Genetic Heterogeneity between some recently detected strains of Chicken Anemia Viruses in commercial young chick's flocks.

Hanaa A. Elsamadony
Animal Health Research Institute
Hoda M. Mekky
National Research Centre
Hanaa. S. Fedawy
National Research Centre
Dalia M. Sedeek
National Research Centre
Aalaa Saad (alaa.samir87@yahoo.com)
Animal Health Research Institute https://orcid.org/0000-0003-4740-4419

Research Article

Keywords: Chicken Anemia Viruses, VP2, young chicks, genetic characterization

Posted Date: March 28th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1455803/v1

Abstract

Chicken anemia virus (CAV) is a serious viral infection that causes considerable impairment of immunity and severe anemia in chickens all over the world. In young chicks, significant illness and mortality are observed (2–3 weeks). The vertical route of transmission enhances the chance of infection and virus persistence among diseased flocks. This research aimed to confirm the existence of CAV in young chicks (at the age of 1–7 days) in Egypt during 2021, genetic divergence between the spreading viruses and vaccinal strains in order to improve virus control. DNA was isolated from samples that were collected, used in a targeting VP2 gene by PCR, and then characterized VP2 on a molecular level in the detected viruses. Five CAV were detected, sequenced, and submitted to Genebank under accession no MZ574095, MZ574096, MZ476999, MZ477000, MZ477001. The strains were classified into genogroups I, II, and IIIb, and one detected strain was congregated with the vaccinal strain 26P4 and Nobilis P4. Also, the VP2 protein of the five CAV isolates and Egyptian CAV showed a high Conservation score. In silico analysis of the secondary structures of VP2 proteins of Cux-1 (reference strain) and the five strains showed that the a-helix and ß-strand architectures were quite comparable in number and position. As well as, VP2 of the five CAV isolates showed the absence of an insertion or a deletion in comparison to Cux-1 in the sequenced part.

Introduction

Chicken anemia virus (CAV), belongs to the family Anelloviridae the unique individual in genus Gyrovirus. CAV is a single-stranded, circular DNA (closed) its length of nearly 2300 nucleotides, containing three overlapped open reading frames (ORFs) [1], that coding three different viral proteins (VP): VP1 the major capsid protein, VP2 (non-structural (a dual-specific protein phosphatase) and VP3 (non-structural protein also, called apoptin) [2, 3].

That, VP1enhances the production of the host's antibody is also implicated in virus multiplication and pathogenicity, and hypervariability was noticed in its sequence [4, 5]. The VP2 is considered as a bricklayer's protein to emphasize the correct translation of VP1 [6, 7]. VP3 activates programmed cell death in thymus' cells and lymphoid T cells of infected chicken. [8)]

CAV is spreading in chicken-producing countries; infection may be symptomatic (2–3 weeks) or subclinical (beyond three weeks) [9]. The virus targets bone marrow, blood-cell stem cells (erythrocytes and leukocytes), the thymus, and T lymphocytes [10]. Chicks less than 2–3 weeks generally suffer from bloodlessness, stunted growth, sluggishness, gloominess, drooling of wings, ruffled feathers, paleness of the soft tissues, comb, wattle, and beak. Bone marrow and thymus impairment accompanied by an increase in deaths, and hemorrhages (intramuscular, and subcutaneous) were observed. [8, 11], The severity of the disease relies on the status of the immune of the chick; meaning, maternally antibodies blood levels. The infection in chickens more than two weeks old generally be a subclinical case.

In general, CAV causes immunosuppression, which is frequently followed by subsequent infections with other infectious agents and weakened immune responses to vaccines [9]. In flocks infected with CAV, infections with MD and IBD viruses have been documented, bacterial and viral respiratory and cutaneous illnesses are also common. [12, 13].

This research aimed to confirm the existence of CAV in young chicks (at the age of 1–7 days) in Egypt during 2021. Genetic divergence between the spreading viruses and vaccinal strains in order to improve virus control.

