Characterization and Pathotyping of Egyptian Field Strains of Rabbit Haemorrhagic Disease Virus (2005-2010)

Metwally, AY*; Arafa, A; Hegazy, A M*; Hussein, EG*** and Soad, A Abd El-Waneis****

*Animal Health Research Institute, Kafr El-Sheikh Prov. Lab.
**National Lab. for Vet. Quality Control on Poultry Production
***Animal Health Research Institute, Damanhor Prov. Lab.

ABSTRACT

Rabbit haemorrhagic disease (RHD) is highly contagious acute fatal viral disease of rabbits and widely distributed in Egypt. Vaccination considered to be the most important tool for controlling the disease. The present work was planned to study some aspects related to epidemiology of Rabbit Haemorrhagic Disease Virus (RHDV) outbreaks, which occurred among vaccinated rabbitary flocks from 2005 to 2010. A total of 25 suspected RHD outbreaks were investigated for the disease history, virus detection by haemagglutination test(HA) for clarified representative liver homogenate and serological examination by haemagglutination inhibition test (HI). In addition, pathotyping and molecular characterization of some recovered circulating field strains was determined and protection studies were conducted. The field survey revealed variable mortality rate from 15 to20% for Cunipravac (Laboratories Hipra) vaccinated flocks and 22-30% for SVRI (Veterinary Serum and Vaccine Research Institute) vaccinated flocks. The end point of HA titer expressed as log 2 ranged from 2^7 to 2^{10}. Antibody response in rabbitary flocks under investigation during the course of RHD revealed HI titer(Log_2) from 1:80 to 1: 640. At assessing protection efficacy of available RHD vaccines, the complete protection was recorded in rabbits vaccinated with circulating RHD field virus vaccine. Egyptian circulating field RHD viruses are not closely related to some of the available commercial vaccinal strains and mutation continues to occur and the emergence of new variant strains should be considered for strategic plan for controlling RHD outbreaks in order to reduce economic losses.

INTRODUCTION

Rabbit haemorrhagic disease (RHD) is highly contagious acute fatal disease of rabbits. RHD is one of the major viral diseases which threatens rabbit population ,outbreaks were first reported in China, *(1)* and later recorded in other countries of Asia, different European countries, Mexico and elsewhere, *(2)*. The disease was introduced into Egypt in 1992 *(3,4)*. Subsequently ,outbrcaks were recorded in several governorates *(5-8)*.

RHD is characterized by high morbidity and mortality rate from 40- 90 %. Infection occurs in rabbits of all ages but clinical disease is only observed in adults and young animals older than 40-50 days *(9)*. Clinical manifestation including non specific symptoms pyrexia and dullness are often encountered accompanied by nervous signs, but respiratory signs as dyspnea and frothy bloody nasal discharge may be observed in later stages of the disease. The most severely affected organs were lung and liver, which is brownish red and fragile, often with a marked lobular pattern *(10)*.

The causative agent of RHD is calcivirus, a positive-sense single stranded RNA virus.. Virion is 30±40 nm in diameter, shows a characteristic morphology and is composed of a major protein of 60 kDa (VP60). The 7±5 kb positive-sense ssRNA genome encodes a large precursor polyprotein which undergoes proteolytic cleavage to yield mature proteins *(11, 12)*. The C-terminal region of the polyprotein gives rise to the polypeptide p60, which is detected by antibodies against the capsid protein, viral particles also encapsidate an abundant VP60-linked poly adenylated sub genomic RNA of about 2.2 kb *(13)*.

Control policy mainly depends on vaccination using inactivated formalized vaccine

prepared from clarified liver suspensions of infected rabbits ,that have been developed in several countries including Egypt *(14-16).*

