**ORIGINAL ARTICLE**

# Molecular characterization of some isolates of rabbit viral hemorrhagic disease (VHD) in Egypt from 2014 to 2019

Hanaa Awad El-Samadony[1] (iD), Hoda Mohammed Mekky[2] (iD), Aly Mohammed Ghetas[2] (iD), Aalaa Samir Saad[3] (iD)

[1]Poultry Diseases Department, Animal Health Research Institute, ARC, Giza, Egypt
[2]Poultry Diseases Department, Veterinary Research Division, National Research Centre, Giza, Egypt
[3]Food Hygiene Department, Animal Health Research Institute, ARC, Giza, Egypt

**ABSTRACT**

**Objective:** Rabbit viral hemorrhagic disease (VHD) is a transmittable and lethal viral illness of rabbits. In this study, genetic identification and genetic analysis of the rabbit hemorrhagic disease virus (RHDV) was made in three governorates in Egypt from 2014 to 2019.

**Materials and Methods:** Livers from 18 freshly dead rabbits, which was guessed to be VHD epidemics in Egypt (Giza, Menofia, and Fayoum governorates) from 2014 to 2019, were examined for RHDV. The examination was based on the hemagglutination assay (HA) test against different mammalian (human O-type and sheep) and avian (chicken and pigeon) erythrocytes, reverse transcriptase-polymerase chain reaction (RT-PCR), and sequencing of the segment of VP60.

**Results:** 33% of the examined samples' virus titers were 5 $\log_2$ to 8 $\log_2$ hemagglutination of human O-type erythrocytes when compared to 28%, 11%, and 28% of sheep, chicken, and pigeon erythrocytes, respectively. Four RHDV isolates out of eight RT-PCR positives were sequenced and phylogenetically analyzed. Sequenced isolates were designed and submitted to GenBank with accession numbers MN904506, MN904507, MN904508, and MN904509. These four RHDV isolates were related to classic G3 (GI.1d/RHDV). Twelve amino acid differences were detected between the vaccine strain sequence (Giza-2006) and RHDV isolates. Amino acid differences at 416, 423, and 476 positions seem interesting as they changed polarity that could change the protein structure and affect host interaction.

**Conclusions:** There is antigenic variation between circulating RHVD strains and the vaccinal strain. This may be the leading cause of vaccination failure and may increase the need to check out the vaccination program against RHVD.

## Introduction

The rabbit industry is promising when it comes to increasing Egyptian revenue, which can help cover meat shortage. Egypt is considered among the top five rabbit meat producers worldwide. Small to medium-sized rabbit producers contribute to a high production rate [1,2]. Rabbit hemorrhagic disease (RHD) extends worldwide and is accompanied by high fatality rates [3].

Rabbit hemorrhagic disease virus (RHDV) is included in the genus *Lagovirus* of the family Caliciviridae [4]. RHDV is an RNA virus (single-stranded) non-enveloped, with about 7,437 nucleotides. The genomic RNA consists of two

overlappings (open reading frames, ORFs). ORF1 (7,034 nucleotides) encodes a huge multiprotein. ORF1 is split into seven non-structural viral proteins and a structural VP60. The shorter ORF2 (353 nucleotides) encodes viral protein VP10 [5]. The subgenomic RNA (2.2 kb) encodes VP60 and VP10. The VP60 contains the specific antigenic epitope, which shows the host immunological reaction target versus RHDV [6]. Thus, molecular studies were carried out on partial and complete sequences of the *VP60* gene to detect the genetic variations between RHDV strains [7–9].

RHVD-affected animals in 12–36 h develop a fever followed by sudden death of its onset in peracute type.

---

Animals with acute RHD survive longer, but suffer from general illness such as depression, off food, redness of the ocular mucous membrane, and in several situations from pulmonary dysfunction (e.g., dyspnea, cyanosis, and blood-stained frothy rhinal secretion), ocular or rhinal hemorrhages. Some rabbits also have disorders of nerves as restlessness, backward arching of the head and neck, and paddling [10]. The subacute type has temperate signs, and almost all rabbits live and produce protective antibodies versus RHDV reinfection. The chronic form is characterized by jaundice, which is severe, generalized, off food, and lethargy. Rabbits resort to die after 1–2 weeks, but those who overcome the disease have a solid seroconversion [3].