Material And Methods

Tissue samples

A total of ten chicken flocks (at the age of 1–7 days) were examined during 2021 old from vaccinated breeder flocks. The collected tissue (bursa, bone marrow, thymus, and liver) (approximately 10 chicks from each flock representing one sample that was delivered to our lab for examination) were homogenized in saline (20% W/V) including 2000 IU/ml Penicillin and 200 ug/ml Streptomycin then centrifuged at 3000 rpm for 15 minutes. After centrifugation collects the clear supernatant fluid and holds it at -80°C until used [14].

DNA extraction and CAV Detection: total DNA was extracted from Tissue supernatants fluid with the PathoGene-spin™ DNA/RNA Extraction Kit according to the manufacturer's instructions (iNtRON Biotechnology, Seongnam), and kept at -80°C.

VP1 Amplification of partial gene was carried out by Taqman assays CAV Q5:5'-GCCCCGGTACGTATAGTGTGAG-3' CAV probe 5' - (6FAM)-CTGCCGAACCCCCAATCTACTATGACTATCC-(TAMRA)-3' Cux-1* specific 5'-CCGTGAGAAAGATGACCCCTT-3' [15] in 2.2.2 s in AB Applied Biosystems.

The positive samples were subjected to conventional assay using the primers (F: 5'-CTA AGATCT GCA ACTGCG GA-3'and R: 5'-CCT TGG AAG CGG ATAGTC AT-3') to amplify a 418 bp fragment of CAV genome [16], and were sent for sequencing.

Sequencing of VP2gene amplified fragment:
ABI 3730xl DNA sequencer uses forward and reverse primers to combine the traditional Sanger technology with the new 454 used for performing gene sequencing using an Applied Biosystems 3130 genetic analyzer (ABI, USA) for sequencing VP2 gene amplified fragment. The sequencing data were checked by NCBI Blast search. To assess the genetic relatedness among the CAV and the phylogenetic tree was generated by the distance-based neighbor-joining (NJ) method using MEGA X [17]. Conservation score and Secondary structures of VP2 protein were performed by Jalview program version 1.8.3_1.2.9_JAL program and PSIPRED 4.0 tool[2] [18] protein structure prediction.

Results

Detection of CAV

Out of 10 examined flocks, 5 (50%) were found positive for CAV by both Taqman and conventional assays.

Molecular Characterization, and Phylogenetic analysis of VP2

The five CAV isolates were sequenced and submitted to Genebank under accession no MZ574095, MZ574096, MZ476999, MZ477000, MZ477001.

The Genogroup classification Phylogenetic tree based on partial nucleotide sequences of VP2 Fig. 1. CAV strains are classified into genogroups I, II, IIIa, and IIIb [19], and the detected strains in this study clustered with genogroups I (MZ574095), II (MZ477000), IIIb (MZ574096, and MZ477001). Perceptible, the MZ477001 strain clustered with the vaccine strain 26P4 and Nobilis P4, in genogroup IIIb.

The nucleotide sequences of detected CAV strains showed homology with other previously detected Egyptian CAV strains (Fig. 2) bootstrap values ≥ 70%. Also, the Conservation score of VP2 protein between five CAV isolates and Egyptian CAV was high (Fig. 3).

The patterns of amino acid substitutions in specific amino acid sites, located at positions A153V (GCG—GTG) and D169 G (GAT—GGC) of the VP2 protein of Cux-1 as reference strain are detected (Table 1). In silico analysis of the secondary structures of VP2 proteins of Cux-1 (reference strain) and five CAV strains was performed. The a-helix and ß-strand architectures were quite comparable in number and position. As well as, VP2 of the five CAV isolates showed the absence of an insertion or a deletion in comparison to CUX-1 in the sequenced part (Fig. 4).

Table 1
partial amino acid sequence VP2 comparison among the five detected Egyptian CAV strains in the study and Cux-1 (reference strain).