Comparisons of different RHD viral isolates reveals close overall homology in terms of genome sequence with few or no consequent predicted changes in amino acid composition; viruses from Germany, France, Spain and Egypt differ by between 2% and 5% corresponding to between one and ten amino acid substitutions in capsid VP60 protein from the different isolates *(17).* However, *(18)* characterized a variant strain "Rain ham" isolated from England and antigenically indistinguishable from known isolates. Moreover, a consistent genetic and antigenic RHDV variant has been identified simultaneously in Italy *(19)* and Germany *(20).* This RHDV variant, named RHDVa, presents amino acid changes in the surface-exposed E region (aa 344–434) that contains the main antigenic epitopes of calicivirus, three times higher than in all previously sequenced RHDV isolates. Antigenic analysis on French isolates(1988-1995) demonstrated two new variant isolates *(21)* .Furthermore, *(22)* has been demonstrated the existence of non protective antibodies due to putative RHD like virus. Meanwhile, severe outbreaks with high mortality having the same clinical pictures and post mortem lesions of RHD in vaccinated rabbit flocks was reported *(23)* and proved that the newly isolates RHD not closely related to classical vaccinal strain and may be a variant.

The purpose of this study was to assess the identification and pathotyping of circulating field RHD viruses during the period from 2005 until 2010 and genotyping by RT-PCR and sequencing analysis ,as well as protection studies with available vaccines .

MATERIAL AND METHODS

Specimens

Five rabbit colonies had history of vaccination against RHD and exhibiting symptoms and lesions suspected to be RHD were investigated during March, April and May each year from 2005 to 2010 at Kafrelsheikh,El-Behera and El-Gharbia governorates, as well as cases admitted to National Lab for Vet. Quality Control on Poultry Production .

Clinical and post mortem examination was conducted and recorded . Infected liver tissues(three from each farm) were aseptically collected, frozen and homogenized (20% w/v) with PBS, then the homogenate was clarified by centrifugation at 3000 rpm for 30 min. The supernatant fluid was collected, pooled and stored at – 20 ºC until used for virus detection by haemagglutination test and genotyping.

Sterility test for bacterial contaminants :Small amount of collected supernatant fluids were inoculated into a set of media including: nutrient agar, MacConkey agar, blood agar and tryptic soy agar in order to exclude bacterial contamination.

Haemagglutination test(HA) :It was carried out *(24)* at room temperature using 0.75 % human type (O) washed RBCs

Serum samples: Five serum samples were collected from each investigated rabbitary during RHD outbreaks from apparent healthy rabbits for assessing haemagglutinin inhibiting antibodies by haemagglutination Inhibition test (HI) *(24).*

Protection studies: Eighty (80) three months old New Zealand rabbits were tested and proved RHD seronegative by HI test. Rabbit were allotted into four equal groups and housed in disinfected independent cage units. Feed and water were offered ad lib . Group 1 was vaccinated 1 ml s/c with vaccine prepared from pooled clarified liver homogenate of circulating RHD field isolates (2005-2010), its preparation and safety were carried out *(24)* Group 2 was vaccinated 0.5 ml s/c with Cunipravac-RHD(Laboratories Hipra) derived from a field Spain strain . Group 3 was vaccinated 0.5 ml s/c with RHD virus vaccine obtained from Veterinary Serum and Vaccine Research Institute (SVRI), Egypt. Group 4 was kept as unvaccinated control group. Serum samples were collected from all animals ten days post vaccination for assessing active immune response using HI test, simultaneously all experimental animals were challenged by intranasal inoculation 1ml of pooled clarified liver homogenate of circulating RHD field isolates (2005-2010). All groups were daily

observed for clinical signs and/or mortality along 15 days ,liver samples were collected from dead rabbit for detection haemagglutination activity.

RT-PCR: viral RNAs were extracted from the samples of liver suspension from circulating RHD field isolates from 2005 to 2010(RHD-Egy-05 to RHD-Egy-10) as well as available vaccines Hipra and SVRI by using RNeasy (QIAGEN, Germany) and amplified using a One-Step RT-PCR kit (QIAGEN, Germany). The reactions were carried out in 25 ul volume reaction using 5 ul of 5X-RT-PCR buffer, 1ul of RT-enzyme provided with the kit, 1.25 ul of $MgCl_2$, 1 ul of each primer, 1ul of dNTPs and 10 ul of PCR-water, then 5 ul of RNAs for each sample. The RT-PCR was carried out using oligonucleotide primers *(25)*. The primer sets and their position, numbered according to the RHDV sequence were illustrated in Table 1. It was designed in Metabion Company, Germany.

