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Detection and molecular characterization of fowl pox virus isolated from a Turkey flock

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Abstract
A confirmatory diagnosis of turkey pox was done based on clinical signs and symptoms. A total of 10 poults were investigated and clinical specimens for histopathology as well as scab lesions were collected from clinically suspected field cases for molecular detection of turkey pox. Histopathological examination, Polymerase chain reaction (PCR) and Gene sequencing was performed for confirmatory diagnosis. External gross lesions of infected turkey birds showed cutaneous lesions mainly nodular, papular, pustular, erosion during diseased condition and crust lesions at recovery stages mainly around the eye, head, wattle, snood, beak and neck. All histopathological observation includes infiltration of inflammatory cells. The most pathognomonic histopathological lesions intracytoplasmic inclusions bodies (Bollinger bodies) were found. Outbreak samples gave positive for PCR test of turkey pox virus and phylogenitic analysis revealed 99.9% of homology with other Indian strain of the virus.

Keywords: Fowl pox virus, Turkey, PCR, Bollinger bodies, phylogenetic analysis

Introduction
In modern era, turkey farming in Tamil Nadu is being measured as an substantiate income creating sources for middle-class poultry farmer for entrepreneurship due to its faster growth rate as well as a promising source of good quality lean meat (Samad, 2013) [11]. Turkey farming is affected by the concurrent infection of several viral diseases that causes high mortality and morbidity. Turkey pox is one of the highly contagious diseases that infects all age, sex and breed which is caused by the genus Avipoxvirus having a 188.53 kb size genome (Weli *et al.*, 2011; Tripathy, 2008; Haydar *et al.*, 2017) [14, 12, 5] Similar to avian pox, turkey is more susceptible to turkey poxvirus. This disease is clinically manifested gross lesion as visible papules, pustules, vesicles and finally crustes formation. Predominant pox lesions are observed in head, neck, around the beak, eyelids, wattle, snood, wings, vent and rarely in legs of the turkey (Kabir *et al.*, 2015) [7]. Diphtheritic form is occasionally, may develop in the mucous membrane of mouth, pharynx, and esophagus with fibro-necrotic lesions which is visible after postmortem of dead birds (Joshi, 2011) [6]. In outbreak, low mortality was recorded in cutaneous form, but high mortality was observed in diphtheritic or combined form concurrent infection of as well as with secondary bacterial infections. Since, turkey pox is a contagious disease and the virus is slow transmission but sometimes mechanical vectors such as insects and mosquitoes may increase the infection rate of disease (El-Mahdy *et al.*, 2014) [4]. Since, it causes economics loses due to low growth rates of young birds, loss of production, treatment and vaccination cost as well as mortality of birds (Biswas *et al.*, 2011) [2]. Turkey pox is an emerging disease in Tamil Nadu, but appropriate treatment protocol is not available in our country. Moreover, for the control and prevention of turkey pox confirmatory diagnosis is the first prerequisite. So, the present investigation was conducted to find out the molecular detection of turkey pox and histopathological changes using clinical specimen and PCR with specific primer. Many number of pox outbreaks have been reported from different parts of India. Previous researcher have reported the presence of persistent FPV infection in unvaccinated and vaccinated flocks (Dana *et al*., 2000; Biswas *et al*., 2011) [3, 2]. However, there are very scanty reports on genomic characteristics of FPV circulating in turkey, though needs attention for better understanding regarding its epidemiology. This study reports first time the molecular characteristics of FPV in turkey reared in backyard system of management.

Materials and Methods
The present study was conducted in the Veterinary University training and research centre (VUTRC-TANUVAS). The necessary PCR and sequencing was performed at the Central

University Laboratory (CUL), TANUVAS, Madhavaram, Chennai-51.

Sample collection

In a village of Madurantakam block of Chengalpet district, Tamil Nadu, the Turkey farms showed mortality during July 2020. A total of 10 poults (500 Nos.) were investigated in the village. The symptoms were noted and postmortem was conducted. In this study, total 6 samples from postmortem of dead birds were collected and immediately preserved in 10% formalin for histopathological and 50% glycerol saline for virological examination.

Histopathological examination

For histopathological examination, after proper fixation of clinical specimens, 5 microns thickness histopathological slides were prepared and stained with hematoxylin and eosin stain for detection of intra-cytoplasmic inclusion bodies (Bollinger bodies) and histological changes as described by Bancroft and Gamble (2002) [1].

DNA extraction

DNA was directly extracted from the collected postmortem lesions trachea, lungs, liver, kidneyl specimens lesions by using a QIAamp DNA extraction kit (QIAGEN). Samples were thoroughly ground with help of sterile mortar and pestle, then, DNA was extracted and eluted with 100 µl elution buffer and stored at −20 °C according to the manufacturer's procedures, for molecular detection of turkey pox.

