Molecular characterization of the chicken anemia virus by using the VP1 gene of CAV/ SK/2017 strains, Iraq

ABSTRACT

Chicken infectious anemia virus (CIAV) is one amongst the numerous pathogens in the poultry industry. CIAV infection can cause restraint of the immune system, the excitement of co-infections, vaccination failures and mortality. chicks. Maternal antibodies not prevent infection but to prevent these symptoms, immunological disorder, or transmission of the virus. Genetic characterizations for five sequences of chicken infectious anemia virus (CIAV) from different flocks of broiler industrial chickens were a consideration for the primary time in Iraq. Phylogenetic analysis of the viral protein 1 (VP1) gene, as well as the hypervariable region of the CIAV genome, indicated that Iraqi CIAV strains have belonged into genotype II. Amino acid comparison exhibit that the diversity of VP1 is indicated that the new strains were extremely pathogenic viruses. Our epidemiological study provided new insights into the prevalence of CIAV strain in recent years in Sulaimani province/Iraq.

Keywords: Viral protein (VP1), viral evolution, phylogenetic analysis

Introduction

Chicken infection anemia virus (CIAV) is a Gyrovirus belonging to the family Circoviridae. The agent is a nonenveloped, initial isolated by (Yuasa et al., 1979; Hailemariam et al., 2008). The genome consists of a single molecule of circular (covalently closed end) negative-sense single-stranded DNA (Gelderblom et al., 1989; Murphy et al., 1999). CIAV is associate degree economically necessary microorganism with a worldwide distribution. CIAV infections are manifested by either clinical or subclinical signs. (Schat, 2004). The clinical symptoms are mainly perceived in young chicks of 10–14 days of age that typically acquire the infection vertically. (Adair, 2000). The arrangement of the genome consists of three overlapping open reading frames (ORFs) encoding three viral proteins: VP2, a dual-specificity phosphatase This was the first dual specificity protein phosphatases gene to be identified in a small viral genome (Noteborn et al., 1998; Peters et al., 2006).

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Research Article

Mohammed Omar baba sheikh

Department of Molecular biology-veterinary laboratory centre, Sulaimani veterinary directorate
ORCİD - 0000-0002-0503-5267

Correspondence

Sulaimani Veterinary directorate, Shekh mhedin, Sulaimani, Kurdistan region, Iraq
muhamad.omer@ymail.com
009647701571458

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VP3, additionally called poptin, that has been shown to possess apoptotic activity in transformed cell lines (Jeurissen et al., 1992), and VP1, the foremost capsid protein, a larger region of the genome that contains the hypervariable region in additionally to upstream amino acid differences; so, it's typically used for genetic characterization and molecular studies. (Craig et al., 2009; Renshaw et al., 1996). In classical CIAV, has three genetically distinct genotypes (I, II, and III) have been recognized through phylogenetic analysis based on the VP1 gene (Islam et al., 2002; Snoeck et al., 2012). Genotypes II and III are rumored to be distributed worldwide, whereas genotype I consist alone of isolates from Australia (Kim et al., 2010). In Iraq, the primary has reported the detection of CIAV in broiler chicken (Al-Al-Mohana, al., 2013) but, genetic characterization from Iraq isolates has not been reported. This study describes the CIAV in 4, 10, 14 day-old commercial broilers and also the detection of CIAV DNA in tissues. A virus strain was genetically characterized in a part of the VP1 gene for the first time in Iraq, and the extent of genetic variation among the current strains and Global relationship with other CIAV strains was analyzed. Moreover, we tend to compare the particular amino acids region in VP1 that determined the virulence of CIAV.

MATERIAL METHODS

Samples and DNA extraction

Tissue (liver, spleen, and bursa) samples were obtained from clinical and subclinical 10 broiler flock in Sulaimani province between the ages of (4, 10 and 14) days. The samples were collected during the April 2016 to June 2017 and directly sent them to Sulaimani veterinary diagnostic laboratory. The CIAV DNA was extracted from pooled samples of each flock using extraction kit for tissue (Genet-Bio Republic Korea) from the homogenized liver, bursa, and spleen of chickens.

