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Molecular characterization of chicken anemia virus outbreaks in Nagpur province, India from 2012 to 2015

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ABSTRACT

Chicken anemia virus (CAV) is one of the important poultry pathogen. CAV infection can cause immunosuppression, aggravation of co-infections, vaccination failures and mortality. We are reporting the CAV outbreaks from the Nagpur province of India, between the years 2012–2015. The breeds included cockerel and Black Australorp of age varying from 29 to 50 days. The mortality rate observed among poultry was from 20% to 62.5%. Clinical symptoms like anemia, subcutaneous hemorrhages, growth retardation, abnormal feathers and hind limb paralysis suggested CAV infection. Postmortem analysis showed hemorrhages in thigh muscle and atrophy of the thymus and bone marrow. Seven out of 11 samples showed positive amplification of the CAV genome upon PCR. Phylogenetic analysis of all the seven isolates based on VP1 gene nucleotide sequence suggested circulation of genotype A strains in Maharashtra. The study will help us understand the circulating genotype of CAV in India and formulate its diagnosis and vaccination.

1. Short communication

Chicken anemia is an important disease of poultry. First reporting of the disease was in Japan in the year 1979 [1]. The disease is ubiquitous in all major chicken producing countries of the world [2–13]. Chicken anemia virus (CAV) gained global attention as an immunosuppressive agent which targets hemocytoblasts in bone marrow and precursor lymphocytes in thymus [14]. The virus shows both vertical and horizontal transmission by contact exposure to infected chickens [15,16]. Although birds of all age groups are susceptible to infection, the clinical signs are observed in detail in young birds [16]. Adult birds develop subclinical infections leading to significant economic losses [17]. CAV causes clinical disease with high mortality characterized by anemia, subcutaneous hemorrhages, growth retardation, abnormal feathers, leg paralysis and atrophy of the thymus and bone marrow [1,18]. CAV targets the thymus impairing the maturation of T lymphocytes leading to compromised cell-mediated immunity response [14]. Immunosuppression caused by lymphocyte depletion increases susceptibility to various bacterial and viral infections [19] and also deteriorates the response to vaccines [20,21].

Isolates from different countries subjected to cross neutralization test suggested the presence of only one serotype [22,23]. Later a study proposed a possible second serotype [24,25]. CAV is divided into genotypes A and B based on VP1 gene nucleotide sequence [26,27]. In addition, a small cluster of CAV is considered as a possible genotype C and consists of recombinant viruses [28].

CAV is a member of genus *Gyrovirus* in the family *Anelloviridae* [29]. CAV is a small, non-enveloped icosahedral virus of 25 nm diameter and has a single stranded circular genome of about 2.3 kbp [30,31]. The genomic organization of CAV comprises of three major overlapping open reading frames (ORF) encoding viral protein 1 (VP1), viral protein 2 (VP2) and viral protein 3 (VP3) [32,33]. VP1 is the major viral capsid protein with a hypervariable region spanning 13 amino acids from 139 to 151. In addition, it plays a vital role in the growth and spread of the virus [34]. VP1 protein carries epitopes responsible for generating the neutralizing antibody response [35]. VP2 is a non-structural protein with phosphatase activity and helps in scaffolding during virion assembly. Moreover, VP2 has been modulated for attenuation of the CAV

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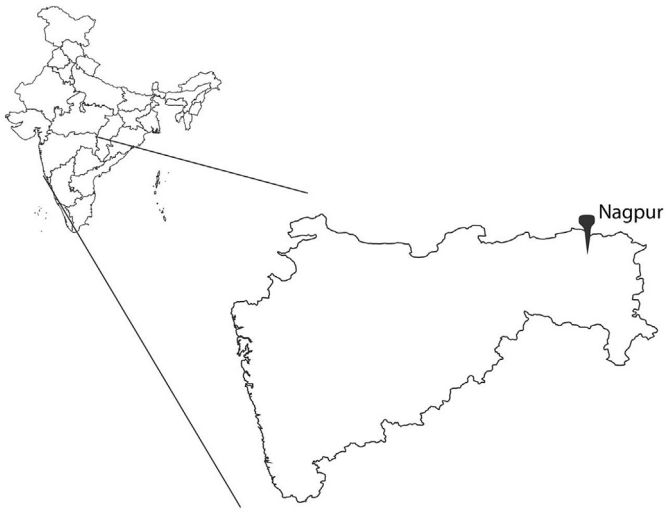

Fig. 1. Geographical location of chicken anemia virus outbreak in India.

Table 1
Details of the chicken anemia virus outbreak in the state of Maharashtra, India during 2012–15.