Molecular detection

For Polymerase chain reaction (PCR) selective primers that were used from previously described by Lee and Lee (1997) [8]. Primer for amplification of *P4b* gene avian pox virus as forward 5'-CAGCAGGTGCTAAACAACAA-3' (F) and reverse 5' CGGTAGCTTAACGCCGAATA-3' (R) were used for 578 bp amplicons of poxviruses. To performed PCR a total of 25- µl reaction mixture was prepared which was consisting of 1x PCR buffer (Invitrogen Vienna Austria) supplemented by 1.5 mM $MgCl_2$ (Invitrogen), 0.2 mM dNTP mix, 1.25 µl of each primer, 1.5 U of Taq DNA polymerase and 1.5 µl of prepared DNA as template. For PCR amplification the cycle were used as the initial denaturation was performed at 90 °C for 7 minutes, followed by 34 cycles of denaturation continued at 95 °C for 40 seconds (s), annealing at 60 °C for 35 s and elongation at 75 °C for 35s and final extension at 72 °C for 5 minutes in a thermocycler. Then PCR products were electrophoresed at 2% Agarose gel for 1 h at 100 V. Finally, DNA was visualized under a U.V. transilluminator.

Electrophoresis, purification and sequencing of PCR product

PCR samples (5 µl) were loaded onto 1% agarose gels 90 mA for 30 min in TBE buffer. PCR products were purified by Purification kit (Qiagen) and resuspended in 50 µl H2O. These products were then sequenced on both strands, using the amplification primers and internal primers in an automated sequencer

Results and discussion

Avian pox is a contagious and slow spreading viral disease, generally observed in chickens, but other avian species, *viz*. turkey, quail, pigeons etc. are also found to be susceptible to Avi-poxviruses (Tripathy and Reed 2003) [13]. Serological, histopathological and viral isolation methods for diagnosing the pox diseases are generally time consuming, labor intensive and less sensitive due to extensive cross reactions observed between the members of Avi-*poxvirus* genus. The conventional detection methods are perceived to be having limitations and moreover require *in-vivo* or *invitro* assay systems.

In gross lesion turkey birds showed cutaneous lesions mainly nodular, papular, pustular, erosion during diseased condition and crust lesions at recovery stages mainly around the eye, head, wattle, snood, beak, and neck (Figures 1 and 2). The nodular lesion of pox virus infection varies from birds to birds and recorded up to 1 to 3mm in diameter mainly in head regions. This clinical signs of turkey pox is similar with previous several findings of Yoshikkawa *et al.* (2002) [15] and Prukner-Radovcic *et al.* (2006) [10]. Chronic cases as well as recovery stages of turkey pox the most pathognomic histopathological lesions intracytoplasmic inclusions bodies (Bollinger bodies) with different size were noted in infected tracheal lesions in wet pox conditions.

PCR is a highly sensitive molecular technique for detection of particular organism-specific gene, here all the clinical specimens were subjected to PCR for molecular detection and confirmation of turkey pox. The PCR was done based on amplification of 578 bp pox virus using a specific primer and confirmation of *P4b* gene-specific products. These specific primers have previously been used successfully in various studies for the detection of avipoxvirus gene by several workers (Kabir *et. al.*, 2015) [7]. Turkey pox was showed specific bands on 578 bp on 2% NA Agarose gel electrophoresis and this finding is strongly supported by Lee *et al*. (1997) [8] who found 80% samples of fowlpox were positive during molecular detection in his study.

Sequence analysis of the isolates reveals 99% similarity with the previously isolated strains of India from sparrow, silver pheasant, Indian peacock and golden pheasants. This findings show that the present isolated virus strains is widely prevalent in different avian species in different agro-climatic regions. This finding corroborates the report of Luschow *et al.* (2004) [9] where no differentiation among poxvirus genome of turkey and sparrow and FPV upon phylogenetic analysis and sequencing. For effective methods to confirm FPV in backyard turkey, PCR in combination with clinical signs, histopathology and sequence analysis is used. More elaborate studies needed in future, for better understanding the epidemiology of FPV in backyard system of management using these parameters are suggested.

