



Molecular Characterization of Chicken Anaemia Virus Circulating in Commercial Poultry Flocks in Egypt during 2020

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ABSTRACT

Chicken Anemia Virus (CAV) is an extremely contagious immunosuppressive disease causing high economic losses in poultry production. In the present study, tissue samples (bone marrow, thymus, and spleen) were collected from 86 different broiler chicken farms located in fourteen governorates in Egypt during 2020. They suffered from retard growth, weakness, and a drop in egg production with an observed mortality rate ranged 5-15%. A total of 26 samples were positive for CAV using PCR in six governorates in Lower Egypt with a 30% incidence rate, especially in Sharkia (78%), Ismailia (62.5%), and Alexandria (60%). The viral protein1 (VP1) gene of CAV was genetically characterized by sequencing of 10 selected viruses in six governorates, revealing that all Egyptian strains were clustered into two groups (A, B) that was distinct from vaccine strains (Del-Ros, Cux-1, and 26PA) which were clustered in group C. The seven Egyptian viruses in this study (A-Egypt-AN1-2020 to A-Egypt-AN7-2020) were clustered with the viruses from Japan, Argentina, and Malaysia in group A, and the other three viruses (A-Egypt-AN8-2020, A-Egypt-AN9-2020, A-Egypt-AN10-2020) were clustered with the viruses from Nigeria, and India in group B. The Egyptian viruses in the current study acquired new specific mutations clustering them into new subgroups (2A, 2B). By mutation analysis comparing with Del-Rose reference strains, V75I, M97L, and K139Q, E144Q were recorded in all viruses in the group A and B. All Egyptian viruses in the current study had specific new mutations at Y13N, H22N. Moreover, mutation at G74E in Egyptian viruses recorded in the current study was related to sub group 2A, I83V in three strains (A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020), and S140A in the hypervariable region was found in four strains (A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and A/Egypt/AN5/2020) in subgroup 2A. Furthermore, Q139 and Q144 amino acid substitutions, which are important in viral replication, were observed in all viruses. The field viruses in the study were distinct from the vaccinal strains by phylogenetic analysis and A.A. identity. In conclusion, the CAV was continuously circulating in Egypt from different genotypes. It acquired new specific mutations clustering them in a new subgroup, and it was distinct from vaccinal strains. Therefore, it is important to conduct continuous monitoring on the genetic evolution of CAV and further studies on the pathogenicity of the virus and the vaccine efficacy.

Keywords: Chicken Anemia Virus, Egypt, Genetic evolution, Viral protein 1 gene

INTRODUCTION

Chicken Anaemia Virus (CAV) is an immunosuppressive pathogen leading to a barrage of economic losses for poultry breeders (Islam et al., 2002). The first detection of CAV was in Japan in 1978 (Yuasa et al., 1979). Then it was recorded and spread in different governorates in Egypt leading to high economic losses (Erfan et al., 2018).

The Chicken Anaemia Virus causes high economic losses, especially in broiler chickens. Chickens of all ages are vulnerable to infection, but the chickens less than two weeks old commonly represent the clinical diseases (Miller and Schat, 2004; Cheng et al., 2019). The infection is characterized mainly by anemia, weight loss, and lymphoid atrophy accompanied by immunosuppression that leads to an increase in the susceptibility of secondary bacterial and viral infection. The mortality and morbidity rates due to CAV infection reach 55-80% (Lai et al., 2018).

Chicken Anemia Virus (CAV) is a non-enveloped virus and has a negative-sense genome (Peters et al., 2006) that consists of three overlapping open reading frames encoding to three viral proteins (VP1, VP2, and VP3). The VP1 capsid protein is an important major structural protein. The VP2 protein is a dual-specificity protein that acts as a scaffold to phosphatase activity to assist the correct assemblage of VP1 protein (Craig et al., 2009; Rosario et al., 2017). The VP3 protein called apoptin is the primary factor of virulence of CAV inducing the apoptosis of the thymocytes and hemopoietic cells in infected chickens (Castaño et al., 2019).

