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Pathology and molecular characterization of chicken parvovirus in desi chickens of Chhattisgarh

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Abstract
Enteritis is one of important disease of chickens characterized by diarrhoea, poor weight gain and in some flocks, high mortality. *Chicken Parvovirus* (ChPV) is one of the important viral agents involved in enteric disorders. The present study was aimed to detect the ChPV associated with enteritis in desi and kadaknath chickens of Chhattisgarh. Total of 151 desi and kadanath birds (1-18 weeks old) died due to enteritis with symptoms of diarrhea, vent pasting, ruffled feathers and poor growth were subjected to necropsy. Intestinal tissue samples collected from 151 birds were subjected to PCR assay for detection of ChPV by amplifying 561 bp sequence of NS gene. ChPV was detected in 31.78% (48/151) of intestinal samples collected chickens of Durg, Rajnandgaon, Balod and Raipur district of Chhattisgarh which suffered from enteritis. Lesions consisted of catarrhal enteritis, dilated, inflamed or pale intestinal wall with watery and gas filled contents, atrophy of immune organs and pancreas. Sequences of NS gene of ChPV showed 98.37%-99.19% nucleotide similarities with other previously published sequences. Phylogenetic analysis revealed that detected Indian isolate is grouped with Poland 1 and USA 2 isolates. The results indicated the presence of ChPV in Indian chicken flocks and its close association with enteritis.

Keywords: Chicken parvovirus, enteritis, prevalence, pathology, molecular characterization

Introduction
Enteritis is one of the important diseases of chickens characterized by diarrhoea, poor weight gain and in some flocks, high mortality (Saif *et al.*, 2008) [17]. Enteritis due to enteric viruses can cause damage to the gastro-intestinal tract of birds thereby providing conductive environment for bacteria and/or protozoa to grow and cause further damage to the gut. It is possible that damage caused to the gut initially by enteric viruses and subsequently by secondary pathogens may lead to irreversible changes to the host, which may result in irreversible damage to the flock (Saif *et al.*, 2008) [17]. Further any change in delicate and balanced microenvironment *i.e.*, microflora of intestine leads to poor vitamin production, immune-suppression and increased growth of harmful bacteria and viruses in the intestine of chickens (Yegani and Korver, 2008) [20].

The enteric viruses reported to cause enteric diseases are found in single and multiple infections which include the *Fowl Adenovirus* of group-I (FAdV-I), *Chicken Parvovirus* (ChPV), *Avian Reovirus* (AReoV), *Avian Rotavirus* (ARtV), *Chicken Astrovirus* (CAstV) and *Avian Nephritis Virus* (ANV) (Moura *et al.*, 2013; Mettifogo *et al.*, 2014) [9, 10]. The causes of enteric disease have never been definitely established because they are complex and polymicrobial and similar disease signs can likely be caused by different pathogens (Baxendale and Mebatsion, 2004) [1]. Some studies also have shown that ChPV is an important viral agent involved in enteric disorders (Nunez *et al*, 2013) [11].

ChPV are small, spherical, non-enveloped viruses of about 18-25nm diameter that belong to subfamily *Parvovirinae* of *Parvoviridae* family under genus *Aveparvovirus* (Cotmore *et al.* 2014) [4]. The genome of the icosahedral virus consists of linear single-stranded DNA molecule of about 5kb length and encodes two major proteins, the nonstructural (NS1) protein, essential for replication of the viral genome and a structural viral protein (VP) encoding the capsid of the virion. The NS gene appears to be the highly conserved among ChPV and often used as a target for molecular nucleic acid based diagnostic methods (Zsak *et al.* 2009; Koo *et al.* 2015) [7, 22].

Presence of the ChPV was reported from few countries such as USA (Zsak *et al.* 2009) [22], Hungary (Palade *et al.* 2011) [14], Croatia (Bidin *et al.* 2012) [2], Poland (Tarasiuk *et al.* 2012)

[19], South Korea (Koo *et al.* 2013) [7], Brazil (Mettifogo *et al.* 2014) [9] and China (Feng *et al.* 2016) [5]. However, scanty data about prevalence of ChPV are available from India (Pradeep *et al.*, 2020) [15]. There is a lack of documented information and no baseline data is available regarding the prevalence of ChPV in Chhattisgarh. Keeping in-view the above facts, the study was aimed to investigate the prevalence, pathological study and molecular characterization of ChPV in desi chickens of Chhattisgarh.

