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Isolation and Molecular Characterization of Turkey Pox Virus from Maharashtra, India

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ABSTRACT

Avian pox diseases are contagious and slow spreading viral infections in birds. The present study was aim to, isolate and molecular characterization of turkeypox virus from a clinical case. Ten out of the twelve scab lesions sample collected from clinically suspected cases were positive for avian pox viurs (APV) based on virus isolation and polymerase chain reaction. We conducted genetic characterization of the APV strain. The phylogenetic analyses of P4b gene APV genome indicated that, avian poxviruses fragments sequenced in this study clustered along the A clade of avipoxviruses, genetically related to Indian fowl pox virus isolated from chicken, showing 99% homology.

Key words: PCR, Phylogenetic, Turkey pox virus.

The Poxviridae family comprises of enveloped DNA viruses that have a characteristic brick-shaped or ovoid morphology. Avian pox is a highly contagious viral disease caused by avipoxviruses (family *Poxviridae*, subfamily *Chordopoxvirinae*) known to infect a wide range of avian species with a worldwide distribution (King *et al*., 2012; Bányai *et al*., 2015). Infected birds manifest disease in two major forms. The first is a milder cutaneous form (dry form), characterized by proliferative nodular skin lesions on the featherless parts of the skin and is self-limiting. The second is a diphtheritic form, characterized by fibrino-necrotic lesions that appear in the upper respiratory and gastrointestinal tracts of birds and is often fatal (Tripathy and Reed, 2019).

The diagnosis of avipoxvirus is primarily performed by characteristic skin lesions, histopathological examination, electron microscopy, virus isolation on chorioallantoic membranes (CAM) of embryonated chicken eggs and by serological methods. Recently, molecular biological methods have facilitated the rapid, specific and highly sensitive detection of viral DNA (Singh *et al*., 2007). The highly conserved loci of the viral genome, such as the gene encoding the P4b core protein (FPV167) was targeted for PCR based diagnosis and phylogenetic analysis (Gyuranecz *et al.,* 2013; Bányai *et al.,* 2015; Sahu *et al*., 2020). Phylogenetic analyses have revealed, three major clades of Avipoxvirus, namely clade A (FWPV-like), clade B (canarypox virus) and clade C (psittacinepox virus). Recently, two additional clades, D and E, were proposed (Mapaco *et al.,* 2018; Ribeiro *et al.,* 2020).

In India, the re-emergence of avipoxviruses in various avian species such as fowl, turkey, pigeon, duck, quail, peacock, golden pheasant and silver pheasant have been recorded (Singh *et al*., 2003; Pawar *et al.,* 2011; Sharma *et al*., 2019 and Sahu *et al*., 2020). In spite of, vaccination the avipoxviruses outbreak has been observed among the domesticated poultry (Sharma *et al*., 2019). In Maharashtra, avipoxvirus has been reported in desi birds, Indian peacocks and wild pigeon from different parts of Maharashtra (Pawar *et al.,* 2011; Sawale *et al.,* 2012; Thorat *et al*., 2019). Apart from these report, the infection status of avipox virus turkey in this region was unreported. The present study focused on the isolation and molecular characterization of the avian poxvirus isolates from turkeys in Maharashtra state of India.

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During the year 2017-2018, turkeys housed in poultry Farms in and around Satara region demonstrated lesions on the neck and eyelids of the turkeys. The apparently diseased birds displayed roughened feathers and stunted growth. On the basis of the clinical signs and gross lesions observed, it was suspected for avipoxvirus infection. Twelve clinical samples including scabs, skin biopsies from affected birds were collected aseptically and stored at -20°C for further use.

The collected materials comprising of tissue lesions were homogenized (1:10) in phosphate buffered saline (pH 7.2) containing antibiotics (penicillin @10,000 IU/ml, streptomycin @500 µg/ml and gentamicin @250 µg/ml) by using sterile mortar and pestle. It was centrifuged at 1300 g at 4°C for 30 min and the supernatant was collected and passed through a 0.45 µm filter (Millipore).

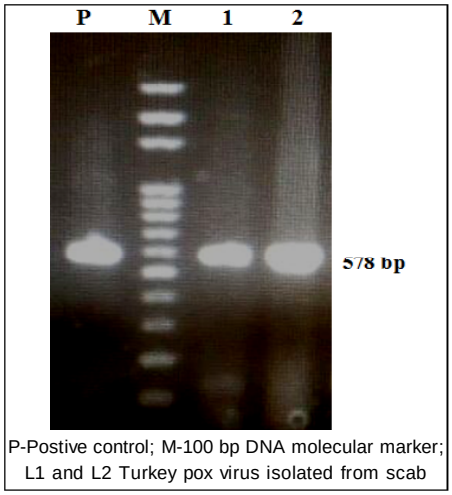
Filtrated samples (100 µl) were used for inoculation of specific pathogen free (SPF) embryonated chicken eggs of 10-11 days old *via* route chorioallantoic membrane (CAM). The eggs were incubated at 37°C and observed for 5-6 days.

DNA was extracted from CAM and scabs tissue lesions using the standard proteinase K/phenol: chloroform

protocol of Sambrook *et al.* (1989). DNA was dissolved in TE (10 mM Tris-HCL, 1.0 mM EDTA, pH 8.0) buffer and stored at -20°C until use. PCR based amplification of avian pox virus P4b gene was carried out using primer pair FP CAGCAGG TGCTAAACAACAA/RP CGGTAGCTTAA CGCCGAATA to amplify 578 bp amplicons. Reaction was performed in volume of 25 µl containing 1.5 units of Taq polymerase,10 mM Tris-HCL (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each dNTP, 10 pmol of each primer, 100 ng DNA and nuclease-free water up to 25 µl for 35 cycles. After initial denaturation at 94°C for 5 min, each cycle consisted of denaturation at 94°C for 30 sec, annealing at 52°C for 45 sec and extension at 72°C for 1 min. PCR amplified products (5 µl) were loaded onto a 2% agarose gel and stained with ethidium bromide.