	Number of amino acid in VP2 gene	
	153	169
M55918.1 CAV/ Cux-1	A	D
M81223.1 CAV/ Cuxhaven 1	V	D
MZ476999.1 CAV/ Beheira.1	V	G
MZ477000.1 CAV/ Damanhour.1	V	G
MZ477001.1 CAV/ S.1	V	G
MZ574095.1 CAV/ Beheira/2	V	G
MZ574096.1 CAV/ Beheira/3	V	G

Discussion

VP2 and VP3 genes are responsible for the induction of apoptosis in infected cells [21, 21]. Furthermore, VP2 protein could be used for manufacturing monoclonal antibodies by being a target antigen [3]. however, there are low studies about VP2. So, expanding the knowledge about this protein may help in enhancing CAV infections control.

Molecular analysis of VP2 nucleotides in the current study isolates are intimately related to other detected CAV isolates from Egypt.

Molecular analyses of VP2 gene partial sequencing revealed that the found strains are congregated in different genogroups (I, II, and IIIb), indicating molecular divergence of the CAV strains spreading in Egypt affecting the young chicks.

The strain MZ477001 congregated with the vaccinal strain 26P4 and Nobilis P4, in genogroup IIIb. The finding of strains with molecular sequences near to the vaccinal strain (Del-Ros vaccine) was mentioned before in Italy (**18**), leading us to believe that a vaccinal strain or a strain in the field with a molecular sequence close to that vaccine be possible to spread.

CAV vaccines are made from field strains that have been sequentially inoculated in tissue culture cells or embryonated chicken eggs for attenuation [22]. The degree of attenuation, on the other hand, does not always prohibit vaccinations from being transmitted vertically or horizontally to and in between young chicks [23]. While attenuated CAV strains were noticed to have the capacity to return to virulent phenotypes following chicken-to-chicken transmission in the field, this vaccination behavior could pose a risk to young chicks [24, 25].

There were 3 Nucleotide changes in the sequenced part of VP2 in the 5 examined strains at position 458 from C to T, position 506 from A to G, and position 507 from T to C. These Nucleotide changes were also previously mentioned in Turkish isolates. As the result of amino acids mutation analysis, the isolates had mutations at A153V and D169G when compared to Cux-1 and Cuxhaven 1 as reference strains. A153V mutation was noticed frequently in Turkish isolates. However, the D169G mutation is not commonly seen in Turkish isolates. [26]

Conclusions

In conclusion, the CAV was detected in young chicks. The phylogenetic tree of the partial VP2 gene sequencing during this study indicated the Egyptian CAV strains recovered from young chicks were classified into genogroups I, II, and IIIb. one detected strain in this study was congregated with the vaccinal strain 26P4 and Nobilis P4. Regular testing of CAV's genetic development is essential, in addition to more research into the impact of new genetic changes on the virus's virulence and vaccination effectiveness.

Declarations

Author contributions

Hanaa A. El-Samadony: methodology and data curation; Hoda M. Mekky: editing and data curation; Hanaa. S. Fedawy[2], Dalia M. Sedik: editing; Aalaa Saad: methodology and writing.

Acknowledgments

The authors thank the Poultry Diseases Departments in both National Research Centre and Animal Health Research Institute, Giza, Egypt, for their help.

Compliance with ethical standards

Funding

No funding.

The authors didn't receive any funds from any organization.

Conflict of interest.

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed

FINAL APPROVAL for the study under number: (14712012022)