RHDV strains are classified into three subtypes: RHDV classic (G1–G5), RHDVa variant, and RHDVb variant [11]. Lately, thay have been categorized into two genotypes: GI.1 that has different forms, GI.1a (G6/RHDVa), GI.1b (G1), GI.1c (G2), and GI.1d (G3–G5), and GI.2 [12]. These forms show antigenic, genetic, and pathogenicity differences, affecting virus adaptation and spread [8,9]. RHD outbreaks caused by GI.1a are characterized by a 70%–90% mortality rate in rabbits older than 4–6 weeks. But younger rabbits are less susceptible. Clinical signs and mortality rate (5%–70%) were determined in adults and even in rabbit kittens from 15 to 20 days old infected with GI.2 [10]. These variants also have hemagglutinating, non-hemagglutinating, or variable hemagglutinating activity [13].

The immunogenic protein and the main viral structure of RHDV are VP60 [14]. It comprises three parts: the N-terminal arm, S, and a short hinge, and P splits into two portions P1 and P2. The VP60 protein variance caused differences in epidemiological diversity, molecular, immunizing agent, and body defenses between three RHDV subtypes. [15,16].

In Egypt, the first reported HVD outbreak was in 1991 [17]. Subsequently, the disease outbreaks were reported in different governorates [18,19]. Despite several vaccination programs, different RHDV variants belonging to GI.1 were identified in vaccinated [20,21] and non-vaccinated rabbits [22]. Recently, RHDV strains relating to GI.2 (RHDV2/b) have been identified from outbreaks in vaccinated rabbit flocks [23]. For effective control of RHDV outbreaks, continuous monitoring of RHDV circulating in Egypt is in demand. Thus, the study goal was to molecular characterize RHDV from 18 suspected RHDV-infected farms in three different governorates in Egypt (Giza, Menofia, and Fayoum) from 2014 to 2019.

## Materials and Methods

### Ethical approval

The study was carrid out according to 10 principles of the Declaration of Helsinki [24]. All examined tissues were disposed of according to biosafety procedures under the committee's supervision.

### Sample collection

Livers from freshly dead rabbits [approximately 5%–10% livers from each farm representing one sample (clinical case) which were delivered to our lab for examination] were collected from 18 suspected RHDV cases [3 unvaccinated (20 examined livers) and 15 vaccinated (100 examined livers)] in 3 different governorates in Egypt (Giza, Menofia, and Fayoum) during the period from 2014 to 2019, with average age of 1–3 months and from various breeds (New Zealand, Albino, and Baladi) with the history of vaccination (killed vaccine). Suspected rabbits were those suffering from lacrimation, ocular hemorrhages, depression, off food, reddish conjunctiva, blood-stained frothy nasal discharge, and fragile liver with focal lobular necrosis, accompanied by an enlarged congested hemorrhagic spleen and bloody kidneys, as well as hemorrhagic tracheitis, congested edematous, and hemorrhagic lungs. The collected samples were kept at −80°C for diagnosis.

### Sample preparation

10% suspension of liver homogenate in phosphate buffered saline (PBS) at pH 7.2–7.4 was prepared, filtered through a cheesecloth, and centrifuged at 5,000× for 15 min [25]. The supernatant was stored at −80°C.

### Erythrocyte suspension

Four parts of freshly collected blood samples from human O-type, chicken, sheep, and pigeon were added to one part 4% sodium citrate (as an anticoagulant). For washing the erythrocyte, the volume of PBS (pH 7.1) equal to the volume of erythrocytes was added and then the suspension was centrifuged as 500× for 5 min. The supernatant was decanted. The washing step and decanting were repeated. Then, 0.5% erythrocyte suspension was prepared (0.5 ml packed cells to 100 ml PBS at pH 7.0–7.2) [26].

### Hemagglutination assay (HA) test

The HA test was carried out to identify RHVD according to [27] in 96 V-shaped wells microtitration plate. The HA titer that correlated to the highest dilution, producing complete agglutination of erythrocytes, was taken.

### Reverse transcriptase-polymerase chain reaction

Extraction of RHVD mRNA was carried out using the PathoGene-spin™DNA/RNA Extraction Kit following the manufacturer's instructions. The used primers were P33: 5′-CCA CCA CCA ACA CTT CAG GT-3′ and P34: 5′-CAG GTT GAA CAC GAG TGT GC-3′ targeting 538 best pair (bp) [28]. The reactions were performed in one-step reverse

transcriptase-polymerase chain reaction (RT-PCR) (System Biolab Company), that were warmed up at 50°C for 15 min and then at 95°C for 15 min; then the 35 cycles of 95°C for 1 min, 56°C for 1 min, 72°C for 2 min, and finally 72°C for 10 min [28].