Materials and Methods

Dead birds suspected to have died of enteritis with history of diarrhea, vent pasting, ruffled feathers and poor growth were subjected to postmortem examination. Total of 151 desi and kadaknath birds (1-18 week old) from 16 different farms with capacity of 500-1500 were included in the present investigation. Detailed post-mortem examination of birds which received at Department of Veterinary Pathology was carried out. Similarly, poultry farms located in the different districts of Chhattisgarh i.e., Durg, Rajnandgaon, Raipur and Balod were visited and dead birds were subjected to necropsy.

Study of prevalence: Prevalence of ChPV in enteritis affected chickens of Durg, Balod, Rajnandgaon and Raipur districts of Chhattisgarh will be calculated on the basis of numbers of samples found positive for ChPV out of total number of dead birds screened by polymerase chain reaction (PCR) assay.

Clinical signs and pathomorphological study: The ailing birds were examined for clinical signs, if any. Dead birds were subjected to detailed post mortem examination and gross pathological lesions were recorded. Tissue samples of intestine, thymus, bursa, spleen and pancreas were collected in 10% buffered formalin and processed for histopathological study by paraffin embedding technique. Sections were cut at 5-6 µ thickness and stained with routine haematoxylin and eosin (H and E) staining (Luna, 1968) [8].

Molecular detection of ChPV: Intestinal tissue samples from the dead birds of various farms having history or clinical symptom of diarrhea, enteritis, vent pasting, ruffled feathers and poor growth were collected and stored at -20 ºC for molecular detection of ChPV by PCR assay.

Detection of ChPV by polymerase chain reaction (PCR): For detection of viral DNA by PCR assay, the genomic DNA from intestinal tissue sample was extracted by using the Himedia DNA purification kit as per the manufacturer's protocol. Extracted DNA was stored at -20º C for further use.
PCR was carried out to amplify the 561 bp sequence of NS gene of ChPV by using forward (F5'TTC TAA TAA CGA TAT CAC TCA AGT TTC 3') and reverse primers (5'TTT GCG CTT GCG GTG AAG TCT GGC TC3') (Zsak *et al.*, 2009) [22]. For amplification, 3µl of DNA was incubated in total volume of 20 µl reaction mix containing 10 µl PCR master mix, 1µl of each forward and reverse primer and 5 µl of nuclease free water. PCR was carried out following initial denaturation at 94°C for 3 min and then 30 cycles at 94°C for 30 sec, 55°C for 30 sec and 72°C for 1 min and further extension at 72°C for 5 min. The PCR products were separated in 1.5% agarose-gel and visualized in Geldoc (Biorad).

Molecular characterization of ChPV

Nucleotide sequencing : PCR product of NS gene segment of ChPV was outsourced for sequencing at PDRC (Poultry Diagnostic and Research Centre), Pune, to get both forward and reverse sequences to generate consensus sequences. The sequences were initially analyzed using NCBI online BLAST server to identify the sequence specificity. Based on the BLAST results, sequences were further compared with other nucleotide sequences of ChPV in GenBank database.

Phylogenetic analysis: The sequence was aligned, analysed and compared using CLUSTAL W with other publicly available sequences of isolates from various countries like Brazil (MG846437, KY069111, MH176305, MG846435, MG846436, MG846440, MF784849, KU569162, MG846439, MF784850, KY649251, KY649263, MH176313, MG846438, KY649250), Poland (JQ178300, JQ178301), USA (GQ260159, KM598416), Turkey (MN717246, MN717251), Switzerland (OM469252) and Hungary (GQ281296). Phylogenetic tree was constructed using neighbour-joining algorithm with1000 bootstrap replicate in the MEGA-6.06 software (Tamura *et al.* 2013) [18].