Table 1. Oligonucleotide primers according to the RHDV sequence:

Primer	Sequence (5'to 3')	Location	Size of amplicon
RHDV- P33(F)	CCACCACCAACACTTCAGGT	6473–6492	538 bp
RHDV- P34(R)	CAGGTTGAACACGAGTGTGC	6992–7011	

(F): Sense. (R): Antisense. bp: base pair.

The amplification reaction was performed using the following temperature profile: an RT step at 50 °C for 30 minutes followed by initial denaturation at 95°C for 15min, then 40 cycles of 95°C for 1 min, then 56°C for 1 min, and 72°C for 2 min, then a final extension step at 72°C for 10 minutes on thermocycler (Biometra, Germany). The amplified PCR reaction mixture is loaded into 1.5% agarose gel (molecular biology grade) electrophoresis on, visualized by ultraviolet transillumination stained with ethidium bromide.

Partial VP60 gene sequencing:

Nucleotide sequence analysis of the VP60 gene was conducted to identify the genotype of the circulating RHD field isolates from 2005 to 2010(RHD-Egy-05 to RHD-Egy-10). The PCR products of the VP60 gene fragments (500 bp) were directly sequenced. Partial nucleotide sequencing of the VP60 gene was carried out. The amplified VP60 products were purified using a PCR purification kit (Qiagen, Hilden, Germany) and sequenced by using the forward primer (RHDV- P33) by using BigDye Terminator v3.1 Cycle Sequencing Kit on an automatic sequencer (ABI-3130; Applied Biosystems). The sequenced fragments of VP60 gene of the Egyptian isolates were compared to other strains isolated worldwide by using BLAST tool of NCBI.

Phylogenetic analysis was carried out on VP60 gene. Multiple and pairwise sequence alignments were constructed using the ClustalV algorithm and a phylogenetic tree were constructed using the neighbour-joining of MegAlign program from LaserGene Biocomputing Software Package (DNASTAR, Madison, WI).

RESULTS AND DISCUSSION

Rabbit haemorrhagic disease virus (RHDV), a member of the Caliciviridae, is an important pathogen that causes a highly contagious disease in rabbits The disease is responsible for high economic losses in rabbitries. Infected rabbits usually die within 48 to 72 hours of necrotizing hepatitis and haemorrhagic syndrome. In Egypt, RHD was first reported in Sharkia governorate *(3)* . Since, spring of 1991 severe outbreaks of RHD were recorded allover the country in rabbitary flocks of all breeds with high morbidity and mortality rates up to 90% in adult and 50% in young rabbits *(6)*. Subsequently, several attempts were carried out to produce effective ,save and potent vaccine for controlling the disease *(15,16)*. However, vaccination failures against RHD have been recorded in some rabbit colonies and the newly isolated RHD not closely related to classical vaccinal strain and may be a variant *(23)* .Therefore, the characterization and pathotyping of circulating

RHD viruses appears very important for the strategic plan for controlling the disease.

Epidemiological studies on investigated flocks:

A total number of 25 suspected RHD outbreaks were investigated at Kafrelsheikh, El-Behera and El-Gharbia governorates ,Egypt during the period in between 2005 and 2010 .The capacity of infected flocks ranged from 250 to 1500 rabbit with age groups from suckling until mature dams. Rabbit breeds were mostly New Zealand , some California and mixed inbreeding, also 2 colony V- line . All investigated flocks had been vaccinated twice per year at August and February by using local vaccine (SVRI) or one shot annually by Cunipravac (Hipra).The clinical symptoms were sudden deaths, depression, convulsion , cyanosis of lips and nostrils ,dyspnea accompanied by abdominal respiration and cries. Frothy bloody nasal discharge was occasionally observed. Sometimes anal sphincter appeared loosen with mucoid faecal discharges.

At necropsy, all infected rabbit showed an enlarged, pale, friable liver with a fine lobular appearance .Kidneys and spleen were severely congested. Multiple petechial haemorrhages were prominent on renal surface. Lungs showed congestion, oedema and subpleural haemorrhages. Mortality rate ranged 15-20% for Cunipravac vaccinated flocks and 22-30% for SVRI vaccinated flocks (Table,2).

