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Studies on Pathogenicity of Chicken Infectious Anaemia Virus Isolated From a Poultry Flock of Chhattisgarh, India

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ABSTRACT

The present study reports the effects of a recent isolate of chicken infectious anaemia virus (CIAV) designated as Durg CIAV, recovered from a poultry flock of Raipur, Chhattisgarh state, India. In experimentally infected day old chicks, the isolate produced consistent clinical signs, low haematocrit values and generalized lymphoid atrophy particularly of thymus, bursa and spleen along with depletion of lymphocytes from the lymphoid organs in almost all infected chicks. Haematological studies revealed significant decrease in haemoglobin ($p \leq 0.01$), PCV ($p \leq 0.01$), total leucocyte count ($p \leq 0.01$) and lymphocyte ($p \leq 0.05$) values. Results revealed that Durg CIAV isolate is pathogenic and immunosuppressive in nature. Therefore, suitable prevention and control strategies need to be formulated to prevent the virus infection in avian flocks in the country.

Keywords: Pathogenicity, Chicken infectious anaemia virus (CIAV), PCR

Indian poultry industry has progressed from backyard activity to an organized scientific and vibrant industry. Due to high demand of safer protein source, poultry industry has always been faced with challenges in the form of various diseases. The diseases could be caused by viruses, bacteria, fungi, protozoa, metazoan parasites and arthropods and can lead to losses in the form of mortality, production loss, condemnation and the cost of medication. Many viral agents cause economically significant clinical conditions in poultry. One of the most significant conditions is Chicken infectious anaemia (CIA) posing a serious threat to the industry and has assumed significance due to its immunosuppressive effects.

CIA is a disease characterized by aplastic anemia, generalized lymphoid depletion, subcutaneous and intramuscular hemorrhages, and immunodepression (McNulty, 1991). Because of the immunosuppression, increased mortality due to secondary complications is often observed. The causative agent of CIA is chicken infectious anemia virus (CIAV). Chicken is the only recognized natural host, but serological survey has revealed prevalence of this disease in domestic and wild birds (Farkas *et al*., 1996). The clinical disease is mainly noticed in young chicks of 10–14 days of age, which usually acquire the infection vertically. The disease is characterized by increased mortality, reduced weight gain, anaemia, aplasia of bone marrow and atrophy of thymus (Rosenberger and Cloud, 1998; Todd, 2000; Senthil Kumar *et al*., 2002). The major economic loss caused by this virus is associated with severe immunosuppression and increased mortality due to secondary infections. CIAV isolates are serologically similar, although variation in severity of lesions has been reported (Dhama, 2002; Dhama *et al*., 2002; Dhama *et al*., 2008). Apathogenic CIAV isolates does not exist. Scarce resource is available on pathogenic potential of Indian isolates of CIAV to susceptible chicks (Natesan *et al*., 2006). Present situation demands constant monitoring of this virus in the field for emergence of any new variants and consequent change in pathogenicity. Hence, the present study was carried out to find out the pathogenic potential of a recently recovered field isolate of CIAV from poultry flocks of Chhattisgarh.

MATERIALS AND METHODS

Experimental infection

Clinically healthy day old Vencobb commercial broiler chicks (20) were obtained from Indian Broiler group, Rajnandgaon for pathogenicity test of Durg CIAV isolate and were randomly divided into two groups: Group 1 and 2. Group 1 consisted of 5 chicks and was kept as control. Group 2 consisted of 15 chicks and kept as Test Group. Polymerase chain reaction (PCR) positive tissue samples (bone marrow) were homogenized in phosphate buffered saline (PBS) disrupted by two freeze/thaw cycles and clarified by centrifugation. The supernatant was collected and all the 15 birds of test group were inoculated with 0.2 ml of the supernatant fluids into the leg muscle intramuscularly. Control chicks were inoculated with 0.2 ml of PBS intramuscularly into the leg muscle. Necessary approval from Institute Animal Ethics Committee was obtained for experimentation.

PCR Identification

Bone marrow from CIAV suspected birds were collected and stored at -20ºC for PCR. Tissue was collected and processed (Zhou *et al*., 1997). For PCR, DNA was extracted from bone marrow using HiPurA Mammalian genomic extraction kit according to manufacturer's instructions. The VP2 gene in CIAV from suspected CIAV birds were detected by using the forward primer 5' AAT GAA CGC TCT CCA AGA AG 3' and reverse primer 5' AGC GGA TAG TCA TAG TAG AT -3' which amplify the 587 bp DNA fragment of CIAV (Tham and Stanislawek, 1992). The PCR was performed in an automated thermal cycler (Applied Biosystems). All the reactions were carried out in a volume of 50µl in 0.2 ml PCR tubes. The PCR amplification was carried out using PCR mix containing 25 µl of 2X Mastermix (HiMedia), 1µl of 10þMol of each primer set, 5µl of DNA template and sterilized molecular grade water to make up the required reaction volume. The cycling profile included were initial denaturation of 95°C for 2 min, followed by 30 cycles of denaturation, annealing and extension at 95°C for 1 min, 56°C for 2 min and 74°C for 2 min respectively and the final extension was carried out at 74°C for 10 min and short time storage at 4°C. The amplified products were analyzed using electrophoresis unit. It was loaded to 2% agarose, stained by ethidium bromide, visualized under ultraviolet light, and photographed by a gel documentation system and the data was analyzed using computer software (Sambrook and Russel, 2001).