References

1. - Natesan S, Kataria JM, Dhama K, Rahul S, and Baradhwaj N (2006) Biological and molecular characterization of chicken anaemia virus isolates of Indian origin. Virus Res 118(1–2):78–86. http://doi:10.1016/j.virusres.2005.11.017
2. - Oluwayelu D, Todd D, Olaleye O (2008) Sequence and phylogenetic analysis of chicken anaemia virus obtained from backyard and commercial chickens in Nigeria. Onderstepoort J Vet Res 75:353–357. http://doi:10.4102/ojvr.v75i4.111
3. -Hiremath C, Jhala M, Bhanderi B, Joshi C (2013) Cloning and sequence analysis of VP1, VP2 and VP3 genes of Indian chicken anemia virus. Iran J Vet Res 14:354–357. https://ijvr.shirazu.ac.ir/article_1835_2ba7adeb2b2b8af37555743398ae9d2d.pdf
4. - Noteborn MHM, De Boer GF, Van Roozelaar DJ, Karreman C, Kranenburg O, Vos JG, Jeurissen SHM, Hoeben RC, Zantema A, Koch G et al (1991) Characterization of Cloned Chicken Anemia Virus DNA That Contains All Elements for the Infectious Replication Cycle. J Virol 65:3131–3139. http://doi:10.1128/JVI.65.6.3131-3139.1991
5. - Todd D, Creelan JL, Mackie DP, Rixon F, McNulty MS (1990) Purification and biochemical characterization of chicken anaemia agent. J Gen Virol 71:819–823. http://doi:10.1099/0022-1317-71-4-819
6. - Noteborn MH, Van Der Eb AJ, Verschueren CA, Koch G (1998) Simultaneous expression of recombinant baculovirus-encoded chicken anaemia virus (CAV) proteins VP1 and VP2 is required for formation of the CAV-specific neutralizing epitope. J Gen Virol 79:3073–3077. http://doi:10.1099/0022-1317-79-12-3073
7. - Peters MA, Jackson DC, Crabb BS, Browning GF (2002) Chicken Anemia Virus VP2 Is a Novel Dual Specificity Protein Phosphatase. J Biol Chem 277:39566–39573. http://doi:10.1074/jbc.M201752200
8. - Ganar K, Shah M, Kamdi BP, Kurkure NV, Kumar S (2017) Microb Pathog 102:113–119. https://doi.org/10.1016/j.micpath.2016.11.017
9. - Schat KA, van Santen VL (2008) Chicken infectious anemia virus and other Circovirus infections. Diseases of Poultry; Saif. (eds) Wiley-Blackwell Ames, IA, USA
10. - Hoerr FJ (2010) Clinical Aspects of Immunosuppression in Poultry. Avian Dis 54:2–15. http://doi:10.1637/8909-043009
11. - Adedeji AJ, Sati NM, Pewan SB, Ogbu KI, Adole JA, Lazarus DD, Ijiwo SJ, Okpanachi A, Nwagbo IO, Joannis TM, AbduPA (2016) Concurrent Infections of Chicken Infectious Anemia and Infectious Bursal Disease in 5 Weeks Old Pullets in Jos, Plateau State, Nigeria Vet. Sci Res Rev 2:60–65. http://dx.doi.org/10.17582/journal.vsrr/2016.2.3.60.65
12. - Davidson I (2019) Biotic concerns in generating molecular diagnosis matrixes for 4 avian viruses with emphasis on Marek's disease virus. J Virol Meth 274:113708
13. - Gimeno IM, Schat KA (2018) Virus-Induced Immunosuppression in Chickens. Avian Dis 62:272–285