The CAV's Amino Acid (A.A.) composition is extremely restrictive, with major differences in some regions in the VP1 gene called Hyper Variable Region (HVR) at 139-151 A.A. (Zhang et al., 2013). The VP1 gene is responsible for the genetic characterization of the virus, and It encodes a highly immunogenic protein responsible for the potential and

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virulence of infection (Lien et al., 2012). Three genotypes of CAV (I, II, and III) are genetically distinct and recognized based on the phylogeny of VP1 gene (Snoeck et al., 2012). Genotypes II and III are worldwide distributed, while genotype I consists alone of Australian isolates (Kim et al., 2010). Only minor mutations in amino acid sequences have been recorded in CAV viruses from various geographical positions (Ducatez et al., 2005; Kim et al., 2010). The Commercially used CAV vaccines (Cux-1N/Germany and CAV/Nobilis®P4) are derived from wild-type CAVs that are not completely attenuated; therefore, the virus can revert to virulent, and be transmitted horizontally and vertically causing clinical symptoms in young chickens (Tseng et al., 2019)

The objective of the present study was to disseminate the situation and molecular characterization of CAV circulating in Egypt during 2020 and to determine the relationship between current viruses and vaccine strains for improving the control of the virus.

MATERIALS AND METHODS

Tissue specimen

The samples were collected from 86 commercial broiler chicken farms (aged two to four weeks) located in 14 Egyptian Governorates (Beheira, Sharqia, Qalyubia, Dakahlia, Ismailia, Gharbia, Giza, Matruh, Cairo, Alexandria, Minya, Beni Suef, Sohag, Faiyum) during 2020 (Table 1 and Figure 1). Most tested broiler chicken farms were vaccinated. The samples were collected from different tissues (bone marrow, thymus, and spleen). The samples were homogenized by grinding. Twenty-five mg of various affected tissues were grounded with phosphate buffer saline (PBS) and the mixture of one mg streptomycin sulphate/ml and 1000 I.U. penicillin/ml using a mortar and pestle. The Freezing and thawing were carried out three times, and then it was centrifuged at 3000 rpm for 20 minutes. Next, the supernatant was collected in a new tube and stored at -20 till used (Hirai and Shimakura, 1974).

Ethical approval

The handling of chickens was based on the ethical role of the National Animal Health and Research Institute, Giza, Egypt. The tissue samples were collected from dead chickens suffering from an infection of CAV in commercial farms. Both handling of the chickens and sampling were performed with respect to animal rights, and immediately after the confirmation of death by a veterinarian.

Table 1. The location, number, and the result of evaluated samples by PCR for detection of chicken anemia virus

Governorate	No. of collected samples	No. of CAV positive farms
Beheira	10	5
Sharqia	9	7
Qalyubia	9	4
Dakahlia	8	0
Ismailia	8	5
Gharbia	7	0
Giza	7	0
Matruh	5	0
Cairo	5	2
Alexandria	5	3
Minya	5	0
Beni Suef	3	0
Sohag	3	0
Faiyum	2	0
Total	86	26 (30%)