Virus DNA amplification by PCR

The extracted DNA was amplified using the primers CAV1 and CAV2 for PCR covering a 387-nucleotide region in the highly conserved overlapping sequence of VP2 (Cardona et al, 2000,; KUMAR. 2007), anemia f and anemia r for PCR product of 675 bp (Marin et al, 2012; AboElkhair et al, 2014) of partial VP1 genes (Table 1). The PCR amplification was performed in a 20 µl volume by using PCR Premix (2X). This kit provides a complete system for fast, high yield and reliable single tube one (Genetbio, Korea). The amplification was performed with the Thermal Cycle (Hercuvan, USA). The PCR profile of CAV1 and CAV2 primers was pre-denaturated at 94°C for 5 min, followed by 35 cycles of denaturation, annealing and extension at 94°C for 30 sec, 60°C for 30 sec and 72°C for 30 sec respectively. and a final extension of 72°C for 5 min. The reaction of Anemia f and Anemia r primers was performed by denaturation at 94°C for 5 min, followed by 40 cycles of denaturation, annealing and extension at 94°C for 30 sec, 60°C for 30 sec, 72°C for 40 sec and a final extension 72°C for five min. The PCR products were analyzed by electrophoresis on a 1% agarose gel and visualized on Gel Documentation (UVtec, UK).

Table: 1 Primer oligonucleotide employed for CIAV

Primer	sequences	bp	position	target gene
CAV1-F	CAAGTA ATT TCA AAT GA A CG	387		VP2
CAV2-R	TTG CCA TCT TAC AGT CTT AT			
anemia-F	GAC TGTAAGATGGCAAGACGAGCTC	675 bp	833–1508	VP1
anemia-R	GGC TGA AGG ATC CCT CAT TC			

Sequences analysis and Phylogenetic tree

Nucleotide sequence alignments, emendation, and amino acid prognosis of just about the partial region of the VP1 gene were performed using Clustalw (Thompson et al., 1994). The predicted amino acid and nucleotide sequences of 5 CIAV were determined and compared. The MEGA 7.0 software system using for phylogenetic trees were generated by the neighbor-joining method (Tamura et al., 2013) with 1000 bootstrap replications. Sequences data were submitted to GenBank with accession numbers MH095973 to MH095977, KY399853, and KY399854

RESULT

CIAV Identification

7 samples were screened by PCR assay using cav1 & cav2 primer pairs and expected size 387bp (Figure 1). Only five samples were found to be positive using the anemia primer pair and expected the size of 675 bp (Figure 2). The positive case as well as all age of the flock that employed in this study.

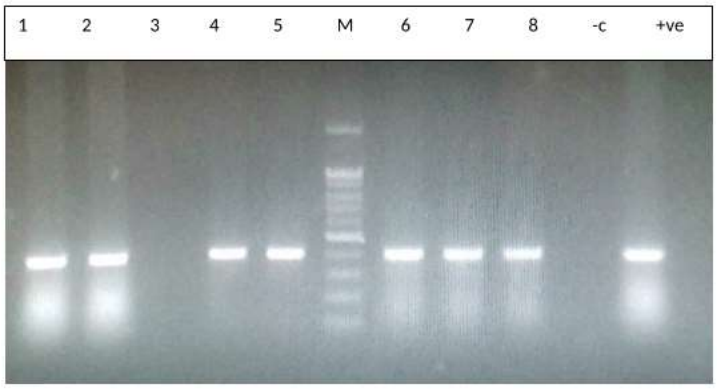

Fig 1: Detection of CAV by PCR and amplification of CAV VP2. Specific PCR product (387 bp) detected in CAV-infected chicken; (M) 100 bp DNA ladder .lanes 1-8, positive samples, except lane, 3 Negative samples. Lane –c : Negative control. Lane +ve: Positive control.

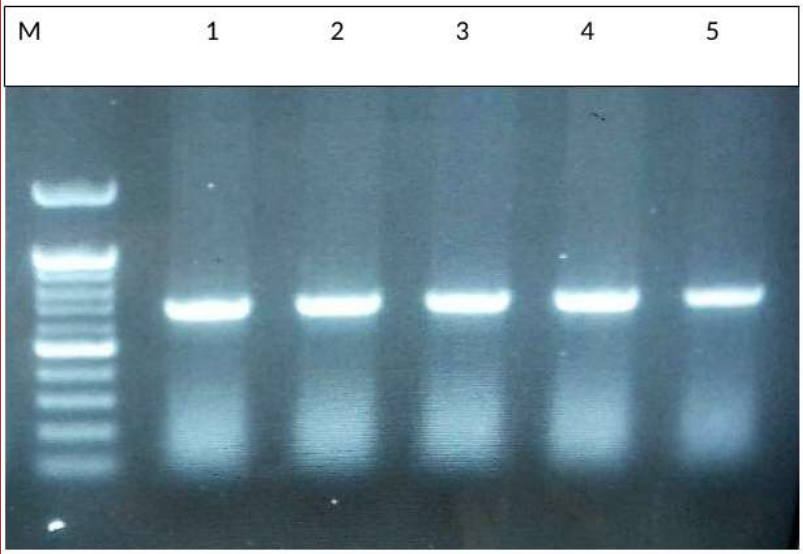

Fig 2: Detection of CAV by PCR and amplification of CAV VP1. Specific PCR product (675 bp) detected in CAV-infected birds; (M) 100 bp DNA ladder .lanes 1-5, positive samples.