Table 2. Epidemiological data on investigated rabbitary flocks and HA activity of infected liver homogenate.

Farm No.	Date	breed	capacity	Governorate	vaccine	Mortality %	HA (log2) titer
1	March 05	N	900	kfs	SVRI	22	7
2	March 05	N	400	Kfs	SVRI	23	7
3	March 05	N	300	Kfs	SVRI	25	9
4	April 05	C	250	Kfs	SVRI	30	10
5	May 05	M	500	Kfs	SVRI	30	10
6	March 06	C	500	Kfs	SVRI	23	8
7	March 06	M	700	Kfs	SVRI	27	8
8	April 06	M	400	Kfs	SVRI	25	7
9	April 06	N	600	GH	SVRI	24	8
10	May 06	N	1000	Kfs	SVRI	22	7
11	March 07	N	1200	kfs	SVRI	22	8
12	April 07	N	500	Kfs	SVRI	25	8
13	May 07	C	250	GH	Cuniparv	17	9
14	May 07	M	600	Kfs	SVRI	27	8
15	Sep.07	N	800	GH	Cuniparv	15	9
16	March 08	N	1500	Kfs	SVRI	22	8
17	April 08	M	800	Kfs	Cuniparv	18	7
18	May 08	M	900	Kfs	Cuniparv	16	8
19	May 08	V	250	GH	Cuniparv	15	8
20	Jun. 08	N	500	BH	SVRI	30	9
21	March 09	N	1300	Kfs	Cuniparv	20	10
22	April 09	N	1400	BH	SVRI	30	8
23	May 09	M	700	BH	Cuniparv	20	10
24	May 09	M	800	Kfs	SVRI	30	9
25	Sep. 09	V	300	Kfs	Cuniparv	15	9
	2010						

N=New Zealand C=California M=mixed breeds

Kfs=Kafrelsheikh GH=El-Gherbia BH=El-Behera

We have observed dramatic mortality in advanced pregnant does or shortly after parturition especially the first time , as well as at the end of fattening period of growing rabbit (6-8 weeks old) frequently associated with large temperature fluctuations in short periods of time. This observation may explained the high prevalence of RHD outbreaks during spring season .Similar results and observation were recorded in several studies *(3,26).* All breeds were highly susceptible ,although lowest mortality was recorded in V- line breed.

Haemagglutination test (HA): . The end point HA titer expressed as log 2 was ranged from 2^7 to 2^{10} (Table,2) The first test developed for detecting RHDV was the haemagglutination test *(27),* which is based on the ability of RHDV to agglutinate human erythrocytes *(1).* This test was widely used by Chinese and European scientists as a screening method *(28).* However, the sensitivity and specificity of this method appear to be unsatisfactory. Investigations estimated the specificity of the test to be 92%, and its sensitivity to be in the order of 80 to 90% *(9).* The most sensitive and specific method for detection of RHDV is the use of the reverse transcriptase polymerase chain reaction (RT-PCR) to detect viral RNA. Liver samples of rabbits were positive with RT-PCR at dilutions up to 10^{-10} *(29)* RT-PCR , also it has been used successfully to detect viral RNA in rabbit serum.

Haemagglutination inhibition (HI) : Antibodies responses in rabbitary flocks under investigation during the course of RHD revealed HI titer (Log_2) from 1:80 to 1: 640 (Table.3) .

The haemagglutination inhibition test (HI) was the first test reported to detect RHDV antibodies *(27).* HI is commonly used and are an inexpensive and simple method for RHDV antibody detection *(30,31).* However, to avoid false positive results it is necessary to remove natural agglutinins in the serum, and a range of procedures for serum pre-treatment have been used *(32).* False positive test results for RHDV were also observed in rabbit infected with Pasteurella species. *(33).* Another disadvantage of the test is its inability to detect low concentrations of antibodies, which has been observed by some researchers *(34).*

RHDV specific antibodies (IgM ,IgG and IgA) can be determined by using isotype ELISA *(35)* to distinguish between different classes of immunity (maternal antibodies, recent and past infection, re-exposure to RHDV and exposure to RHDV-like agent). Vaccinated and naturally exposed rabbits ,as well as specify the infection process in single animals were compared in more detail *(36)* . These tests are an important tool to clarify test results based on the competition ELISA.