CAV: Chicken anemia virus, No: Number

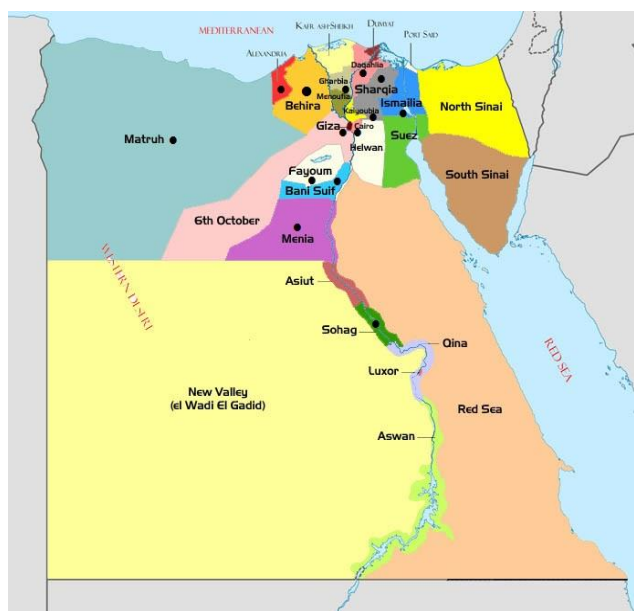


Figure 1. Distribution of chicken anemia virus in Egyptian map in 2020

Detection of chicken anemia virus by PCR

DNA was extracted from tissue homogenate by QIAmp DNA mini kit (Qiagen Inc., Valencia, Calif., USA) following the manufacturer's instructions. Amplification of partial VP1 gene was carried out by using EmeraldAmp® GT PCR kit (TaKaRa Bio, Inc., Shiga, Japan), gene-specific oligonucleotide primers (5-GAC TGT AAG ATG GCA AGA CGA GCT C-3 and 5-GGC TGA AGG ATC CCT CAT TC-3) were used (Todd et al., 1991) according to manufacturer's instructions. The specific amplified PCR product was detected by agarose gel electrophoresis.

Sequencing of partial VP1 gene

Ten positive samples were selected for partial sequencing of the VP1 gene (Table 2). The positive amplified PCR product was purified using QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). The sequence reaction was carried

out by using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA), then it was purified by using Centrisep spin column, (Thermo Fisher, USA), and the sequence collection was performed by ABI 3500 Genetic Analyzer (Life Technologies, USA).

Genetic and phylogenetic studies

The DNA sequence of the partial VP1 gene for Egyptian CAV in this study was aligned with 23 different viruses from different countries and vaccine strains (Del-Ros, Cux-1, and 26PA) from the National Centre in Biotechnology Information (NCBI) by using Bio-Edit software clustal W (Hall, 1999). The Phylogenetic tree was made by using the Neighbor-Joining method in MEGA 6 with 1,000 bootstrap replications (Tamura et al., 2013). The genetic distance among different viruses was carried out using DNASTAR Lasergene 9 (Madison, WI, USA). The viruses in the present study were published in National Center for Biotechnology Information (NCBI) under accession number (Table 2).

Table 2. Governorates and Genbank accession number of partial VP1 gene sequence of 10 chicken anemia virus selected samples

No.	Code	Governorate	Accession number
1	A-Egypt-AN1-2020	Beheira	MW286460
2	A-Egypt-AN2-2020	Sharqia	MW286461
3	A-Egypt-AN3-2020	Alexandria	MW286462
4	A-Egypt-AN4-2020	Cairo	MW286463
5	A-Egypt-AN5-2020	Ismailia	MW286464
6	A-Egypt-AN6-2020	Sharqia	MW286465
7	A-Egypt-AN7-2020	Qalyubia	MW286466
8	A-Egypt-AN8-2020	Beheira	MW286467
9	A-Egypt-AN9-2020	Beheira	MW286468
10	A-Egypt-AN10-2020	Sharqia	MW286469

RESULTS

Clinical signs and gross pathology

The gross examination showed that there were infected flocks suffering from stunting, depression, poor growth, weakness, mild drop in egg production with mortality rates ranging from 5% to 15%. While the complete necropsy examinations revealed paleness in bone marrow and liver, atrophy of bursa of Fabricius and thymus. Subcutaneous hemorrhage was also observed in some cases. It was similar to clinical signs findings of Swayne et al. (2019).

Detection of chicken anemia virus by PCR

Out of 86 tested tissue samples, 26 samples were CAV positive at the correct size band of 675 bp with an incidence rate of 30% (Table 1). All positive samples were recorded in six governorates (Beheira, Sharqia, Alexandria, Cairo, Ismailia, Qalyubia) in Lower Egypt with the highest incidence in Sharqia, Ismailia, and Alexandria (78%, 62.5%, and 60%, respectively), and the lowest incidence in Cairo (40%).

