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Molecular Detection and Phylogenetic Analysis of Chicken Parvovirus Associated with Poultry Enteritis

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ABSTRACT

Chicken parvovirus (ChPV) is of many viruses related to enteric diseases, which usually associated with enteritis and Runting and stunting syndrome (RSS). Aim of the present study was to find out the occurrence of ChPV associated with poultry enteritis in four districts of southern Rajasthan, and their phylogenetic analysis. Total 604 dead poultry birds affected with enteritis, from commercial poultry farms were examined for presence of ChPV. Intestinal samples of four birds were pooled to make one biological sample. ChPV were detected by PCR using non-structural protein (NSP) gene specific primers. Phylogenetic analysis was carried out by partial gene sequencing. ChPV was detected in 18.54% (28/ 151). Mixed infection of FAdV+ChPV were detected in 15.23% (23/151) pooled samples. The partial molecular characterization of the ChPV partial molecular characterization revelled high level of nucleotide 98.95-100% and amino acid similarity 98.95-99.98% with other ChPV sequences from Brazil and other parts of the world.

HIGHLIGHTS

- ChPV was detected in 33.77% (51/ 151) in pooled samples from four districts of Southern Rajasthan.
- Hhigh level similarity was found with other ChPV sequences from Brazil and other parts of the world.

Keywords: Chicken Parvovirus, PCR , Poultry enteritis, Phylogenetic analysis

Enteritis is an economically important multifactorial disease that affects birds in the first few weeks of life and is characterized principally by diarrhoea, poor weight gain and, high mortality (Saraswat *et al.* 2021). It involves many etiologic agents, including enteric viruses such as parvovirus, adenovirus astrovirus, rotavirus, and reovirus, bacteria, such as *Salmonella spp., E. coli, Clostridium spp., and* protozoa, such as *Eimeria spp*. (Mettifogo *et al*., 2014). *Chicken parvovirus* belongs to Parvoviridae Family. Virion is non-enveloped ssDNA particles about 20 nm in diameter with icosahedral symmetry assembled from 32 capsomers that are 20-22 nm in diameter (Zhang *et al.,* 2020). Genome size is 4-6 Kb encoding three opened reading frames (ORFs) - 5'ORF, 3'ORF and the third small ORF localized between junctions of these two ORFs. 5'ORF encodes non-structural protein (NS) taking a part in viral replication while 3'ORF encodes structural proteins of capsid (VP_1, VP_2 and VP_3). The function of the third ORF is not explained so far (Tarasiuk *et al*., 2012). The NS gene appears to be highly conserved among parvoviruses, and it is often used as target for nucleic acid based diagnostic tests (Koo *et al*., 2015).

In present study, ChPV form poultry enteritis in unexplored areas of Rajasthan state of India, was detected, described the partial molecular characterization.

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MATERIALS AND METHODS

Present study has been conducted at Department of Veterinary Microbiology CVAS, Navania Udaipur Rajasthan, to investigate the problem of enteritis and heavy mortality in commercial poultry farms in four districts *Viz.* Ajmer, Dungarpur, Sirohi, and Pratapgarh of southern Rajasthan during the period of January 2019 to February 2020. A total of 604 dead birds having signs of enteritis, rough feather, and poor weight gain were included in this study. The birds were subjected to post-mortem and molecular screening for ChPV.

Molecular detection

Pooled Intestinal samples were subjected to molecular detection of the ChPV using PCR. Tissue samples were processed as described (Saraswat, 2019). Viral nucleic acid was extracted using Gene JET Viral DNA and RNA Purification Kit (Thermo Scientific). For PCR nuclease-free water was used as a negative control. While Positive samples, stored in our lab were used as a positive control.

Polymerase chain reaction (PCR)

ChPV detected using NS gene specific primers with forward and reverse sequences 5'-TTCTAA TAACGATATCACTCAAGTTTC-3 and 5'-TTTGCGCT TGCGGTGAAGTCTGGCTCG-3' respectively (De la Torre *et al.,* 2018). The PCR reaction used 20µl of mixture that contained 0.5 µl of each forward and reverse Primers (10 pmol): Adding 10 µl 2x PCR master mix (Thermo scientific): **2**µl DNA as Template and 7µl nuclease free water. PCR amplification was performed under the following temperature conditions a cycle of 95°C for 5 min, 35 cycles at 95°C for 30 Sec, and 55°C (ChPV) for 1 min, and 72°C for 1 min, lastly final extension was done at 72°C for 10 min. The amplified product (561-bp of ChPV) was submitted to electrophoresis in 1.5% agarose gel.