Clinico-pathological observations

All the chicks of both control and test group were observed for any clinical signs of CIAV. Blood samples were collected 12 days post infection (d.p.i) by jugular venipuncture. Three Chicks from control and six from test group were sacrificed on day 12 and post mortem examination was conducted to examine the presence of lesions. Bursa of Fabricious, thymus, spleen, liver, lung and kidney were collected in 10% formalin saline for microscopic examination. The formalin fixed tissues were routinely processed to obtain 4-5 µm thick, haematoxylin & eosin stained sections for histopathological examination.

RESULT AND DISCUSSION

Clinical signs and mortality

Chicks of group 2 infected with Durg CIAV isolate showed sub-acute course of disease. Infected chicks exhibited signs of anorexia, anaemia and weakness. None of the chicks died during the course of study in control and test birds. The haematological values of CIAV infected chicks showed significant decrease in Hb ($p \leq 0.01$), PCV ($p \leq 0.01$), TLC ($p \leq 0.01$) and lymphocyte ($p \leq 0.05$) values as compared to control groups (Table 1).

Table 1: Mean Haematological parameters of broiler birds experimentally infected with CIAV

Parameter	Control (n=5)	Test (n=15)	Significance
Hemoglobin (g/dl)	10.8 ± 0.37	6.6 ± 0.36	**
Packed cell volume (%)	32.6 ± 0.50	22.2 ± 0.29	**
Total Leucocyte count (10^3/ul)	16.8 ± 0.48	12.4 ± 0.38	**
Total Erythrocyte count (10^6/ul)	5.02 ± 0.44	2.8 ± 0.14	**
MCV(fl)	67.4±6.27	82.6±4.87	NS
MCH(pg)	22.06±1.33	24.83±2.36	NS

MCHC(g/l)	33.15±1.18	29.78±1.64	NS
Heterophil (%)	33.80 ± 0.37	40.66 ± 0.23	**
Lymphocyte (%)	63.0 ± 0.44	56.55 ± 0.30	*
Monocytes (%)	1.4 ± 0.28	1.33 ± 0.15	NS
Eosinophils (%)	1.4 ± 0.24	0.80 ± 0.2	NS
Basophils (%)	0.40 ± 0.24	0.66 ± 0.18	NS

Note: ** signifies P< 0.01, * signifies P< 0.05 and NS – non-significant

PCR Identification

The experimental CIAV infection was confirmed by PCR testing of the tissue samples of CIAV infected experimental chicks, using VP2 gene specific primers which yielded an amplicon size of 587 bp (Fig. 1). No amplification was seen in control birds and was found negative for the virus.

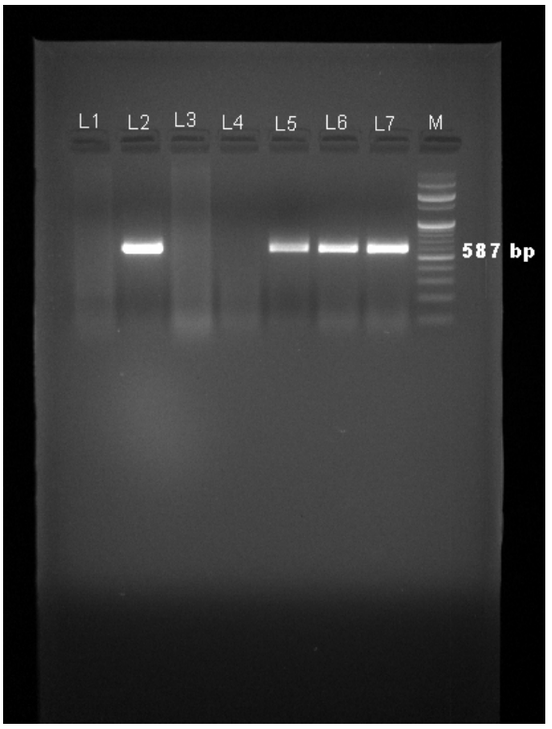

Fig. 1: Amplification of VP2 gene (587 bp) of CIAV. L1: Negative control; L2: Positive control; L3 & L4: Control group; L5-L7: Test group; M: 100 bp Ladder

Gross Lesions

The gross lesions observed in the CIAV infected birds include paleness of the liver, thymic and bursal atrophy, mild to moderate atrophy in spleen and muscular haemorrhages (Fig. 2).