Genetic characterization of VP1 gene of chicken anemia virus

The nucleotide and amino acid sequence of VP1 gene of selected 10 positive samples of CAV located in six governorates (Beheira, Sharqia, Alexandria, Cairo, Ismailia, Qalyubia) were aligned with different CAV viruses and vaccine strains from the National Centre in Biotechnology Information (NCBI) By using Bioedit clustal W software (Hall, 1999), and The Phylogenetic tree was made by using the Neighbor-Joining method in MEGA 6 with 1,000 bootstrap replications (Tamura et al., 2013). The phylogenetic tree showed that VP1 gene of CAV was divided into two groups (A, B) distinct from vaccine strains (Del-Ros, Cux-1, and 26PA) that were clustered in group C. The seven Egyptian viruses (A/Egypt/AN1/2020 to A/Egypt/AN7/2020) were clustered with G6/Japan/AB119448.1, ArgA0021-3/Argentina/EU871783.1 and SMSC-1/Malaysia/AF285882.1 in group A in a new subgroup 2A, but the other three viruses (Egypt/AN8/2020, Egypt/AN9/2020 and Egypt/AN10/2020) were clustered with NIE/19.04/118/Nigeria and CAV-B/India in the group B in a new subgroup 2B as shown in Figure 2.

By mutation analysis of partial VP1 gene sequence including the hypervariable region (139-151) comparing with Del-Rose reference strains, the V75I, M97L, K139Q, and E144Q mutations were recorded in all viruses related to group A and B. The last two mutations were included in the hypervariable region. All Egyptian viruses in the current study had specific new mutations at Y13N and H22N. In addition, the seven Egyptian viruses related to group 2A had G74E and I83V in A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and S140A in A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and A/Egypt/AN5/2020. The last one is included in the hypervariable region as shown in Table 3.