The obtained nucleotide and amino acid sequences of five CIAV partial VP1 genes during this study were compared with different CIAV reference strain in GenBank by multiple alignments with the ClustalW enclosed in MEGA.7 software. The five nucleotide sequences of detected CAV displayed a restricted diversity were closely associated with one another with identity 97–99% whereas amino acid identity 97-100% among them. The nucleotide sequence identities between the five CIAV isolate partial VP1 and 35 CAV isolates retrieved from GenBank ranged between 93.36%and 98.24%. The highest identity (98.24%) was found between the chicken CIAV isolates and IR4-CIAV (accession no. KU195692, isolated from Iran, in 2013). The lowest identity (93.36%) was found between the chicken CIAV isolates and CAU269/7 (accession no. AF227982, isolated from Australia in 2000). Interestingly, compare amino acid sequences of those isolate with three industrial vaccines (Del rose and Cux-1, NobilP4vac) strains, exhibit homology around (95.5-97%, 95-96.5%, and 94.5-96%) respectively. See (Table 2). Multiple sequences alignment of five CIAV strains with six references strains as well as 3 industrial vaccines within the (positions 139–151), and virulence-associated motif (Kye et al, 2013; Todd et al., 2002) positions 75, 89, 125, 139, 141, and 144 in the VP1 protein, all Iraqi CIAV that were conserved among genotype II viruses and given I, T, I, Q,Q,Q respectively as the majority of virulent strains (Figure.4). Phylogenetic analysis of the VP1 nucleotide sequences by neighbor-joining separated the CAV strains into 3 distinct genotypes: I, II, and III, (Ducatez et al.,2006; Snoecket al., 2012). The phylogenetic analysis of the partial VP1 gene sequence of CIAV and strains in this study showed that five field virus sequences belonged to (Genotype II) and were clustered with (CAVb/Brzl, /IR5-CAV and /GD/China) isolates, (Figure.3), however Five field virus amino acid sequences it's to create divergence when compared with three genotypes (II, III and I) ranged around (2.4%, 4%, and 4.5%) respectively.

DISCUSSION

CIAV is an economically important pathogen worldwide due to its highly immunosuppressive effect. In Iraq, CIAV was first reported in 2012 (Al-Mohana et al., 2013). within the current study, Out of 10 tissue homogenate were examined and investigated for the presence of CIAV DNA, seven samples were positive with proportion seventieth, It confirmed that CIAV is widely distributed among chicken flocks in Sulaimani province, This result suggests that CIAV can be concerned in inflicting subclinical infections. Attributable to the widespread application of vaccination for breeders and therefore the presence of maternal antibodies, the clinical type of CIAV is rare (Sommer and Cardona, 2003).

Phylogenetic analysis of the VP1 sequences classifying CIAV genetically into 3 distinct genotypes (I, II, and III), all isolated during this study clustered with genotype II (Figure 3), this result indicated that Genotype II more prevalence in Iraq. The genetic variations of CIAV are determined depending on the sequence of the VP1 gene which contains the hypervariable region in between the amino acid residues 139–151 (Kye et al, 2013), additionally to be the presence of the genetic determinates of CAIV virulence. The amino acid motif of the highly pathogenic CIAV as I75, T89, I125, 139, Q141, and Q144 strains (Abdel-Mawgod et al., 2018). A comparison with the VP1 sequence of Iraqi CIAV in this study, it had been found that the five field virus sequences contain the motif of highly pathogenic viruses (Figure. 4). Vaccination of poultry trade presently isn't promptly obtainable, the topology of the phylogenetic tree showed that the CIAV Iraqi sequences are

in a distant relationship with different vaccine strains commercially used, for example, Del-Ros, Nobilis P4, and Cux-1. All five isolate CAV sequences are enclosed in cluster II, whereas vaccine strains are located in cluster III. Nevertheless, Del-Ros vaccine strain is considered as an appropriate one of the best choice strains for induction in vaccine when compared to the other two vaccine strains (Nobilis P4, and Cux-1).