Results and Discussion

Prevalence of ChPV: By PCR assay, out of 151 samples screened, ChPV was detected in 31.78% (48/151) of intestinal samples collected from 1 to 18 week old desi and kadaknath chickens of Durg, Rajnandgaon, Balod and Raipur district of Chhattisgarh which suffered from enteritis. ChPV was speculated to cause enteritis after its initial identification from the intestines of malabsorption syndrome affected chickens (Kisary *et al.* 1984) [6]. Subsequently, several isolates of ChPV are being reported from around the world in chickens with enteritis by researchers (Zsak *et al.* 2008; Palade *et al.*, 2011, Bidin *et al.*, 2012 Mettifogo *et al.*, 2014) [2, 9, 14, 21]. The present findings are also in agreement with Blicharz *et al.*, (2012) and Koo *et al.*, (2013) who also reported prevalence of 22.2% and 26.5% ChPV respectively in chickens. However, Pradeep *et al.*, (2020) [15] reported prevalence of 100% for ChPV in enteritis along with runting and stunting syndrome affected flocks.

Clinical signs: Clinical signs recorded in enteritis affected birds include reduced feed intake, depression, diarrhea, dehydration, vent pasting, ruffled feathers, partially digested food material in faeces and stunted and poor growth. Similar symptoms were also reported by Zsak *et al.*, (2013) [23] and Nunez *et al.*, (2020) [13] during experimental ChPV infection in chickens. Altered growth performance may be attributed due to impairment in enzymatic digestion and absorption of nutrients from the gastrointestinal tract as the virus targets the pancreas and intestinal tract (Rebel *et al.*, 2006) [16].

Pathomorphological study: Intestines from enteritis affected birds revealed characteristic gross lesions like dilated, inflamed or pale intestinal wall and most of them showed watery contents and gas bubbles (Fig. 1). Atrophy of the immune organs (bursa of Fabricius, thymus and spleen) was observed. Further, the mild atrophy of pancreas was also evident in few cases (Fig.1). In some cases, undigested feed in thin walled swollen intestines and catarrhal enteritis was evident. Similar lesions were reported in enteritis due to ChPV (Zsak *et al.*, 2013; Pradeep *et al.*, 2020) [15, 23].
Microscopically, the prominent changes were observed in duodenum, jejunum and caeca. Intestine revealed degeneration and vacuolation of epithelium of villi and increased infiltration of mononuclear cells in the lamina propria of duodenum, jejunum and caeca. Desquamation and

sloughing of villi is also noticed (Fig. 2). Hyperplasia of lymphoid follicles and foci of hemorrhages in small and large intestine was also evident (Fig. 3). Moderate to severe distension of crypts was noticed in duodenum and jejunum (Fig. 2). Crypt hyperplasia was characterized by elongation and proliferation of mitotic cells. Necrosis of crypt cells was observed in duodenum and jejunum.

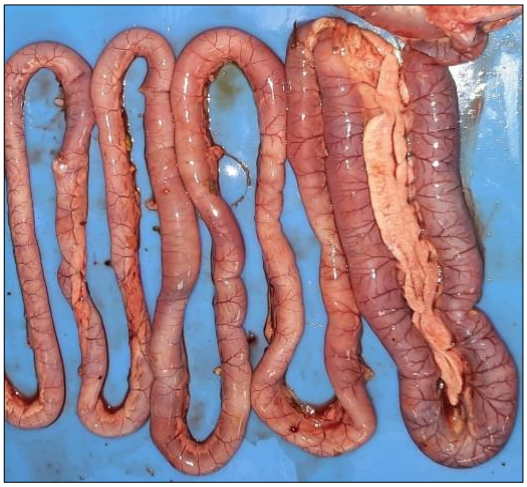

Fig 1: Dilated and inflamed duodenum and jejunum with atrophy of pancreas at distal end.

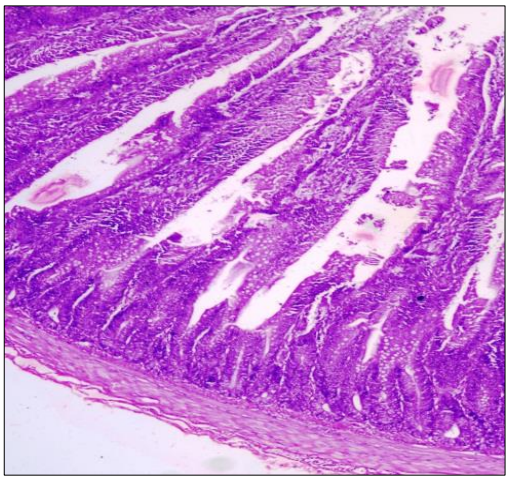

Fig 2: Duodenum showing distention of crypts with vacuolar degenerative changes and sloughing of villi (H&E, 100x).