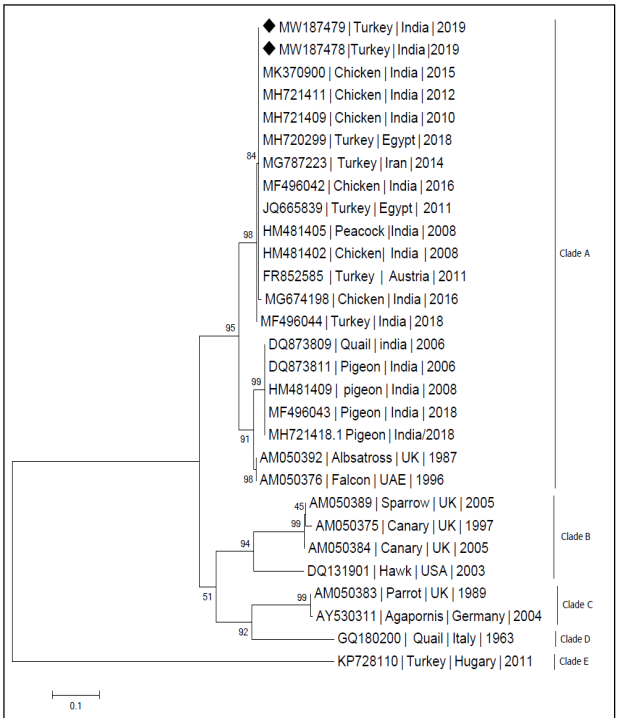
For molecular characterization, PCR amplified products of P4b gene (578 bp) of two isolates, were subjected to nucleotide sequencing. The amplicons were gel-purified by using PCR AccuPrep® PCR Purification Kit (Bioneer Co., Korea) and sequenced commercially. DNA sequences were edited by BioEdit v7.0.5 and submitted to the GenBank database under Accession numbers: MW187478 and MW187479. To assess the phylogenetic clustering and relationship among the different APV, the P4b sequences were aligned with APV sequences available in Gen Bank, using the neighbour-joining method. All phylogenetic analyzes were run by MEGA 6.0.6 (Tamura *et al*., 2013).

In present study, turkey pox virus was isolated from a suspected clinical case of turkey pox. Suspected samples were subjected for virus isolation in chicken embryos. CAM materials of the inoculated embryos were found thickened and oedematous as compared to control suggesting growth of virus. To confirm the presence of avipox in scab materials and on CAM, avipox specific PCR was performed and an



P-Postive control; M-100 bp DNA molecular marker; L1 and L2 Turkey pox virus isolated from scab

Fig 1: Agarose gel analysis of PCR amplified product of 4b core protein gene of turkey pox virus isolates from scab showing the expected band size of 578 bp.



The tree was analyzed by neighbor-joining (N-J) analysis with boot strapping (1000). Reference sequences obtained from Gene Bank. All sequences are named with accession number, host species and year of isolation. Clades are indicated on the right hand side of the tree.

Fig 2: Phylogenetic analysis of APVs based onfpv167 (P4b) gene nucleotide sequences. Phylogenetic tree was constructed *via* multiple alignments of 428-bp nucleotide sequence of P4b gene from 43 APV strains.

expected size amplicon of 578 bp was obtained (Fig 1). The P4b gene Sequence of the two turkey pox virus isolates were submitted to Gen Bank with accession number (MW187478 and MW187479). The NCBI BLAST analysis, exhibited 100% similarity with cutaneous form of avian pox viruses isolated from commercial chicken (MK370900) and golden pheasant from wild bird (HM481402). The phylogenetic analysis results revealed that our turkey virus isolates clustered with FWPV-like viruses which belonged to the major clade A (Fig 2).

Turkey pox virus (TPV) causes severe economic losses in terms of meat condemnation, weight loss and drop in egg production in Indian turkey flocks. Although turkey farming in Maharashtra state in its infancy or limited to experimental purpose. The source of infection amongst turkeys was unknown, but the susceptibility of these turkeys to pox infection was recorded (Bányai *et al*., 2013). The present study confirmed the presence of turkey pox virus by PCR in turkey. Phylogenetic studies showed that the majority of avipoxvirus strains are grouped into WPV-like group (clades A), CNPV-like group (clade B) and the psittacinepox (clade C) (Jarmin *et al*., 2006; Manarolla *et al*., 2010). Our turkey virus isolates clustered with FWPV-like viruses, showing maximum similarity with the commercial layer chicken and wild golden pheasant. Previous work showed that an APV isolate from a free-ranging bird presented a genetic similarity with FWPV and turkeypox virus (Luschow *et al*., 2004; Ha *et al*., 2011) Moreover, APV indirectly transmitted through bird-biting mosquitoes which act as mechanical vectors of avipoxviruses (Yeo *et al*., 2019). It might be APV strains introduced in turkey by indirectly transmission. Taken together, this study provides the molecular characterization of Indian turkey pox virus isolates. To better understanding about the genetic composition of APVs and their relationship to each other and their host species a larger scale survey needs to be performed.

Conflict of interest

The author declares that they have no conflicts of interest.

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