### Partial sequencing of VP60 gene

The PCR product sequencing was carried out by Sigma Company using the Sanger sequencer in two directions. Sequences were analyzed using BioEdit program version 7.1.5 [29]. Bioedit software was used for comparison between the obtained sequences and the others carried out in the GenBank. The neighbor-joining phylogenetic analysis was carried out by MEGA program version 7 [30].

### Analysis of the sequenced part of VP60 gene

RasWin Molecular Graphics program version 2.7.5.2. was used to predict the three-dimensional structure of the VP60 P domain (PDB ID: 4X1W) to analyze the biological significance of functional divergence-related sites. The N-linked glycosylation sites of VP60 were predicted using http://www.cbs.dtu.dk/services/NetNGlyc/.

## Results

### HA test

HA was carried out on the liver homogenates from the suspended HVD rabbits against different mammalian (human O-type and sheep) and avian (chicken and pigeon) samples. In this test, agglutination of dilution more than 1/16 is considered positive [24]. As shown in Table 1, a high percentage of positive samples (33%) had 5 $\log_2$ to 8 $\log_2$ HA titer by human O-type erythrocytes compared to 28%,

11%, and 28% sheep, chicken, and pigeon erythrocytes, respectively.

### Reverse transcriptase-polymerase chain reaction

In this study, applying one-step reverse transcriptase-polymerase chain reaction (RT-PCR) on liver samples using specific primers targeting the VP60 gene of RHVD revealed the presence of the amplified band at the expected size 538 bp in 8 (44%) samples out of the 18 examined samples. The positive cases were five from vaccinated farms and three from unvaccinated farms.

### Sequencing of the amplified part of the VP60 gene

Sequenced isolates were designed and submitted to the GenBank with accession numbers MN904506, MN904507, MN904508, and MN904509.

### Phylogenetic analysis

Four RHDV strains out of eight RT-PCRs were positive (selected randomly) sequenced. The phylogenetic tree containing the 4 identified strains and 33 reference strains was constructed based on a partial sequence of VP60. As shown in Figure 1, the four strains identified in this study are related to GI.1d/RHDV. Yet, the used RHDV vaccine in Egypt, Giza-2006 (JQ995154), belongs to GI.1a/RHDVa. Furthermore, we reported amino acid differences between RHDV strains identified in this study and the vaccinal strain (Giza-2006) based on partial VP60 sequence. We found 12 amino acid differences (Table 2): 7 differences between Giza-2006 and the 4 strains (RHV-1, RHV-2, RHV-3, and RHV-4) identified in this study (I 412 G, T 416 A, P 423 S, V 434 I, A 476 S, S 480 T, L534 F), 2 differences between Giza-2006 and MN904507 (RHV-2) strain (N 414 S, V 467 I), 2 differences between Giza-2006 and MN904508 (RHV-3)

**Table 1.** HA titers $\log_2$ of suspected RHVD suspected samples against different mammalian and avian's erythrocytes.

| | Number of samples/(HA titer to human O type $\log_2$) | Number of samples/(HA titer to sheep RBCs $\log_2$) | Number of samples/(HA titer to chicken RBCs $\log_2$) | Number of samples/(HA titer to pigeon RBCs $\log_2$) |
|---|---|---|---|---|
| | 1/0 | 2/0 | 5/0 | 2/0 |
| | 2/1 | 2/1 | 2/1 | 0/1 |
| | 2/2 | 4/2 | 3/2 | 4/2 |
| | 4/3 | 3/3 | 3/3 | 5/3 |
| | 3/4 | 2/4 | 3/4 | 2/4 |
| | **3/5** | **4/5** | **2/5** | **1/5** |
| | **1/6** | **1/6** | **0/6** | **3/6** |
| | **2/8** | **0/8** | **0/8** | **1/8** |
| Total positive | (6/18) 33%[a] | (5/18) 28%[a] | (2/18) 11%* | (5/18) 28%[a] |