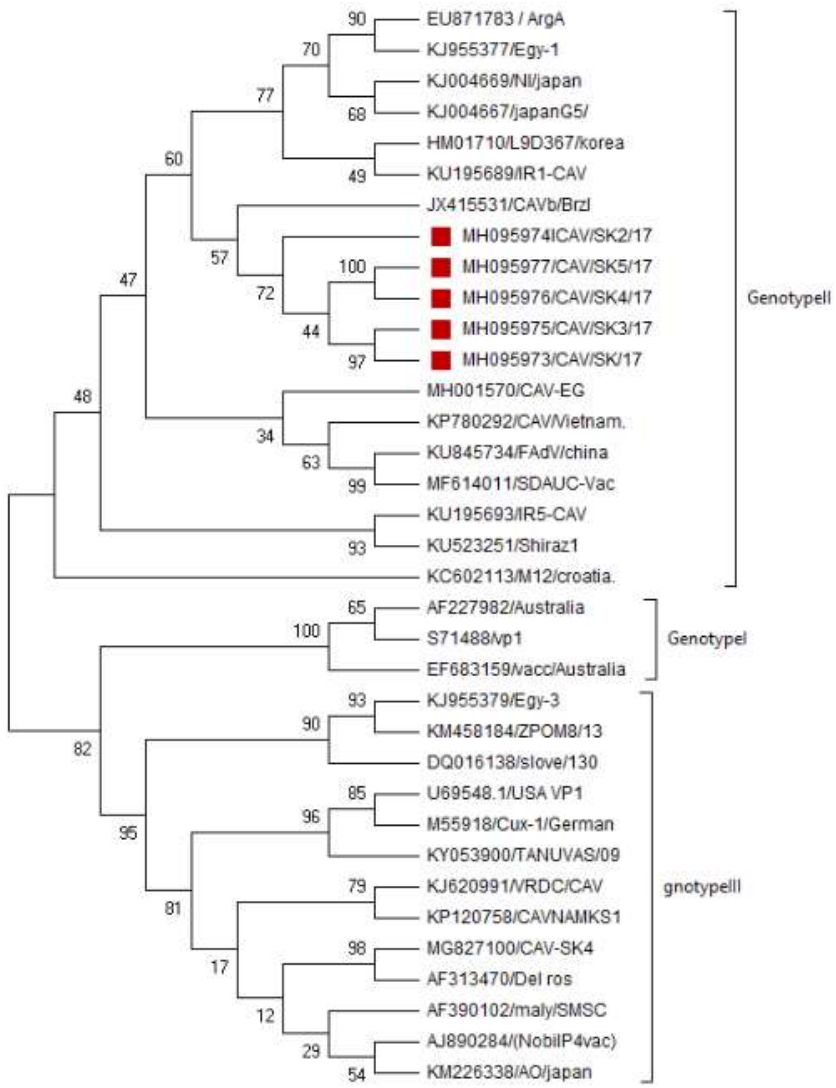

Fig 3. Phylogenetic trees of Cambodian chicken anemia virus (CAV) isolates based on comparison of the partial viral protein1 (VP1) gene sequences of representative CAV strains (n = 35). Tree construction was done using the neighbor-joining method with 1,000 replicates. The Iraqi sequences obtained in the present study are red square .

Table 2. Identities between CAVL/SK/2017 strains and different Genotype related isolates obtained from sequence comparison

ACCESION NO.	STRAIN	GENOTYPE	DNA Identity	a.a identity
AJ890284	NobilP4vac	III	94-95%	94.5-96%
AF313470	Del_ros	III	94.5-95%	95.5-97%
M55918	Cux-1	III	94.30%	95-96.5%
EU871783	ArgA	II	96-97%	97.5-99%
KU845734	FAdV/china	II	97%	96.5-98%
JX415531	CAVb/Brzl	II	97.5%	97.5-99%
KU195693	IR5-CAV	II	97.5%	97.5-99%
KC691411	CMB11090	II	96.5-975	97.5-99%
EF683159	vacc/Australia	I	94.55	95-97%
AF390102	Malaysia/SMSC	III	94%	94.5-96%
DQ016138	slove/130	III	95%	97-99%

According to the history, the virus strain was imported through a trade of eggs from unvaccinated breeders. Veterinarians and breeders ought to perceive the need for vaccination to eliminate economic losses caused by CIAV infections. Additionally, imported eggs and breeders should come from flocks vaccinated against CIAV.

Management assessment is critical, because CIAV is a very resistant virus to the environment, and CIAV has worldwide distribution. It's terribly troublesome to eradicate the disease, however, a vaccination-primarily based strategy and serological tests can facilitate to regulate CIAV infections in young birds. Studies have exhibited the presence of clinical disease associated with CIAV infection in 2-to-4- wk-old broilers from vaccinated parent flocks (Brentano et al., 2005). The information questions the role of maternal immunity in the prevention of transmission and development of clinical illness in young chicks and complicates the role of control measurements.