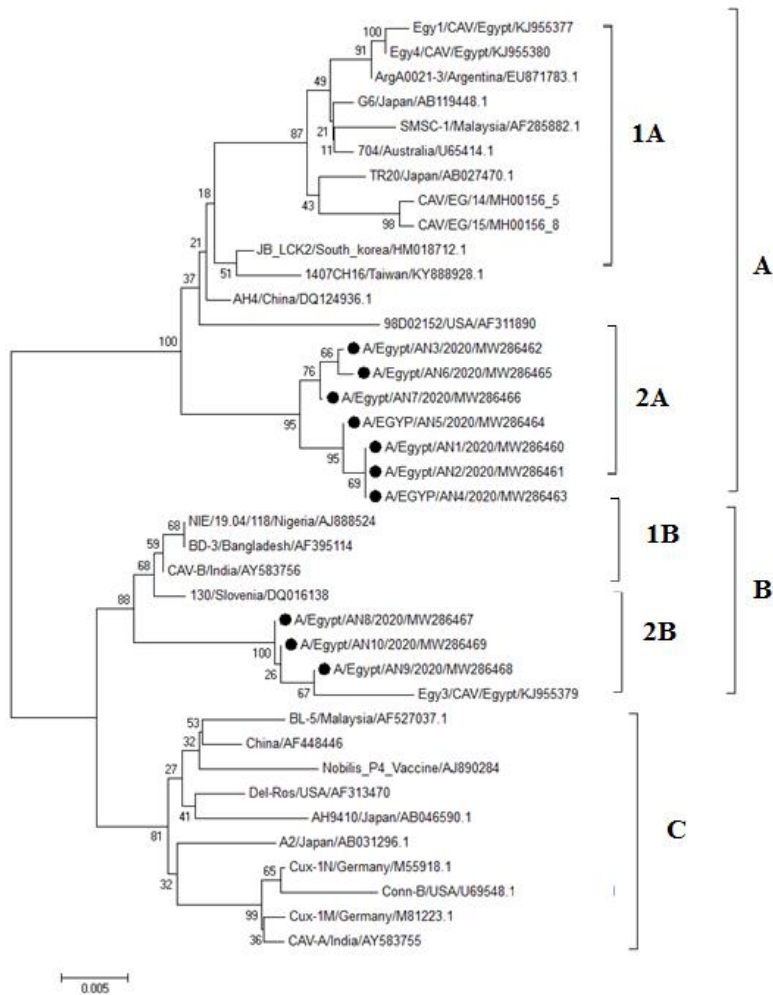


Figure 2. Phylogenetic tree of the partial VP1 gene sequence of chicken anemia virus. FN: The figure shows the phylogenetic analysis of VP1 gene of CAV revealing that all Egyptian viruses were clustered into two groups (A, B) in a new subgroup (2A, 2B) that was distinct from vaccine strains that were clustered in group C. The CAV viruses in the present study are indicated with a black dot.

Table 3. Numbers of amino acid substitution of partial VP1 gene sequence comparing with Del-Rose reference strains

Strain name	Genotype	Number of amino acid in VP1 gene								
		13	22	74	75	83	97	139	140	144
Del-Ros/USA/AF313470 (Reference strain)	C	<u>Y</u>	<u>H</u>	<u>G</u>	<u>V</u>	<u>I</u>	<u>M</u>	<u>K</u>	<u>S</u>	<u>E</u>
SMSC-1/Malaysia/AF285882.1	1A	-	-	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
AH9410/Japan/AB046590.1	1A	-	-	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
ArgA00213/Argentina/EU871783.1	1A	-	-	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
A/Egypt/AN1/2020	2A	<u>N</u>	<u>N</u>	<u>E</u>	<u>I</u>	<u>V</u>	<u>L</u>	<u>Q</u>	<u>A</u>	<u>Q</u>
A/Egypt/AN2/2020	2A	<u>N</u>	<u>N</u>	<u>E</u>	<u>I</u>	<u>V</u>	<u>L</u>	<u>Q</u>	<u>A</u>	<u>Q</u>
A/Egypt/AN3/2020	2A	<u>N</u>	<u>N</u>	<u>E</u>	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
A/Egypt/AN4/2020	2A	<u>N</u>	<u>N</u>	<u>E</u>	<u>I</u>	<u>V</u>	<u>L</u>	<u>Q</u>	<u>A</u>	<u>Q</u>
A/Egypt/AN5/2020	2A	<u>N</u>	<u>N</u>	<u>E</u>	<u>I</u>	-	<u>L</u>	<u>Q</u>	<u>A</u>	<u>Q</u>
A/Egypt/AN6/2020	2A	<u>N</u>	<u>N</u>	<u>E</u>	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
A/Egypt/AN7/2020	2A	<u>N</u>	<u>N</u>	<u>E</u>	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
CAV-A/India/AY583755	1B	-	-	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
BD-3/Bangladesh/AF395114	1B	-	-	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
NIE/19.04/118/Nigeria/AJ888524	1B	-	-	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
A/Egypt/AN8/2020	2B	<u>N</u>	<u>N</u>	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
A/Egypt/AN9/2020	2B	<u>N</u>	<u>N</u>	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
A/Egypt/AN10/2020	2B	<u>N</u>	<u>N</u>	-	<u>I</u>	-	<u>L</u>	<u>Q</u>	-	<u>Q</u>
Nobilis P4 Vaccine/AJ890284	C	-	-	-	-	-	-	-	-	-