Protection studies :All animals were seronegative at the beginning of the experiment before vaccination. Vaccinated animals quickly produced strong humoral immune response against RHD virus as represented by elevated HI titers at 10 days post vaccination. Higher HI titers were recorded in rabbits vaccinated with vaccine prepared from pooled clarified liver homogenate of circulating RHD field isolates (Table,4). Resistance to challenge virus was minimal in the unvaccinated control group, 80% of infected rabbits died 48-96 hours post inoculation. Complete protection was recorded in rabbits vaccinated with circulating RHD field virus vaccine (Table, 4).On the other hand, *(15)* has been recorded that rabbits vaccinated with local or imported vaccines were fully protected against challenge with virulent strain of RHDV 4 days post vaccination and the full protection continued 9 weeks post vaccination. Different RHD virus vaccines induced variable serological responses , meanwhile field isolate RHD virus vaccine induced the highest HI titers starting from 4th day post vaccination, resistance to challenge infection was 100% in rabbits that were vaccinated with field isolate RHD virus vaccine in comparison with 80% and 66.6% in rabbits vaccinated with Izovac Mevax (IZO S.P.A. Italy) and SVRI vaccines respectively *(37).*

Table 3. Sero-epidemiological monitoring for RHD antibodies by HI test on investigated rabbitary flocks.

Farm No.	Date	breed	capacity	Governorate	Vaccine	HI titre distribution(Log_2)			
						1/80	1/160	1/320	1/640
1	March 05	N	900	kfs	SVRI		2	2	1
2	March 05	N	400	Kfs	SVRI		2	2	1
3	March 05	N	300	Kfs	SVRI		2	2	1
4	April 05	C	250	Kfs	SVRI		2	1	2
5	May 05	M	500	Kfs	SVRI		1	2	2
6	March 06	C	500	Kfs	SVRI		1	1	3
7	March 06	M	700	Kfs	SVRI		1	1	3
8	April 06	M	400	Kfs	SVRI		1	2	2
9	April 06	N	600	GH	SVRI		1	3	1
10	May 06	N	1000	Kfs	SVRI		1	2	2
11	March 07	N	1200	kfs	SVRI	1	1	2	1
12	April 07	N	500	Kfs	SVRI	1	1	2	1
13	May 07	C	250	GH	Cuni		2	2	1
14	May 07	M	600	Kfs	SVRI		2	2	1
15	Sep.07	N	800	GH	Cuni		1	2	2
16	March 08	N	1500	Kfs	SVRI	1	1	2	1
17	April 08	M	800	Kfs	Cuni	1	1	2	1
18	May 08	M	900	Kfs	Cuni		2	1	2
19	May 08	V	250	GH	Cuni		2	1	2
20	Jun. 08	N	500	BH	SVRI		1	2	2
21	March 09	N	1300	Kfs	Cuni	2	1	1	1
22	April 09	N	1400	BH	SVRI	2	1	1	1
23	May 09	M	700	BH	Cuni	2	1	1	1
24	May 09	M	800	Kfs	SVRI	1	2	1	1
25	Sep. 09	V	300	Kfs	Cuni	1	2	1	1

N=New Zealand C=California M=mixed breeds Kfs=Kafrelsheikh GH=El-Gherbia BH=El-Behera

Partial VP60 gene sequencing: Results of partial VP60 gene sequencing and phylogenetic analysis of Egyptian RHDV strains compared to other strains from America, Europe and Asia as well as vaccinal strains usually used in the field are illustrated in fig. 1. The results indicated that the Egyptian viruses continuously evolving in different clusters from 2005 till 2010. That also revealed mixing of different viruses circulated in the field that grouped into 2 main groups, one related to the classical RHDV (Egy-05 and Egy-09) and was very closely related to strains isolated from France (01-15=CYM74). The other groups of Egyptian viruses (Egy-07, Egy-08 and Egy-10) was very

closely related to the variant strains of RHDV (like UT-01, Triptis, Rossi-2007, Jx-CHA-97 and Hokkaido-2002) circulated in different countries (like USA, Germany, China and Japan) and vaccinal strains used in the field (Hipra and local Egyptian vaccine from SVRI).