In conclusion, the results of this study indicate that CIAV exists in Iraq broiler farm and could have an economic impact on the poultry trade, Epidemiological studies on an outsized scale are required to evaluate the distribution of CIAV infections in Iraq poultry industry, Moreover, our results counsel that an extra study of the CIAV vaccine is also required to elucidate the relationship between vaccine efficaciousness and local strains in Iraq, in addition as additional studies associated with reversion of vaccine.

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Conflicts of interest

The author declares that there is no conflict of interest.

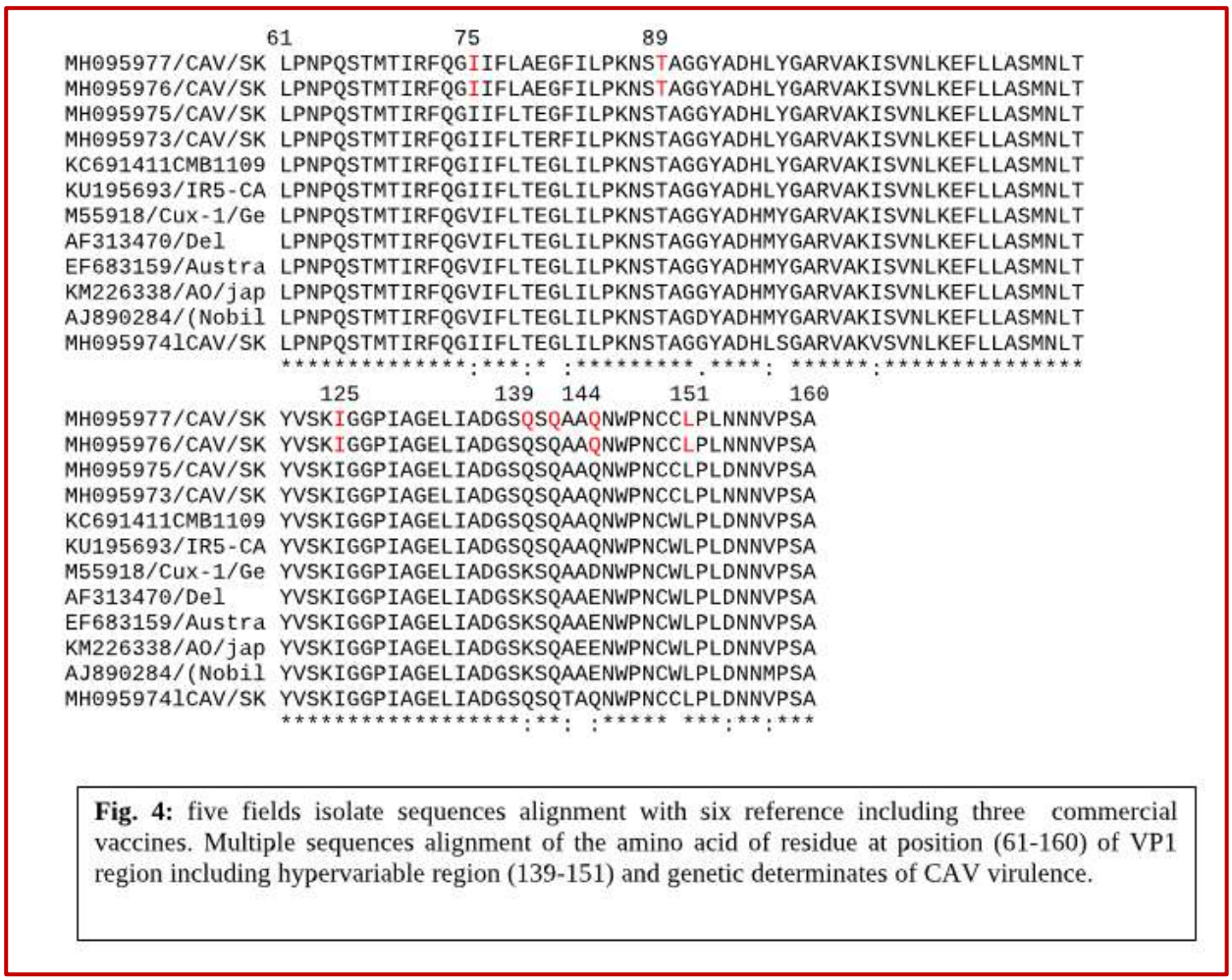

Fig. 4: five fields isolate sequences alignment with six reference including three commercial vaccines. Multiple sequences alignment of the amino acid of residue at position (61-160) of VP1 region including hypervariable region (139-151) and genetic determinates of CAV virulence.

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