14. - Dufour-Zavala L (2008) A laboratory manual for the isolation. identification and characterization of avian pathogens
15. - Markowski-Grimsrud CJ, Miller MM, Schat KA (2002) Development of strain-specific real-time PCR and RT-PCR assays for quantitation of chicken anemia virus. J Virol Methods 101(1–2):135–147. https://doi.org/10.1016/S0166-0934(01)00430-X
16. - Hussein HA, Sabry MZ, El-Ibiary EA, El-Safty M, Abd El-Hady AI (2002) Chicken infectious anaemia virus in Egypt: molecular diagnosis by PCR and isolation of the virus from infected flocks. Arab J Biotechnol 5(2):263–274. https://www.researchgate.net/profile/Hussein-Ahmed-5/publication/266161277_Chicken_infectious_anaemia_virus_in_Egypt_Molecular_diagnosis_by_PCR_and_isolation_of_the_virus_from_infected_flocks/lir infectious-anaemia-virus-in-Egypt-Molecular-diagnosis-by-PCR-and-isolation-of-the-virus-from-infected-flocks.pdf
17. Kumar -S, Stecher G, Li M, Knyaz C, Tamura K (2018)) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol Bio and Evol 35:1547–1549. http://doi:10.1093/molbev/msy096
18. - Buchan DWA, Jones DT (2019) The PSIPRED protein analysis workbench: 20 years on. Nucleic Acids Res 47(W1). https://doi.org/10.1093/nar/gkz297. W402-W407
19. - Quaglia G, Mescolini G, Catelli E, Berto G, Muccioli F, Lupini C (2021) Genetic Heterogeneity among Chicken Infectious Anemia Viruses Detected in Italian Fowl. Animals 11:944. https://doi.org/10.3390/ani11040944
20. - Sun F, Pan W, Gao H, Qi X, Qin L, Wang Y, Gao Y, Wang X (2018) Identification of the interaction and interaction domains of chicken anemia virus VP2 and VP3 proteins. Virology 513:188–194. https://doi.org/10.1016/j.virol.2017.09.014
21. - Lai GH, Lien YY, Lin MK, Cheng JH, Tzen JT et al (2017) VP2 of chicken anaemia virus interacts with apoptin for down-regulation of apoptosis through de-phosphorylated threonine 108 on apoptin. Sci Rep 7(1):14799. https://doi.org/10.1038/s41598-017-14558-8
22. - McNulty MS Chicken anaemia agent: A review (1991)Avian Pathol., 20,187–203
23. - Brentano L, Lazzarin S, Bassi S, Klein T, Schat K (2005) Detection of chicken anemia virus in the gonads and in the progeny of broiler breeder hens with high neutralizing antibody titers. Vet Microbiol 105:65–73
24. - Todd D, Creelan JL, Connor TJ, Ball NW, Scott AN, Meehan BM, McKenna GF, McNulty M (2003) Investigation of the unstable attenuation exhibited by a chicken anaemia virus isolate. Avian Pathol 32:375–382. http://doi:10.1080/037945031000121121
25. Vaziry A, Silim A, Bleau C, Frenette D, Lamontagne L (2011) Chicken infectious anaemia vaccinal strain persists in the spleen and thymus of young chicks and induces thymic lymphoid cell disorders. Avian Pathol 40:377–385. http://doi:10.1080/03079457.2011.586330
26. - Okay S, Aşkar Ş (2021) Molecular characterization of VP2 and VP3 proteins of chicken anemia virus isolates in Turkey. Turkish J Vet Anim Sci 45(2):336–345. http://doi:10.3906/vet-2009-19