A: Alanine, E: Glutamic acid, G: Glycine, H: Histidine, I: Isoleucine, K: Lysine, L: Lysine, M: Methionine, N: Asparagines, Q: Glutamine, S: Serine, V: Valine, Y: Tyrosine. The A.A. identity showed that the seven Egyptian viruses (A/Egypt/AN1/2020 to A/Egypt/AN7/2020) had a high identity percent (97 to 97.9%) with G6/Japan/AB119448.1, ArgA0021-3/Argentina/EU871783.1, and SMSC-1/Malaysia/AF285882, and the other three Egyptian viruses (A/Egypt/AN8/2020, A/Egypt/AN9/2020, A/Egypt/AN10/2020) had a high identity percent (97.3-98.7%) with BD-3/Bangladesh and NIE/19.04/118/Nigeria. The A.A. identity percent among Egyptian viruses in the subgroup 2A and 2B in the current study and vaccine strains used in Egypt (Cux-1, Del-Ros, and 26PA) was 94.8 to 95.7% A.A. and 97-97.8% A.A. respectively as shown in Figure 3.

		Percent Identity																						
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21		
Divergence	1	■	98.4	95.6	95.4	95.1	97.9	97.9	98.4	95.3	95.6	95.3	95.4	95.4	95.7	97.0	97.2	97.0	98.4	98.7	98.4	1	A2-Japan-AB031296.1	
	2	1.6	■	95.4	95.4	94.9	97.9	97.9	99.7	95.1	95.1	95.4	95.1	95.3	95.4	95.6	97.3	97.3	97.3	99.7	98.4	98.1	2	Cux-1M-Germany-M81223.1
	3	4.6	4.7	■	99.5	99.4	96.2	96.2	95.4	97.3	97.3	97.8	97.3	97.5	97.6	97.9	95.4	95.6	95.4	95.4	95.7	95.4	3	G6-Japan-AB119448.1
	4	4.7	4.7	0.5	■	99.2	96.2	96.2	95.4	97.2	97.2	97.3	97.2	97.3	97.2	97.5	95.4	95.6	95.4	95.4	95.6	95.3	4	ArgA0021-3-Argentina-EU871783.1
	5	5.1	5.2	0.6	0.8	■	95.7	95.7	94.9	97.0	97.0	97.2	97.0	97.2	97.0	97.3	94.9	95.1	94.9	94.9	95.3	95.6	5	SMSC-1-Malaysia-AF285882.1
	6	2.1	2.1	3.9	3.9	4.4	■	100.0	97.9	95.9	95.9	96.2	95.9	96.1	96.1	96.4	98.6	98.7	98.6	97.9	98.4	97.8	6	BD-3-Bangladesh-AF395114