Results of amino acids sequence comparison of the Partial sequencing of VP60 gene of the Egyptian viruses and other closely related RHD viruses are illustrated in fig. 2 where we can identify that the Egyptian strains (Egy-07, Egy-08 and Egy-10) which were very closely related to each other and differ than Egy-05 and Egy-09 strains.

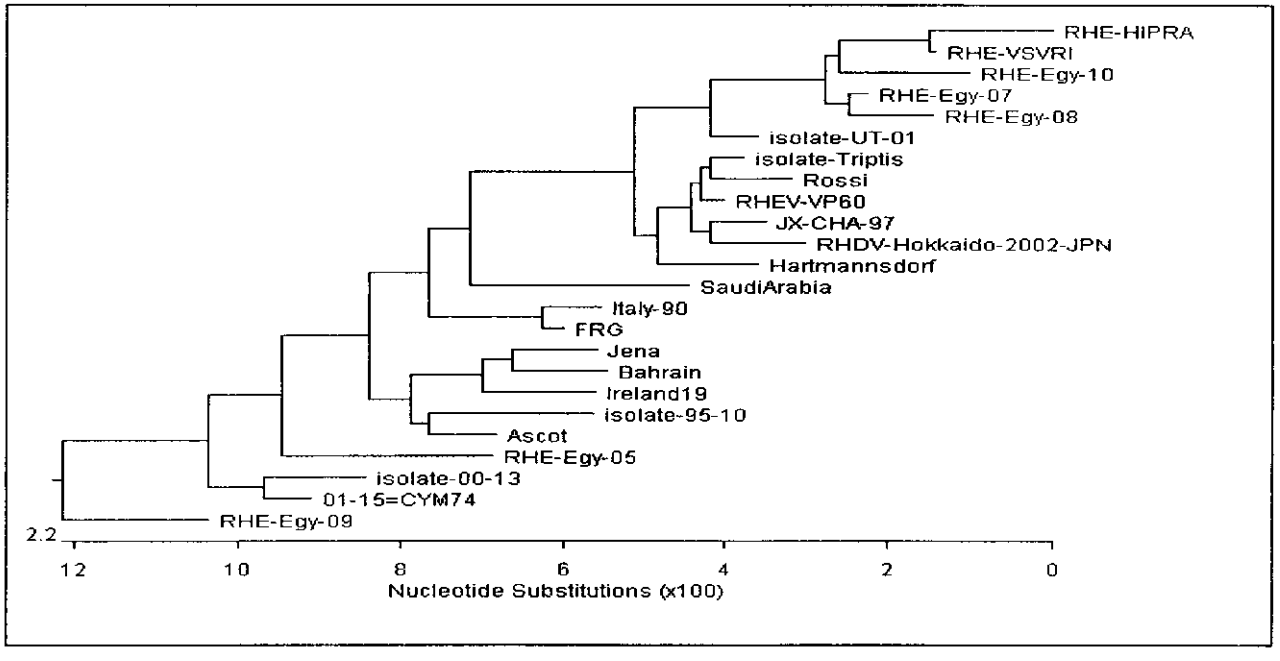
It could be concluded that, there are different RHD viruses circulated in the field and all viruses are not closely related to the available commercial vaccinal strains and the mutation continues to occur. Moreover, the emergence of new variant strains should be considered, so further continues periodic investigation should be applied. Hence, this results may provide an explanation for the occurrence of many RHD outbreaks in rabbitary flocks that have been previously vaccinated with commercial RHD vaccines.