Figures

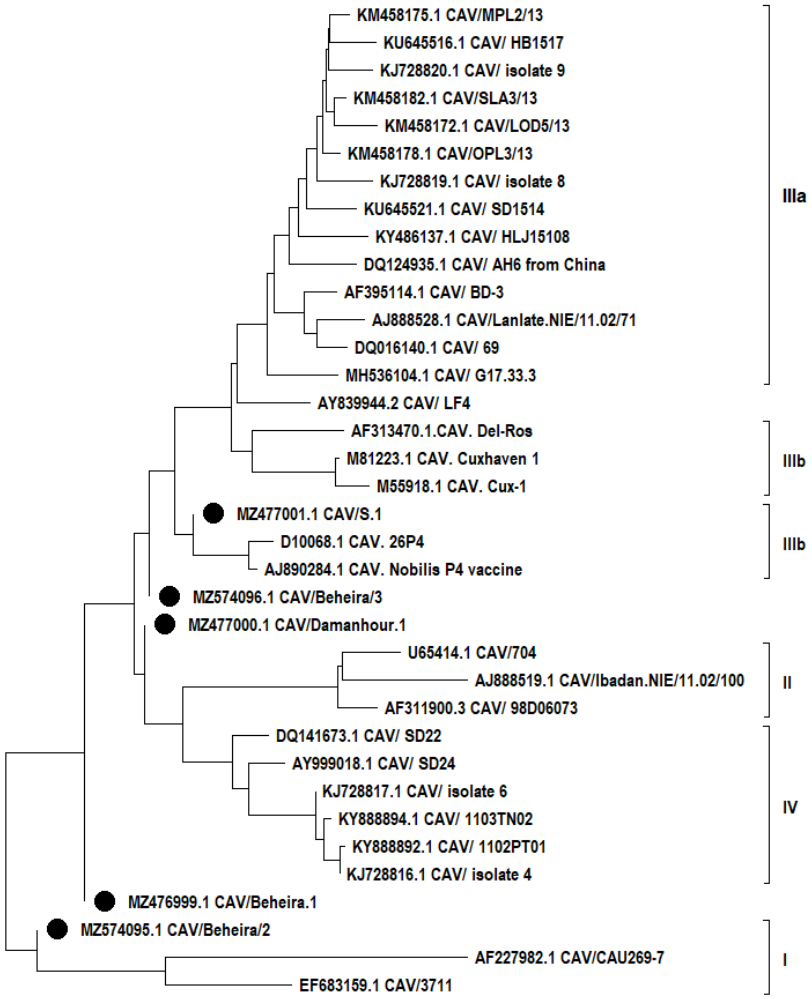

Figure 1

Genogroup classification tree Phylogenetic tree based on the partial VP2 nucleotide sequence of five CAV strains (marked with a black circle) and reference CAV strains got from GenBank.

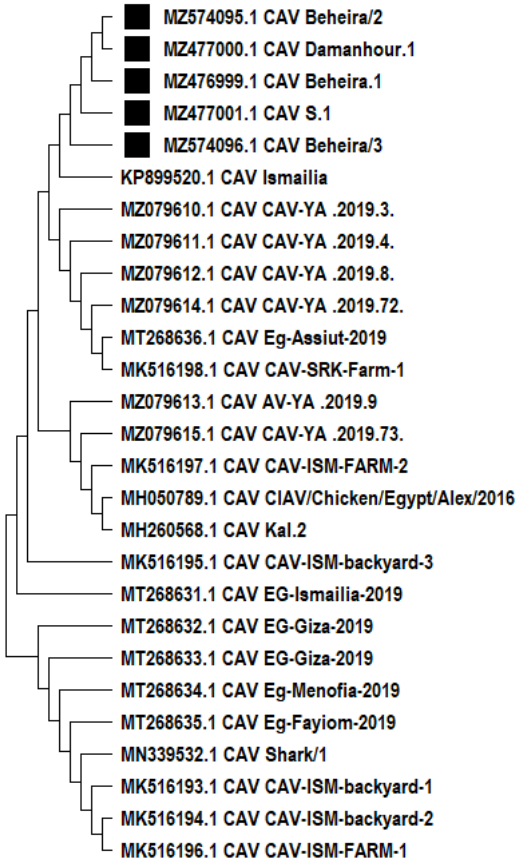

Figure 2

Phylogenetic tree based on the partial VP2 nucleotide sequence of five CAV strains (marked with a black circle) and Egyptian CAV strains previously detected got from GenBank. bootstrap values ≥ 70 % are reported

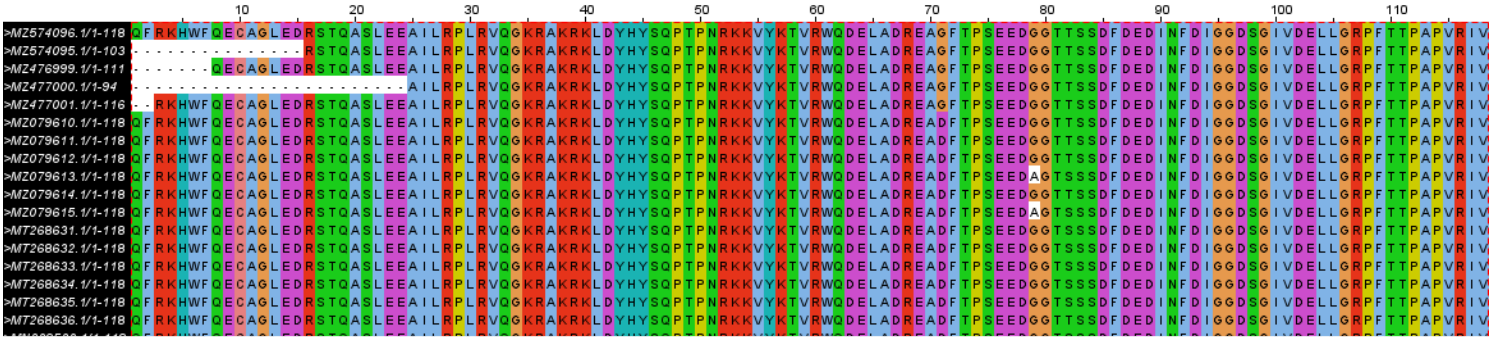

Figure 3

Conservation score of VP2 protein between five CAV isolates and Egyptian CAV strains previously detected got from GenBank