	7	2.1	2.1	3.9	3.9	4.4	0.0	■	97.9	95.9	95.9	96.2	95.9	96.1	96.1	96.4	98.6	98.7	98.6	97.9	98.4	97.8	7	NIE-19.04-118-Nigeria-AJ888524
	8	1.6	0.3	4.7	4.7	5.2	2.1	2.1	■	95.1	95.1	95.4	95.1	95.3	95.4	95.6	97.3	97.3	97.3	99.7	98.4	98.1	8	CAV-A-India-AY583755
	9	4.9	5.1	2.7	2.9	3.1	4.2	4.2	5.1	■	100.0	99.2	100.0	99.8	99.1	99.4	95.7	95.9	95.7	95.1	95.4	94.8	9	A-Egypt-AN1-2020-MW286460
	10	4.9	5.1	2.7	2.9	3.1	4.2	4.2	5.1	0.0	■	99.2	100.0	99.8	99.1	99.4	95.7	95.9	95.7	95.1	95.4	94.8	10	A-Egypt-AN2-2020-MW286461
	11	4.6	4.7	2.2	2.7	2.9	3.9	3.9	4.7	0.8	0.8	■	99.2	99.4	99.8	99.8	96.4	96.5	96.4	95.4	95.7	95.1	11	A-Egypt-AN3-2020-MW286462
	12	4.9	5.1	2.7	2.9	3.1	4.2	4.2	5.1	0.0	0.0	0.8	■	99.8	99.1	99.4	95.7	95.9	95.7	95.1	95.4	94.8	12	A-EGYP-AN4-2020-MW286463
	13	4.7	4.9	2.6	2.7	2.9	4.1	4.1	4.9	0.2	0.2	0.6	0.2	■	99.2	99.5	95.9	96.1	95.9	95.3	95.6	94.9	13	A-EGYP-AN5-202-MW286464
	14	4.7	4.7	2.4	2.9	3.1	4.1	4.1	4.7	1.0	1.0	0.2	1.0	0.8	■	99.7	96.5	96.4	96.5	95.4	95.6	94.9	14	A-Egypt-AN6-2020-MW286465
	15	4.4	4.6	2.1	2.6	2.7	3.7	3.7	4.6	0.6	0.6	0.2	0.6	0.5	0.3	■	96.2	96.4	96.2	95.6	95.9	95.3	15	A-Egypt-AN7-2020-MW286466
	16	3.1	2.7	4.7	4.7	5.2	1.4	1.4	2.7	4.4	4.4	3.7	4.4	4.2	3.6	3.9	■	99.8	100.0	97.3	97.6	97.0	16	A-Egypt-AN8-2020-MW286467
	17	2.9	2.7	4.6	4.6	5.1	1.3	1.3	2.7	4.2	4.2	3.6	4.2	4.1	3.7	3.7	0.2	■	99.8	97.3	97.8	97.2	17	A-Egypt-AN9-2020-MW286468
	18	3.1	2.7	4.7	4.7	5.2	1.4	1.4	2.7	4.4	4.4	3.7	4.4	4.2	3.6	3.9	0.0	0.2	■	97.3	97.6	97.0	18	A-Egypt-AN10-2020-MW286469
	19	1.6	0.3	4.7	4.7	5.2	2.1	2.1	0.3	5.1	5.1	4.7	5.1	4.9	4.7	4.6	2.7	2.7	2.7	■	98.4	98.1	19	Cux-1N-Germany-M55918.1
	20	1.3	1.6	4.4	4.6	4.9	1.6	1.6	1.6	4.7	4.7	4.4	4.7	4.6	4.6	4.2	2.4	2.2	2.4	1.6	■	98.4	20	Del-Ros-USA-AF313470
	21	1.6	1.9	4.7	4.9	4.6	2.2	2.2	1.9	5.4	5.4	5.1	5.4	5.2	5.2	4.9	3.1	2.9	3.1	1.9	1.6	■	21	Nobilis-P4-Vaccine-AJ890284