Bold numbers indicate that HA is positive and that the HA titers are greater than 4 $\log_2$. [a]HA total positive percentage (No. of samples with HA titers more than 4 $\log_2$/total number of samples).
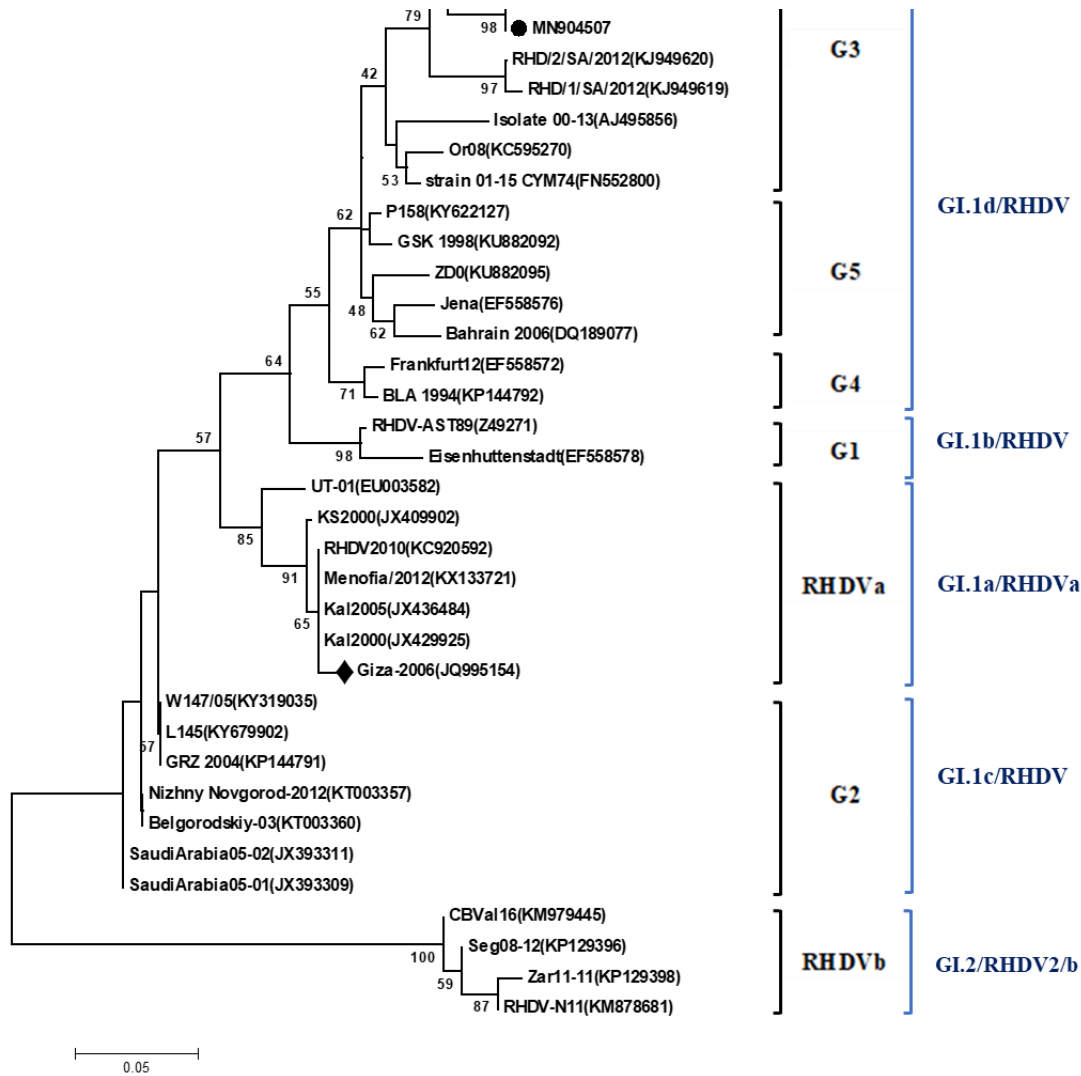
**Figure 1.** Phylogenic tree established on sequences and segments of the RHDV *VP60* gene. • refers to viruses isolated in the current study and ♦ is the Giza-2006 vaccinal strain.

strain (T 449 S, P 450 A), and 1 difference between Giza-2006 and both MN904506 (RHV-1) and Mn904509 (RHV-4) strains (N 481 S). The phylogenic tree of protein (Fig. 2), Giza-2006, is in a different cluster other than the four strains of RHDV under analysis.

### *Amino acids differences*

The three-dimensional structure of VP60 (Fig. 3) and N-linked glycosylation sites of VP60 showed that the MN904506 strain had an N-linked glycosylated site at positions 70 aa and 98 aa. However, MN904507 strain contained N-linked glycosylated sites at 63 aa, 70 aa, and 91 aa. MN904508 strain sites 3 aa, 84 aa, 91 aa, and 112 aa were predicted as N-linked glycosylated sites. However, N-linked glycosylated sites of the MN904509 strain were

3 aa, 84 aa, and 112 aa, while the vaccine strain JQ995154 N-glycosylated predicted sites were 3 aa, 24 aa, 91 aa, and 112 aa.

### Discussion

In this study, genetic characterization of RHDV from 18 suspected RHDV cases in 3 different governorates in Egypt from 2014 to 2019 was carried out.