DNA sequencing and phylogenetic analysis

The amplified products of the gene of ChPV form 2 positive samples were purified using SureTrap Gel Extraction Kit as described by the manufacturer (Genetix biotech Asia Pvt. Ltd.). Each purified product was sequenced in forward and reverse direction using Applied Biosystem by life science technology 3130 XL sequencer (done at the Department of Biochemistry, University of Delhi, South Campus, New Delhi).

Nucleotide sequences were edited in Bio-Edit software. The aligned sequences were analysed on the NCBI website http://www.ncbi.nlm.gov using BLAST to confirm their identity. The nucleotide sequences were then aligned using MEGAX version 5 software by CLUSTAL W method, using partial sequences of ChPV downloaded from the NCBI. The nucleotide phylogenetic tree was inferred using the neighbour-joining maximum composite likelihood method with 1,000 bootstrap replicates that were integrated into MEGA version 5 software (Kumar *et al.,* 2018).

RESULTS AND DISCUSSION

Molecular detection

All the collected intestinal samples were subjected to molecular detection, by PCR which amplified the NS Gene for ChPV and obtained an amplicon of 561 bp (Fig. 2). ChPV was detected in 33.77% (51 /151) samples, out of which 28 (18.54%) were positive for ChPV alone. While mixed viral infection (FAdV-I + ChPV) were found in 15.23% (23/151) samples. The highest prevalence were found as 75.67% (28/37) in flock of Ajmer district and lowest prevalence were found as 46.15% (18 /39) in flock of Dungarpur district. High prevalence of ChPV, indicates that these enteric viruses are widely prevalent in enteritis affected poultry flocks in the study area. And these viruses might play the role in causation of enteritis in affected birds. Recently Saraswat (2019) reported the prevalence of enteric viruses of poultry in and around Udaipur city of Rajasthan state of India. They reported overall prevalence was 73.33% (44/60) enteric viruses in poultry enteritis. In India other report on poultry enteritis by Kaithal *et al.* (2016) but they studied enteric RNA virus's *i.e.* CAstV, ANV and AReoV from Haryana (India).

Recently various researchers from abroad have reported the detection of different enteric viruses from enteritis affected chickens at varied prevalence rate of ChPV 6.6% (De la Torre *et al.,* 2018), 43.75% (Nunez *et al.,* 2016a). Finkler *et al.* (2016) detected ChPV genomes found in all cloacal swab samples (n = 127); Mettifogo *et al.* (2014)

Table 1: Previously published non-structural protein gene sequences of ChPV used for comparison

Gene/ Virus	Strain name	Country	Gen Bank accession number	Host species
Non-structural protein (NS) gene for ChPV	ChPV/USP 162	Brazil	MH176305.1	Chicken
	ChPV/USP 400-1B	Brazil	MH176313.1	Chicken
	ChPV/786-1	Brazil	MF784851.1	Chicken
	ChPV/752-3	Brazil	MF784850.1	Chicken
	ChPV/691-1	Canada	MF784849.1	Chicken
	ChPV/EC/722-34/2016	Ecuador	KY649275.1	Chicken
	ChPV/EC/722-30/2016	Ecuador	KY649271.1	Chicken
	ChPV/EC/722-24/2016	Ecuador	KY649265.1	Chicken
	ChPV/EC/722-22/2016	Brazil	KY649263.1	Chicken
	ChPV/USP 534-6	Peru	KT347548.1	Chicken
	ChPV/Poland/G073/2011	Poland	JQ178301.1	Chicken
	ChPV CAN-13	Canada	JF267316.1	Chicken

Table 2: Prevalence of poultry enteric DNA viruses

Poultry enteric DNA viruses	Ajmer (N = 37)	Pratapgarh (N = 51)	Dungarpur (N = 39)	Sirohi (24 pooled sample)	Overall prevalence (N = 151)
ChPV	17(45.32%)	10 (19.60%)	10(25.64%)	13(54.16%)	50(33.11%)

detected 193 (85.4%) enteric viruses; Koo *et al*. (2013) detected 85.3% of total samples positive for enteric viruses. It indicates that occurrence of enteric viruses varies from area to area and it may depend on geographic climatic conditions.

