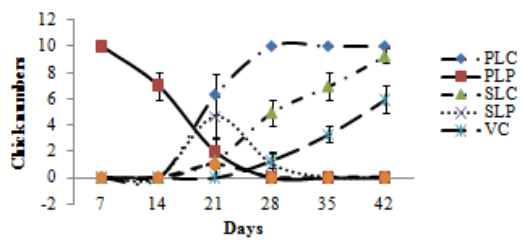


Figure 3. Comparitive analysis of 9 times passaged fowl pox virus innoculated chicks with control on their lesions and viremia



PLP Primary lesions control; PLP Primary lesions passage; SLC Secondary lesions control; SLP Secondary lesions passage; VC Viremia Control; VP Viremia Passage

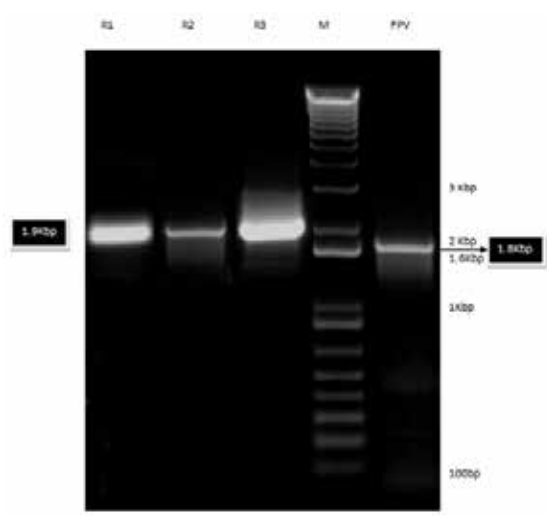


Plate 1. PCR amplified 9 times passaged fowl pox virus

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