Figure 3. Amino acid identities of VP1 gene of CAV compared to other selected viruses and vaccines' strains. FN: The A.A. identity percent among Egyptian viruses in the subgroup 2A and 2B in the current study and vaccine strains used in Egypt (Cux-1, Del-Ros, and 26PA) was 94.8 to 95.7% A.A. and 97-97.8% A.A. respectively.

DISCUSSION

Chicken anemia virus worldwide is an immunosuppressive disease that circulates in several countries (Natesan et al., 2006; Zhang et al., 2013). In Egypt, the CAV was spread in different governorates causing high mortality and economic losses (Erfan et al., 2018). In the current study, 26 positive samples out of 86 samples were collected from different broiler chicken farms located in six out of 14 governorates in Egypt with a high incidence rate (30%) in Lower Egypt, especially in Sharkia (78%), Ismailia (62.5%), and Alexandria (60%) and most of them were vaccinated. It is an indicator for the progressive exacerbation of the disease in vaccinated broiler chicken farms in Egypt during 2020 with a high mortality rate that ranged from 5 to 15 percent.

The VP1 gene sequence is extremely important to determine the virulence of the virus, cell infection ability, viral replication, and the relationship among CAV viruses especially the N-terminal half of VP1 gene that contains the hypervariable region from 139 to 151 A.A. (Renshaw et al., 1996; Islam et al., 2002; Negasi et al., 2008). These hypervariable regions are the most variable region in the VP1 gene of chicken anemia virus as previously mentioned by Islam et al. (2002). Phylogenetically, the VP1 gene was divided into many groups in the world that can easily identify the field and vaccines' strains (Eltahir et al., 2011)

As previously recorded the CAV circulated in Egypt from different genotypes may be due to multiple introductions (AboElkhair et al., 2014; Abdel-Mawgod et al., 2018; Erfan et al., 2018). In the current study, ten viruses were selected from six different governorates to be sequenced for partial VP1 gene and to be compared with viruses isolated from many countries, such as Germany, India, Japan, Nigeria, Bangladesh, USA, Korea, Taiwan, and China (Schat, 2009). All Egyptian viruses in the current study related only to field viruses. The vaccine strains were not recorded. In the present study, the seven viruses were related to the viruses from Japan, Argentina, and Malaysia as previously recorded (AboElkhair et al., 2014; Abdel-Mawgod et al., 2018; Erfan et al., 2018). but the Egyptian viruses in the study were acquired new mutations cluster them in a new subgroup 2A and the other three Egyptian viruses were related to the viruses from Nigeria, India with new mutations cluster them in a new subgroup 2B. These findings indicated continuous circulation of multiple genotypes of CAV in Egypt with multiple evolutions in the VP1.

By A.A. mutation analysis, the viruses related to group A and B had A. A mutation at V75I, M97L, K139Q, E144Q when comparing with DEL-Rose vaccine strain as previously described by AboElkhair et al. (2014) and Erfan et al. (2018). The 139Q and 144Q could decrease the efficacy of the replication in the cell culture (Renshaw et al., 1996). In the current study, the Egyptian viruses had a specific new mutation. The Y13N, H22N were recorded in all Egyptian strains in our study and G74E, were recorded in subgroup 2A and I83V in A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020, and S140A in A/Egypt/AN1/2020, A/Egypt/AN2/2020, A/Egypt/AN4/2020 and A/Egypt/AN5/2020. The H22N was recorded by AboElkhair et al. (2014) in one Egyptian virus that was important in distinguishing the strain of CAV. Further studies are needed to work on the effect of these mutations in the pathogenicity of the virus and they may be the cause of outbreaks in vaccinated farms.

Recently, multiple vaccines are used (mainly Del-Ros, Nobilis P4, and Cux-1 strains) for breeder flocks producing maternal antibodies to newly born chickens which give protection till two to three weeks old (Haridy et al., 2009; Prezotto et al., 2016). The phylogenetic analysis and A.A. identity showed that the field viruses were distinct from the

vaccines' strains as previously recorded by [AboElkhair et al. \(2014\)](#) and [Erfan et al. \(2018\)](#). It explains the occurred infection of the vaccinated flocks by CAV as mentioned by [Abdel-Mawgod et al. \(2018\)](#) and [Erfan et al. \(2018\)](#). Further study is needed to determine the vaccine efficacy against the field stains.

CONCLUSION

The chicken anemia virus was widely circulated with a high incidence in Lower Egypt in six governorates. The phylogenetic tree of the partial VP1 gene during the present study indicated the Egyptian virus were clustered in two groups (A, B) with a newly acquired mutation specific to Egyptian viruses forming new subgroups (2A, 2B). The field virus in the current study was genetically distinct from vaccine strains. Continuous monitoring on the genetic evolution of CAV and further studies are required to work on the effect of acquired mutations on the pathogenicity of the virus and the vaccine efficacy.

DECLARATIONS

Competing interests

The authors declare that they have no conflict of interest.

Authors' contribution

Ahmed Abd Elhalim Mohammed and Abdelhafez Samir carried out tissue specimen collection from the affected flocks, detection of the DNA of Chicken anemia virus and analysis of the data, and wrote the manuscript. Nahed Yehia carried out the sequencing of partial VP1 gene, genetic and phylogenetic analysis, and analysis of the data. All authors read and approved the final manuscript.

Ethical consideration

Ethical issue including plagiarism, consent to public misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy has been checked by the authors

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