Code	Date	Flock	Age	Mortality
NGP-1	28/09/2012	Cockerel,	50 Days	62.5%
NGP-2	4/10/2012	Cockerel,	30 Days	40%
NGP-4	18/10/2012	Black Australarp,	42 Days	50%
NGP-7	19/10/12	Cockerel,	30 Days	20%
NGP-8	20/10/2012	Cockerel,	38 Days	20%
NGP-10	7/8/2015	Cockerel,	45 days	25%
NGP-11	18/9/2015	Cockerel,	29 days	40%

strains [36,37]. In addition, VP2 protein seems to play a minor role in the induction of apoptosis [38,39]. Both VP1 and VP2 proteins of CAV are antigenic and responsible for producing a neutralizing antibody response [40]. The VP3 protein (apoptin) triggers tumor specific apoptosis independent of p53 activation, which makes it a propitious tool for cancer gene therapy [41]. In addition, VP3 is an important mediator of the generalized lymphoid atrophy and anemia that leads to the mortality of CAV infected chicken [42,43].

Challenges for the current CAV vaccines include an inability of the virus to grow in high titre in embryo and cell culture. Subunit vaccines and recombinant viruses can act as potential vaccines for CAV [44]. DNA vaccine for CAV is reported to be safe, stable and induce both humoral and cell mediated immune response [35,45]. Rather, a plant based platform, for the expression of targeted immunogenic proteins, may be a good alternative for oral vaccine [46]. In India, major poultry vaccine failure is due to improper vaccination and inadequate cold chain maintenance [47,48].

Outbreaks of CAV were reported from the Nagpur province in the state of Maharashtra, India between the years 2012–15 (Fig. 1). Reports of outbreaks affecting 29–50 days old birds with a mortality rate of 20%–62.5%, representing different breeds are available in Table 1. An autopsy was carried out on dead birds showing symptoms of CAV infection. Tissue samples like thymus, liver and spleen were collected under aseptic condition from the birds exhibiting clinical signs and lesions indicative of CAV infection. Collected tissue samples were homogenized and stored at −70 °C for further nucleic acid isolation. Genomic DNA was extracted from tissue homogenate. Gene specific primers designed from consensus sequence available from GenBank were used to amplify the CAV genome. The whole genome of CAV isolates from Nagpur was amplified with a pool of six primers viz; CAV1F 3′-GCATTCC-GAGTGGTTACTATTC-5′, CAV1R 3′- CGGAATTCTTACAGTCTTATA-CACC-5'; CAV2F 3′-TAGCTAGCATGGACGCTCTCCAAGAAGATAC-5′ and CAV2R 3′- CAGGGTCATTTGCTTAGGGTG-5'; CAV3F 3′-GTGCCCTCCGCGACACCATCGGC-5′ and CAV3R 3′-GATTGTGCGA-TAAAGCCATTTGC-5'. Amplification was carried out by Phusion polymerase (NEB, USA) and the amplified PCR product was sequenced using the dideoxy chain termination method. The

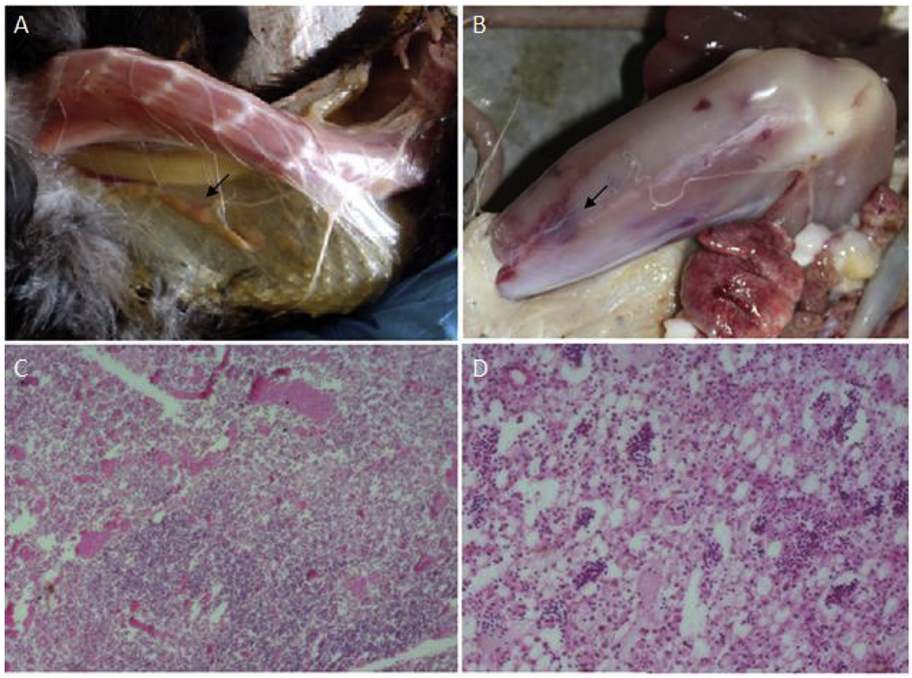

Fig. 2. Gross and histopathological findings of chicken anemia virus infected birds. Gross findings included the atrophy of thymus (A) and hemorrhage in thigh muscles (B). Microscopic lesions showing depletion of cortical thymocytes (C) and hypoplasia of myeloid cells (D).