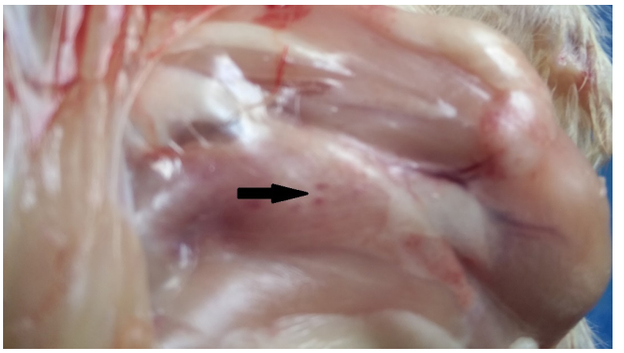

Fig. 2: Muscular haemorrhage in a chick experimentally infected with CIAV

Histopathological Lesions

Histopathological changes include severe depletion of lymphocytes in bursal follicles (Fig. 3). Thymus showed congestion and depletion of lymphoid cells (Fig. 4). Spleen revealed depletion of lymphoid cells (Fig. 5).

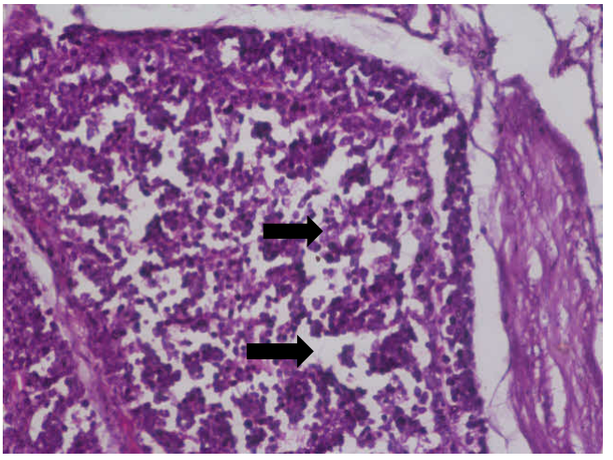

Fig. 3: Microphotograph showing severe depletion of lymphocytes in bursal follicle in a chick experimentally infected with CIAV (H&E X400)

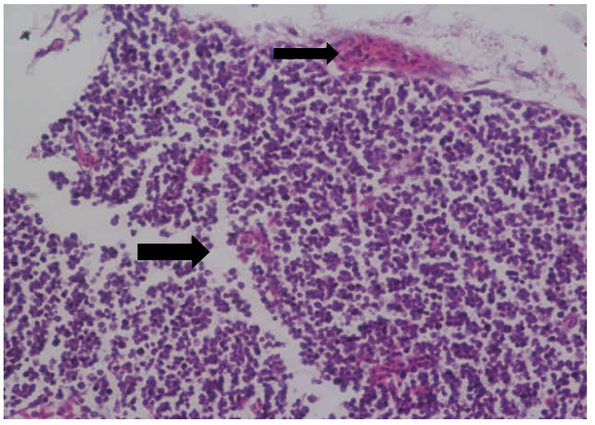

Fig. 4: Microphotograph of thymus showing congestion (thin arrow) and depletion (thick arrow) of lymphoid cells (H&E X400)

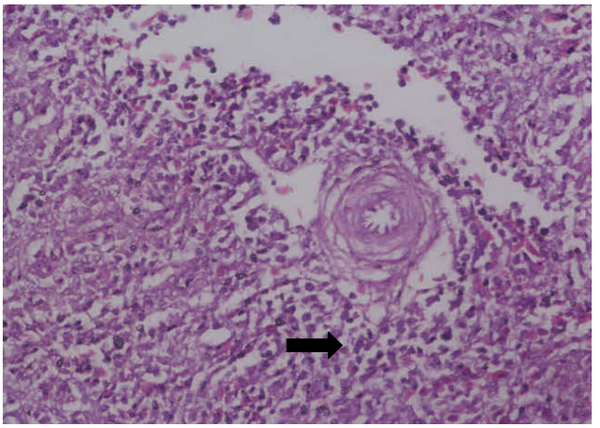

Fig. 5: Microphotograph of spleen showing depletion of lymphoid cells (H&E X400)