Table 4. Results of Protection studies

Group	Vaccine	HI titer	Morbidity	Mortality	Protection percent	HA activity *
1	Field strain	1:640-1:1280	2/20	0/20	100 %	Not applicable
2	Cuniparvac	1:320-1:640	6/20	4/20	80 %	Positive
3	SVRI	1:320-1:640	7/20	6/20	70 %	Positive
4	Control	Not detected	20/20	16/20	20 %	Positive

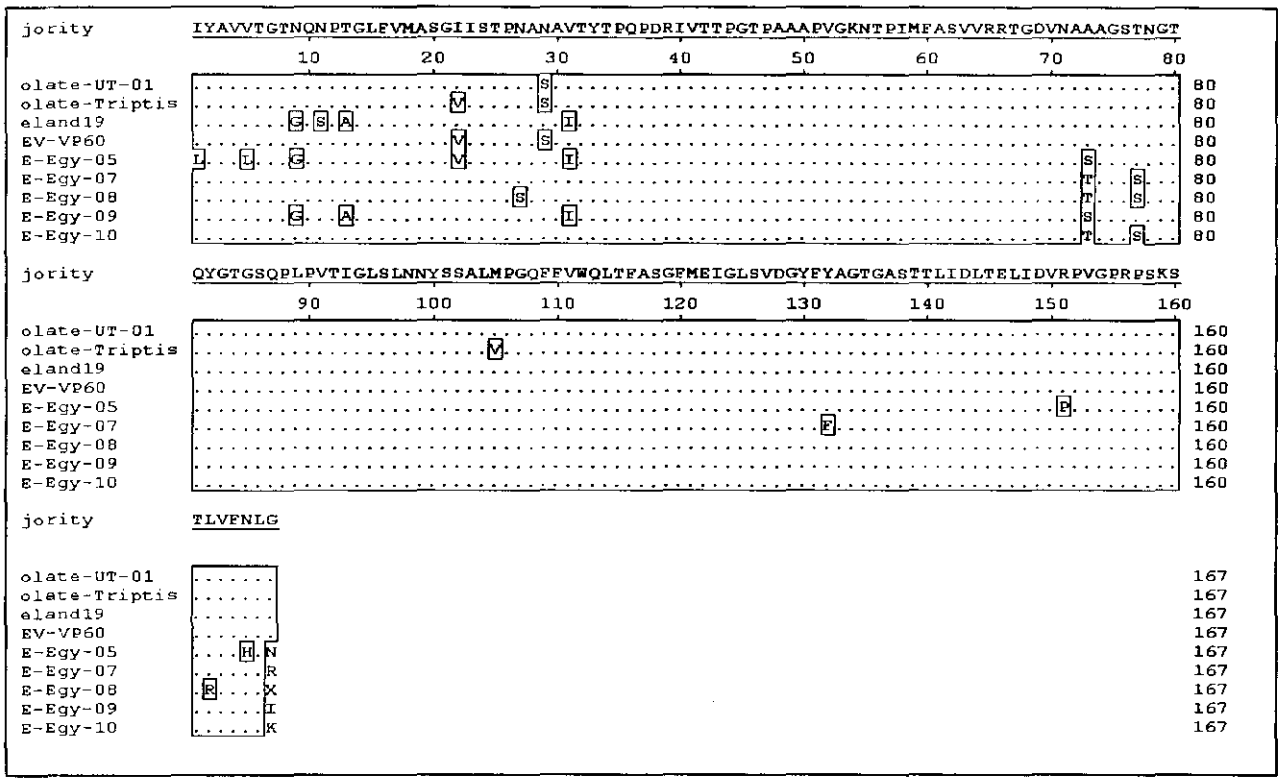
* HA test was carried out on liver homogenate of freshly dead rabbits after challenge.

Fig. 1.



PHYLOGENETIC ANALYSIS OF Egyptian RHDV strains compared to other strains from America, Europe and Asia. The analysis was done by using Clastal V method of DNA star software

Fig. 2.



Amino acids sequence comparison of the Partial sequencing of VP60 gene of the Egyptian viruses and other closely related RHE viruses.

REFERENCES

1.Liu, S J ; Xue , H P ; Pu, B Q ; and Qian, N H (1984): A new viral disease in rabbits. Animal Husbandry and Veterinary Medicine, 16 (6): 253- 255.

2.Gregg, D A ; Wilson, C and House, C (1989): Necrotic hepatitis of rabbits in Mexico: a parvo virus. Foreign Anim. Dis. Rep., 17(2):7-10.

3.Ghanem, I A and Ismail, A (1992): Occurrence of rabbit hemorrhagic disease in Sharkia province. Zag. Vet. J. (EGVMC), 20 (4): 491-502.