Fig 1: Birds affected with FPV showing pustular nodules around the eyes

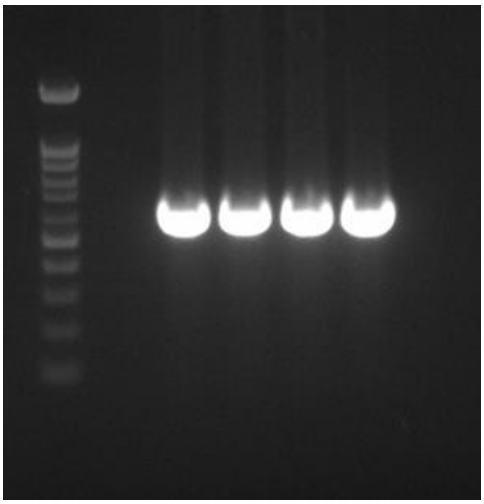

Fig 2: Amplification of FPV 4b core protein gene of FPV using DNA template from FPV field isolates; M- 100 bp DNA molecular marker; Lane 1-4- isolates (578 bp)

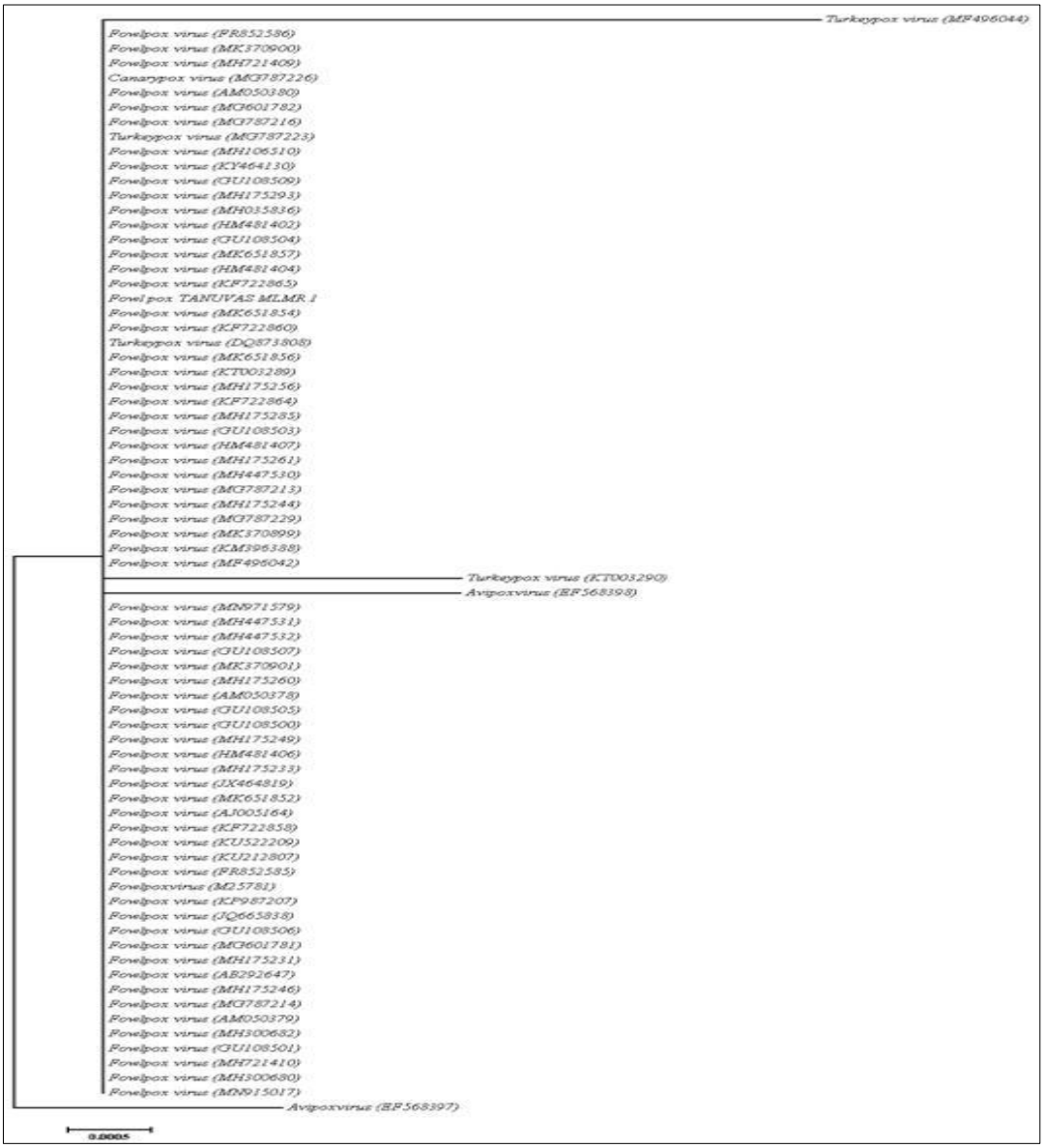

Fig 3: Phylogenetic tree of avipoxvirus P4b constructed

Conclusion

The study revealed that the outbreak in turkey flock was caused by FPV virus closely related to widely prevalent in different avian species virulent virus. The molecular epidemiology of FPV in India is very limited. Hence, more molecular surveillance is required in India to ascertain the circulating strain.

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