HA testing of suspected samples against different mammalian and avian erythrocytes is shown in Table 1. OIE [25] recommended HA test using human type-O erythrocytes [25], but it is not easy to obtain and keep this type of erythrocytes [31]. Usage of different avian erythrocytes were used to test how accurate HA results are

**Table 2.** Amino acid differences between the four RHDV strains that were identified and the Giza-2006 vaccinal strain used in Egypt based on a partial VP60 sequence.

| AA positions | Giza-2006 | MN904506 | MN904507 | MN904508 | MN904509 |
|---|---|---|---|---|---|
| 412 | I | G | G | G | G |
| 414 | N | N | S | N | N |
| 416 | T | A | A | A | A |
| 423 | P | S | S | S | S |
| 434 | V | I | I | I | I |
| 449 | T | T | T | S | T |
| 450 | P | P | P | A | P |
| 467 | V | V | I | V | V |
| 476 | A | S | S | S | S |
| 480 | S | T | T | T | T |
| 481 | N | S | N | N | S |
| 534 | L | F | F | F | F |

A = alanine, F = phenylalanine, G = glycine, I = isoleucine, L = leucine, N = asparagine, P = proline, S = serine, T = threonine, V = valine.
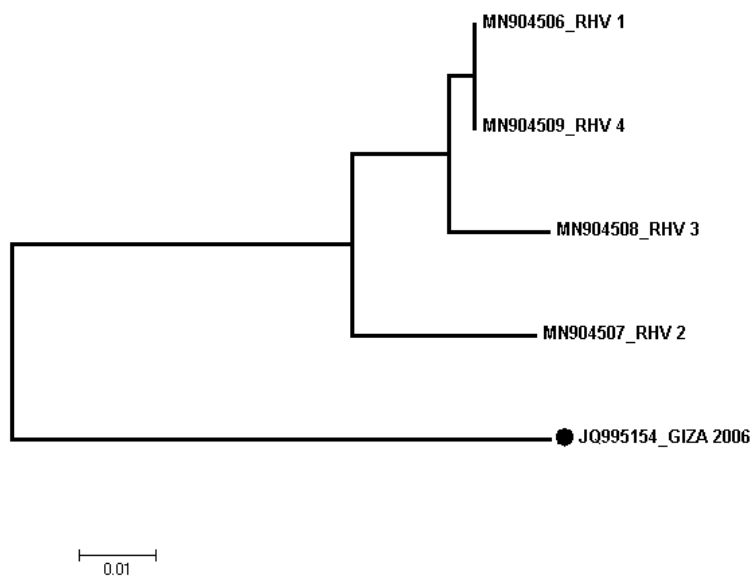


**Figure 2.** Phylogenic tree established on amino acid sequences of the RHDV *VP60* gene between viruses isolated in the current research and the vaccinal strain. The black dot refers to the vaccinal strain.

compared to that obtained by using human O-type erythrocytes. High HA-positive percent and titer were found with human O-type erythrocytes, followed by sheep and pigeon erythrocytes. The result agreed with Sahar's [32] study that human O-type erythrocytes in the HA test had high HA titer, followed by sheep and pigeon erythrocytes. Although the HA test is a preliminary diagnostic test for RHDV [25], it is not accurate since RHDV strains have non-hemagglutinating or variable hemagglutinating activities, which are reported [13].

Prevention of HVD outbreaks depends on the vaccination of rabbits by an effective vaccine closely related to the circulating RHDV strains. In Egypt, an inactivated vaccine based on classic strain has been developed [33]. After the emergence of variant strain in 2008, the classic strain has been replaced [34]. This variant strain was characterized
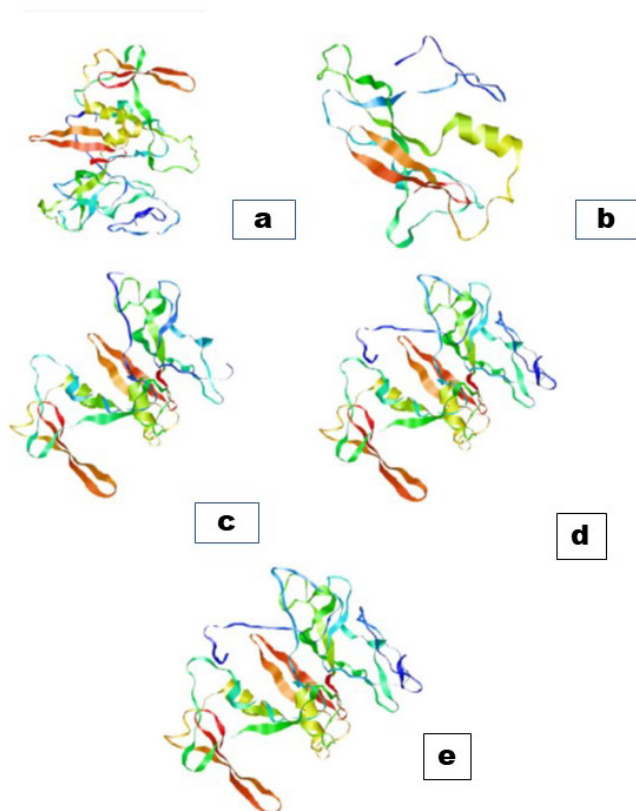
**Figure 3.** Three-dimensional structure of VP60 P domain. Ribbon representation of the VP60 protein structure. (a) (MN904506). (b) (MN904507). (c) (MN904508). (d) (MN904509). (e) Giza-2006 vaccinal strain.