The variation in prevalence among four districts of Rajasthan may be due to size of chicken flocks and number of enteritis affected birds we got. Most of the samples from Ajmer district were from commercial poultry farm which containing large number of chicken reared in a close confined area resulting in high chances of horizontal transmission. Whereas the flock size of poultry farms form Dungurpur, Pratapgarh and Sirohi were small and numbers of farms were also less and scattered.

To find out the relation of prevalence of enteric viruses and age of the affected birds we divided our study population in two categories *i.e.* 0-1 week and 1-12 weeks age group. Among these, high prevalence of ChPV was found in 0-1 week age group (60.56%) and lower in 2-12 week age group 53.75%. Recently Saraswat, 2019 reported highest prevalence of enteric viruses in 21-30 days age group (83.33%) followed by 31-42 day age group (71.42%), 1-10 days age group and 10- 20 days age group (70%). Globally, similar results also reported by De la Torre *et al.* (2018a). Presence of viruses in first week of age gives an indication of vertical transmission of that the virus (Mettifogo *et al.,* 2014).

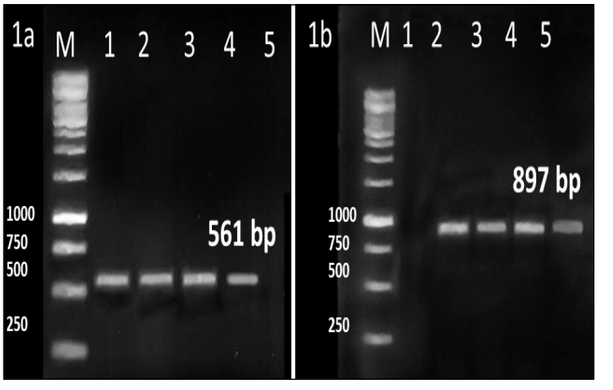

Fig. 1: Agrose gel electrophoresis of PCR product of ChPV (NS Gene): Well no-1: positive control; 2-4 field samples positive for ChPV and 5 negative control

Phylogenetic analysis

Phylogenetic analysis of ChPVs NS gene also has been constructed using 9 previously published ChPVs sequences of Indian origin and 20 from abroad (Fig. 2). Branch I contains most of the sequences using in the analysis and further subdivided into two groups cluster A and B. Cluster A contains the sequences originated from Brazil, Canada, Ecuador and Peru where, cluster B having sequences reported from Poland and Canada. However, the new sequence obtained in this study lies in the branch II which depicted distantly relationship with other ChPVs gene and suggest the new variation adopted in this gene.

The molecular analysis of the obtained sequences of ChPV detected in the present study showed a high level of nucleotide 98.95-100% and amino acid similarity 98.95-99.98% with other ChPV sequences from Brazil and other parts of the world, showing that ChPV around the world shares similar genetic features. Various authors (Nuñez *et al.,* 2016a; Koo *et al*., 2015; Nunez *et al*., 2015b; Koo *et al*., 2013) had also reported similar findings. Niu and co-workers reported were high levels of nucleotide similarity (NT) (98.4 to 100%) and amino acid (AA) (92.1 to 100%) inter-similarity. These sequences also showed high levels of NT (95.8 to 97.1%) and AA (94.1 to 99.2%) similarity when compared with other previous published Brazilian sequences of ChPV.

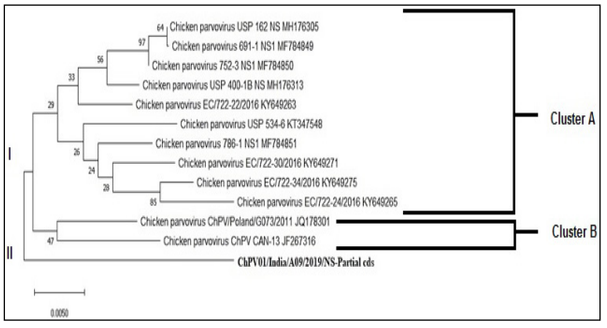

Fig. 2: Phylogenetic tree based on NS gene sequences of ChPV

The evolutionary history was inferred using the Neighbor-Joining method. The evolutionary distances were computed using the Maximum Composite Likelihood method Evolutionary analyses were conducted in MEGA-X software.

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