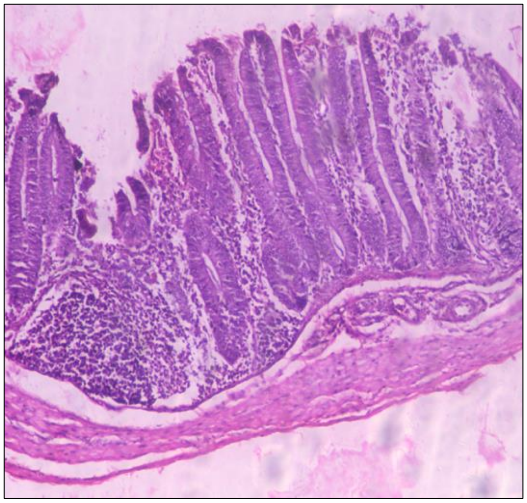

Fig 3: Jejunum showing haemorrhages with lymphoid follicle formation (H&E, 100x).

Bursa of Fabricius showed depletion of lymphocytes in bursal follicles. Proliferation of fibrous connective tissues and cystic bursitis was also evident (Fig. 4). Spleen and thymus also showed depletion of lymphocytes.

Pancreas showed moderate atrophy characterized by areas of necrotic changes in acinar cells with multifocal infiltrates of lymphocytes, presence of lymphoid follicles between the pancreatic acini and perivascular area along with fibrous tissue proliferation (Fig. 5).The microscopic lesions observed in intestine, immune organs and pancreas during the present study are in agreement with Zsak *et al.*, (2013) [23] and Nunez *et al.*, (2016) [12].

Detection of ChPV by PCR: Clinical samples of intestine collected from chickens were confirmed as ChPV by PCR. Amplification of partial NS gene of ChPV revealed 561bp product for 48/151 birds from different farms (Fig. 6). These findings are in accordance with Zsak *et al.*, (2013) [23] and Pradeep *et al* (2020) [15] who also detected ChPV from intestinal samples collected from chickens with enteritis. PCR technique has been routinely used for detection of ChPV in clinical samples without necessitating virus isolation. Conventional methods routinely used for diagnosis of ChPV infection are laborious, time consuming and less sensitive. Hindrance for virus isolation is that, ChPV do not replicate in common tissue culture, whereas for virus neutralization test, the field strains need to be adapted to grow *in vitro* (Zsak *et al.*, 2013) [23].

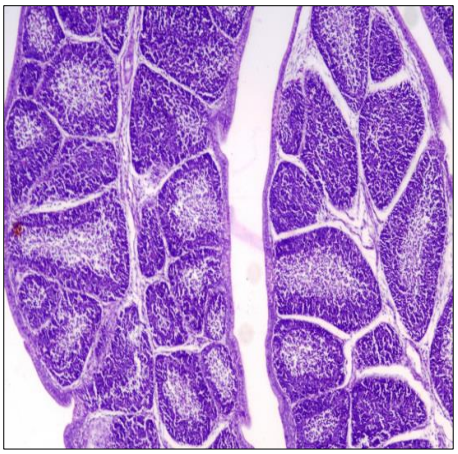

Fig 4: Bursa of Fabricious showing depletion of lymphocytes and proliferation of fibrous connective tissues. (H&E, 200x).

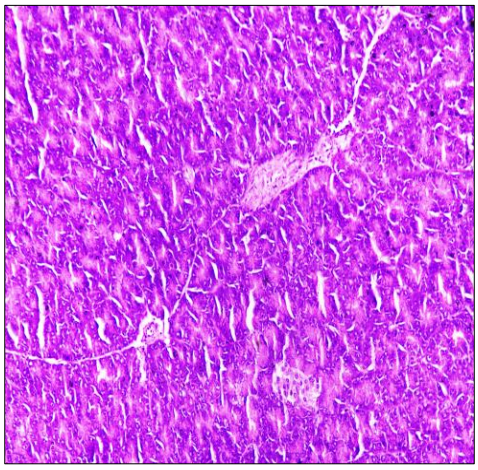

Fig 5: Pancreas: Necrotic changes in acinar cells with fibrous tissue proliferation. (H&E, 200x).