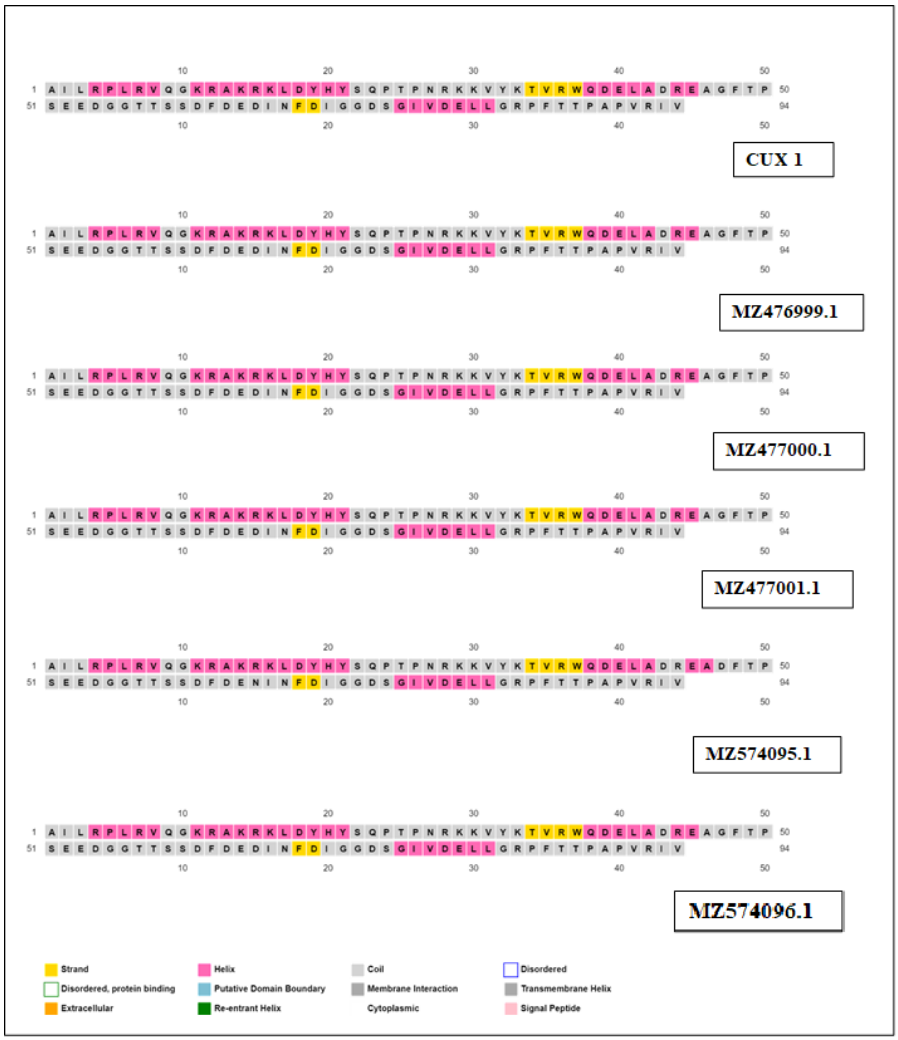

Figure 4

Secondary structures of the VP2 protein of Cux-1 (reference strain) and five CAV strains. A: alanine, C: cysteine, D: aspartate, E: glutamate, F: phenylalanine, G: glycine, H: histidine, I: isoleucine, K: lysine, L: leucine, M: methionine, N: asparagine, P: proline, Q: glutamine, R: arginine, S: serine, T: threonine, V: valine, W: tryptophan, Y: tyrosine.