as an RHDVa variant based on sequence analysis of the *VP60* gene's full length [35]. The four RHDV strains identified in this study belonged to GI.1d/RHD (Fig. 1). Vaccine strain (Giza-2006) used for vaccination of those rabbit flocks under investigation belonged to GI.1a/RHDVa. Also, RHDV stains related to GI.1 and GI.2 were identified in vaccinated and non-vaccinated rabbits [21].

In our study, partial sequences of the *VP60* gene's nucleotide of four RHDV strains (randomly selected) were analyzed with the Egyptian vaccinal strain (Giza-2006) to detect any potential genomic differences. The four RHDV strains belonged to GI.1d/RHD compared to the most commercially used vaccine in Egypt, Giza-2006, which belonged to GI.1a/RHDVa. Furthermore, amino acid differences between RHDV strains identified in this study and vaccinal strain (Giza-2006) were reported based on a partial *VP60* gene sequence. We found 12 amino acid differences (Table 2) that could change the shape of the VP60 protein (Fig. 2). The difference in amino acids positions 416, 423, and 476 are attractive because these differences change amino acid polarity that could affect VP60 protein

folding at these positions [36]. That leads us to deduce that the Giza-2006 vaccine showed a different antigenic index and surface probability profiles from other local circulating variant RHDV strains that may be responsible for emerging new outbreaks despite vaccination. Amino acid position 476 has been described as one of the positively selected codons and potential sites of N-glycosylation, which is principal for protein structure, after analysis of 43 RHDV VP60 complete and pathogenic sequences worldwide distributed and available at GenBank.

N-glycosylation and phosphorylation were mentioned in that they had a principal function in viral infection, different biological functions of proteins, replication, and translation [37,38].

N-glycosylation and phosphorylation mentioned a significant function in viral infection, different biological functions of proteins, replication, and translation [37,38]. Analyses of the biological significance of the functional N-glycosylation sites of different subtypes of RHDV (Fig. 2) revealed some potential active N-glycosylation sites located in the active domain on the outer surface of RHDV VP60.

## Conclusion

There is antigenic variation between the circulating RHVD strains and the vaccinal strain that may be a potent reason for vaccination failure. Thus, continuous monitoring of the existing RHDV strains circulating in Egypt is lso required. A vaccination challenge study is needed to detect how commercially available vaccines can protect rabbits against RHDV strains. Further regular researches are necessary to establish effective vaccination strategies to prevent RHVD outbreaks. RHVD outbreaks overcome builds on the vaccination of rabbits by effective vaccines closely related to the circulating RHVD strains. Also, there is a requirement to analyze the VP60 protein by other molecular methods such as Western blot immunochemistry.

## List of abbreviations

VHD: viral hemorrhagic disease; VP60: capsid protein viral protein; RHDV: rabbit hemorrhagic disease virus; ORFs: open reading frames; NTA: N-terminal arm; S: shell; P: protrusion; PBS: phosphate buffered saline; h: hour; RT-PCR: reverse transcriptase-polymerase chain reaction; bp: base pair.

## Acknowledgments

## Conflict of interests

The authors declare that there is no conflict of interests.

## Authors' contributions

Hanaa Awad. El-Samadony: methodology and data curation; Hoda Mohammed. Mekky: editing and data curation; Aly Mohammed. Ghetas: writing and editing; Aalaa Saad: methodology and writing.