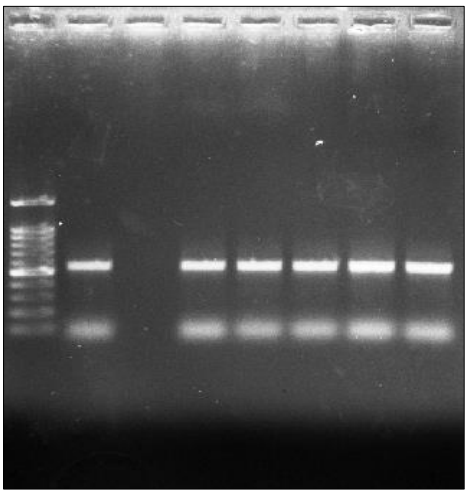

Fig 6: Agarose Gel Photograph showing PCR products of amplified ChPV. Lane 1: 100bp DNA ladder, Lane 3: Negative control Lane 2, 4, 5, 6, 7, 8: positive field sample (561bp).

Sequence and phylogenetic analysis of ChPV: Sequences of NS gene of ChPV showed 98.37%-99.19% nucleotide similarities with other previously published sequences. Nucleotide sequences of the present study showed a high degree of similarity with ChPV sequences detected in Brazil (98.37% -99.19%), USA (98.17%), Poland and Croatia (98.78%), Hungary, Turkey and Switzerland (98.38%). Phylogenetic analysis showed that detected Indian isolate is grouped with Poland 1 and USA 2 isolates (Fig. 7).

The present study revealed close association of ChPV with enteritis. Considering the prevalence status, recent advances in diagnosis, vaccinations and therapeutics, along with appropriate disease prevention and control strategies need to be followed to curtail high losses in the chickens due to this economically important ChPV pathogen in chickens.

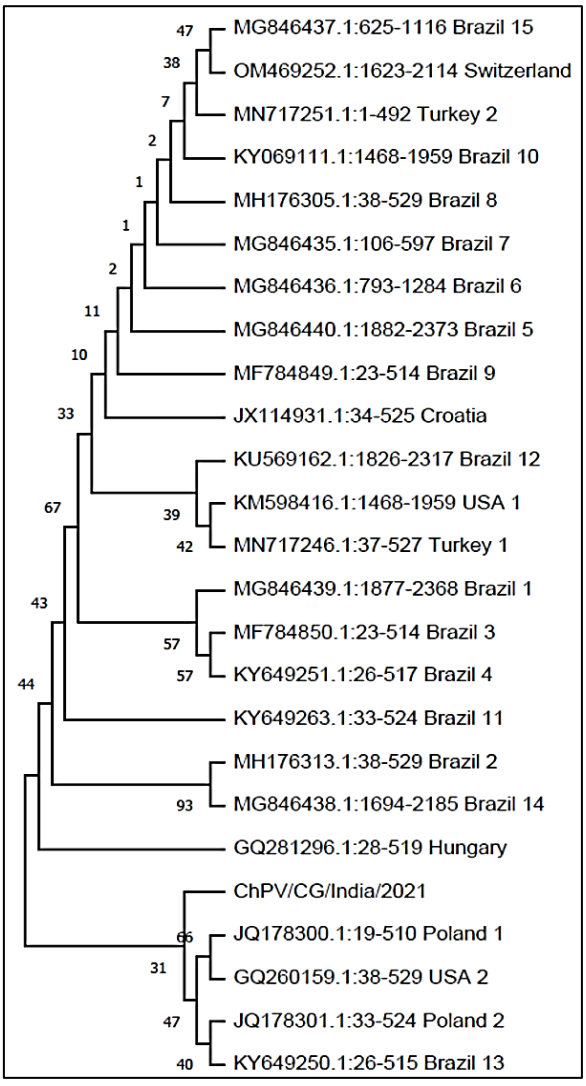

Fig 7: Phylogenetic relationship of Indian ChPV isolate with strains of other countries. (ChPV/CG/India/2021 is from this study).

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