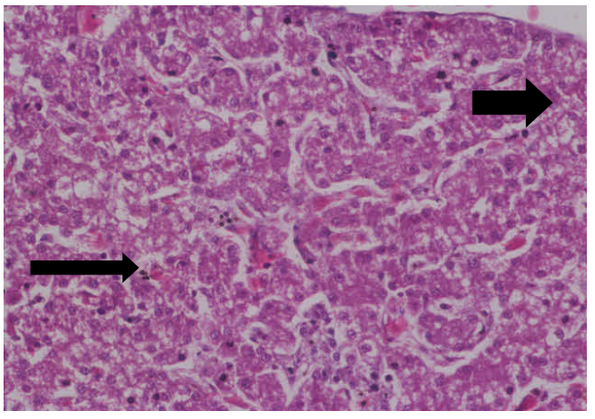

Fig. 6: Microphotograph of liver showing Fatty changes (thin arrow) and necrosis (thick arrow) in experimentally induced CIAV infected chick (H&E X400)

Liver showed fatty changes, congestion and necrosis in experimentally induced CIAV infected chicks (Fig. 6). Lung showed hemorrhages. Kidney showed mild congestion along with degenerative and necrotic changes (Fig. 7) and hemorrhages in some of the sections. All the chicks of uninfected control group remained healthy throughout the experimental period and did not develop any signs or symptoms of CIAV.

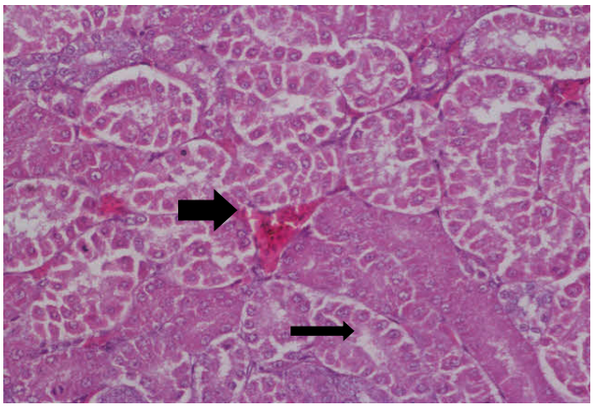

Fig. 7: Microphotograph of kidney showing mild congestion (thin arrow) along with necrotic change (thick arrow) in experimentally induced CIAV infected chick (H&E X400)

Thus, the experimental pathogenicity study revealed that CIAV isolate produced consistent clinical signs, low haematocrit values and generalized lymphoid atrophy particularly of thymus, bursa and spleen along with histopathological lesions of lymphocyte depletion from the lymphoid organs in almost all infected chicks.

The clinical signs, postmortem lesions, and PCV values agreed with the findings of other workers (Yuasa *et al*., 1979; Taniguchi *et al*., 1982 & 1983; Pope, 1991; Ramdan *et al.,* 1998 and Aly, 2001) who stated that hematocrit value below 27% with yellowish changes in bone marrow and thymic atrophy may be indicative to CIAV infection beside other means of diagnosis. Histopathological examinations were in accordance with the findings of (Sakr and Talaat, 1991) who observed marked depletion of the lymphocytes in thymus and bursa of Fabricious. Various degrees of atrophy in the lymphoid follicles with scattered necrotic foci in bursa indicated immunosuppression (Hussein *et al*., 2002). Immunosuppressive effect of the virus may be attributed directly to its destructive effect of hematopoietic and lymphopoietic tissues leading to impaired immune

response (Goryo *et al.*, 1989; Smyth *et al*., 1993 and Hegazy *et al*., 2010). Similar changes were observed by many other workers (Wani *et al*., 2015 and Rimondi *et al*., 2014). PCR assay is proved to be specific and more sensitive since DNA can be extracted from the same tissues as used for virus isolation (Soine *et al*., 1993; Dren *et al.*, 1994 and Miller *et al*., 2001). In this study, PCR yielded positive reactions with correct size as primers (587 bp) for confirmation of CIAV infection in chicken flocks that showed clinical signs, reduced hematocrit value and postmortem lesions. Bone marrow samples were positive because CIAV targets erythroid and lymphoid progenitor cells in the bone marrow and thymus respectively (Adair, 2000). Hailemariam *et al*., 2008 recorded that out of 60 commercial broiler breeder hens tested for the presence of CIAV DNA by nested PCR assay, the highest percentage of positive samples was detected in spleen where 45 samples out of 60 (75%) were positive for CIAV.

CONCLUSION

CIAV isolate produced anaemia and characteristic lesions typical of CIA in almost all the inoculated chicks indicating its pathogenic potential. The results of the present study supported the evidence of other workers about naturally occurring isolates being pathogenic to day old susceptible chicks. Being an emerging pathogen circulating in the poultry flocks of the country, suitable prevention and control strategies need to be implemented for this economically important avian pathogen.

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