## References

[1] Cullere M, Dalle Zotte A. Rabbit meat production and consumption: state of knowledge and future perspectives. Meat Sci 2018; 143:137–46; https://doi.org/10.1016/j.meatsci.2018.04.029

[2] Trocino A, Cotozzolo E, Zomeno C, Petracci M, Xiccato G, Castellini C. Rabbit production and science: the world and Italian scenarios from 1998 to 2018. Ital J Anim Sci 2019; 18(1):1361–71; https://doi.org/10.1080/1828051X.2019.1662739

[3] Abrantes J, van der Loo W, Le Pendu J, Esteves PJ. Rabbit haemorrhagic disease (RHD) and rabbit haemorrhagic disease virus (RHDV): a review. Vet Res 2012; 43:12; https://doi.org/10.1186/1297-9716-43-12

[4] Green KY, Thiel HJ. Taxonomy of the caliciviruses. J Infect Dis 2000; 181(s2):S322; https://doi.org/10.1086/315591

[5] Meyers G, Wirblich C, Thiel HJ, Thumfart JO. Rabbit hemorrhagic disease virus: genome organization and polyprotein processing of a calicivirus studied after transient expression of cDNA constructs. Virology 2000; 276(2):349–63; https://doi.org/10.1006/viro.2000.0545

[6] Capucci L, Fallacara F, Grazioli S, Lavazza A, Pacciarini ML, Brocchi E. A further step in the evolution of rabbit hemorrhagic disease virus: the appearance of the first consistent antigenic variant. Virus Res 1998; 58(1–2):115–26; https://doi.org/10.1016/S0168-1702(98)00106-3

[7] El Bagoury GF, Abd El-Moaty DAM, El-Zeedy SAR, El-Nahas EM, Youssif AA. Molecular identification of Rhdv Egyptian strains based on the highly variable region of VP60 gene. Benha Vet Med J 2014; 26(2):84–100.

[8] McIntosh MT, Behan SC, Mohamed FM, Lu Z, Moran KE, Burrage TG, et al. A pandemic strain of *Calicivirus* threatens rabbit industries in the Americas. Virology J 2007; 4(1):1–3; https://doi.org/10.1186/1743-422X-4-96

[9] Wang X, Hao H, Qiu L, Dang R, Du E, Zhang S, et al. Phylogenetic analysis of rabbit hemorrhagic disease virus in China and the antigenic variation of new strains. Arch Virol 2012; 157(8):1523–30; https://doi.org/10.1007/s00705-012-1340-9

[10] CFSPH. Rabbit hemorrhagic disease and other lagoviruses. 2020. Available via www.cfsph.iastate.edu (Accessed 14 June 2021).

[11] Le Gall-Recule G, Lavazza A, Marchandeau S, Bertagnoli S, Zwingelstein F, Cavadini P, et al. Emergence of a new *Lagovirus* related to rabbit haemorrhagic disease virus. Vet Res 2013; 44(1):1–3; https://doi.org/10.1186/1297-9716-44-81

[12] Le Pendu J, Abrantes J, Bertagnoli S, Guitton JS, Le Gall-Recule G, Lopes AM, et al. Proposal for a unified classification system and nomenclature of lagoviruses. J Gen Virol 2017; 98(7):1658–66; https://doi.org/10.1099/jgv.0.000840

[13] Abd El-Moaty DAM, El-Bagoury GF, El-Zeedy SAR, Salman, OGA. Egyptian non-hemagglutinating isolates of rabbit hemorrhagic disease virus can change to variable Ha profile. Benha Vet Med J 2014; 26(2):71–83.

[14] Mikschofsky H, Schirrmeier H, Keil GM, Lange B, Polowick PL, Keller W. Pea-derived vaccines demonstrate high immunogenicity and protection in rabbits against rabbit haemorrhagic disease virus. Plant Biotechnol J 2009; 7(6):537–49; https://doi.org/10.1111/j.1467-7652.2009.00422.x

[15] Hu Z, Tian X, Zhai Y, Xu W, Zheng D, Sun F. Cryo-electron microscopy reconstructions of two types of wild rabbit hemorrhagic disease viruses characterized the structural features of *Lagovirus*. Protein Cell 2010; 1(1): 48–58; https://doi.org/10.1007/s13238-010-0007-0

[16] Li Y, Yao X, Li Y, Xu F, Yang Z. Adaptive diversification between the classic rabbit hemorrhagic disease virus (RHDV) and the RHDVa isolates: a genome-wide perspective. Microb Pathogen 2017; 110:527–32; https://doi.org/10.1016/j.micpath.2017.07.036

[17] Ghanem IA, Ismail AN. Occurrence of rabbit haemorrhagic disease in Sharkia province. Zagazig Vet J 1992; 20(4):491–502.

[18] Salem B, El Ballal SS. The occurrence of rabbit viral haemorrhagic disease (RVHD) in Egypt. Assiut Vet Med J 1992; 27:295–304; https://doi.org/10.21608/avmj.1992.187190

[19] El Zanaty K. Some investigation on rabbit viral haemorrhagic disease in upper Egypt. Assiut Vet Med J 1994; 30:293–305; https://doi.org/10.21608/avmj.1994.184358

[20] Metwally AY, Madbouly HM. Study on a new isolate of rabbits haemorrhagic disease virus. Beni-Suef Vet Med J 2005; 15:238–243; https://doi.org/10.21608/jvmr.2005.77996

[21] El Sissi AF, Gafer JA. Preliminary diagnosis of non-hemagglutinating strain of rabbit haemorrhagic disease virus. Egypt J Comp Pathol Clin Pathol 2008; 21:161175.

[22] Magouz AF, Elsayed EA, Metwally AY. Detection and characterisation of rabbit haemorrhagic disease virus strains circulating in Egypt. Bulg J Vet Med 2019; 22(4):409–18; https://doi.org/10.15547/bjvm.2085

[23] Abodalal SEA, Tahoon AY. Development and production of a novel bivalent inactivated rabbit haemorrhagic disease virus (RHDV) vaccine. Int J Vet Sci 2020; 9(1):72–7.

[24] World Medical Association (WMA). World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull World Health Organ 2001; 79(4):373.

[25] OIE. Terrestrial manual rabbit haemorrhagic disease, Chapter 3.6.2. pp 1389–406, 2018. Available via https://www.oie.int/app/uploads/2021/03/rhd.pdf

[26] Thayer SG, CW Beard. Serological procedure. In: A laboratory manual for the isolation, identification and characterization of avain pathogens. 5 edition, Quebec, H9P 1H6, Canada, AAAP, 2008, pp: 222–329.

[27] Chasey D, Lucas MH, Westcott DG, Sharp G, Kitchin, A, Hughes SK. Development of diagnostic approach to the identification of rabbit haemorrhagic disease. Vet Reco, 1995; 137(7):158–160; https://doi.org/10.1136/vr.137.7.158

[28] Fahmy HA, Arafa A, Mahmoud AH. Molecular diagnosis of rabbit hemorrhagic disease virus (RHDV). Egypt J Comp Pathol Clin Pathol 2010; 23(1):85–101.

[29] Hall T. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 1999; 41:95–8.

[30] Kumar S, Stecher G, Tamura K. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 2016; 33:1870–4; https://doi.org/10.1093/molbev/msw054

[31] Ahmad ST, El-Samadony HA, Mahgoub KM. Immunological and virological studies on rabbit hemorrhagic disease virus. Glob Vet 2011; 7(6):545–56.

[32] Ahmad ST, El-Samadony HA, Mahgoub KM. Immunological and virological studies on rabbit hemorrhagic disease virus. Glob Vet 2011; 7(6):545–56.

[33] Daoud AM, Khodeir MH, Abbas AM. Preparation of a specific inactivated vaccine against rabbit haemorrhagic disease virus. In 4th Vet. Med. Zag. Congress in Hurghada, Egypt, 1998, vol. 230, p 234.

[34] Salman OGA. Further studies on haemorrhagic viral disease in rabbits in Egypt. Ph.D. Thesis, Department of Bird and Rabbit Diseases, Faculty of Veterinary Medicine, Cairo University, Cairo, Egypt, 2007.

[35] Ibrahim SM, Owees SGA, ElZeedy SA. Sequence analysis of RHDVVP60 gene of Giza-2006 isolate. Egypt J Virol 2012; 9:317–32.

[36] Esteves PJ, Abrantes J, Carneiro M, Muller A, Thompson G, Van der Loo W. Detection of positive selection in the major capsid protein VP60 of the rabbit haemorrhagic disease virus (RHDV). Virus Res 2008; 137(2): 253–6; https://doi.org/10.1016/j.virusres.2008.07.025

[37] Blom N, Sicheritz-Ponten T, Gupta R, Gammeltoft S, Brunak S. Prediction of post-translational glycosylation and phosphorylation of proteins from the amino acid sequence. Proteomic 2004; 4(6):1633–49; https://doi.org/10.1002/pmic.200300771

[38] Maeda Y, Kinoshita T. Dolichol-phosphate mannose synthase: structure, function and regulation. Biochim Biophys Acta 2008; 1780(6):861–8; https://doi.org/10.1016/j.bbagen.2008.03.005