4.Salem, B and El-Ballal, S S (1992): The occurrence of rabbithemorrhagic disease (RHVD) in Egypt. Assiut Vet. Med. J. 27(53):295-304.

5.El-Zanaty, K (1994): Some investigations on rabbit viral hemorrhagicdisease in upper Egypt. Assiut Vet. Med. Journal. 30(60): 293 - 305.

6.Azhar, M A ; Ashgan, M S ; Soliman, A M and Bayoumi, A H (1995): Rabbit viral hemorrhagic disease. Beni-Suef Vet. Med. J. 5(1): 17-22. (RVRD) in Egypt. *Assiut Vet. Med. J., 27 (53): 295-304.*

7.El-Mongy, F A (1998): Studies on viral hemorrhagic disease ofrabbits. Ph.D. thesis, Fac. Vet. Med. Moshtohor, Zag. University. Egypt.

8.Mostafa, A A (2001): Characterization and pathogenicity of locallyisolated virus of hemorrhagic disease in rabbits.Ph.D. Thesis, Fac. Vet. Med. Beni-Suef, Cairo University. Egypt.

9.Capucci , L ; Scicluna, M T and Lavazza, A (1991): Procedures for ELISA tests for the virological and serological diagnosis of virological diagnosis of the European brown hare syndrome. Stlezione Veterinaria. 32 (9): 1381 – 1397.

10.Okerman, L (1994): Diseases of demostic

rabbits. 2nd ed., Black well scientific publication.

11.Alonso, J M , Casais, R , Boga, J A and Parra, F (1996): Processing of rabbit haemorrhagic disease virus poly protein. Journal of Virology 70, 1261-1265.

12.Wirblich, C , Thiel, H J and Meyers, G (1996): Genetic map of the calicivirus rabbit haemorrhagic disease virus as deduced from in vitro translation studies. Journal of Virology 70, 7974-7983.

13.Morales, M , J Barcena, M A Ramirez, J A Boga, F Parra, and J M Moss, S R , Turner, S L , Trout, R C , White, P J , Hudson, P J , Desai, A , Armesto, M , Forrester, N L , and Gould, E A (2002): Molecular epidemiology of Rabbit haemorrhagic disease virus. Journal of General Virology 83, 2461-2467.

14.Gunenkov, V V (1990): Viral hemorrhagic disease of rabbits. Veterinariya (Moskova) 5: 13 -15.

15.Abd El-Motalib, T Y ; Abd El-Gawad, A M and Azzaz, H (1998): Viral hemorrhagic disease of rabbits, Comparative study between theimmune response of local and imported vaccines. Assiut Vet. Med. J.40(79): 150-156.

16.Daoud, A M ; Khodier, M H ; Abbass, A M and Ibrahim S I (1998): Preparation of a specific inactivated vaccine against rabbitviral hemorrhagic disease. 4th Sci. Cong. Fac. Vet. Med. Zag.University, Egypt. 230 - 234.

17.Guittre C, Baginski I, Le Gall G, Prave M, Trepo C,ova L (1995): Detection of rabbit haemorrhagic disease virus isolates and sequence comparison ofthe N-terminus of the capsid protein gene by polymerase chain reaction. Research in VeterinaryScience 58, 128-32

18.Capucci L , Chasey D , Lavazza A and Westcott D (1996): Preliminary characterisation of a nonhaemagglutinating strain of rabbit haemorrhagic disease virus from the United Kingdom. J. Vet. Med. [B], 43,245-250

19.Capucci, L ; Fallacara, F ; Grazioli, S ; Lavazza, A ; Pacciarini, M L and Brocchi, E (1998): A further step in the evolution of rabbithemorrhagic disease virus: The appearance of the first consistentantigenic variant. Virus Res. 58: 115-126.

20.Schirrmeier, H ; Reimann, L ; Kollner, B and Granzow, H (1999): Pathogenic, antigenic and molecular properties of rabbit hemorrhagicdisease virus (RHDV) isolated from vaccianted rabbits: detection and characterization of antigenic variants. Arch. Virol. 144: 719-735.

21.LeGall, R G ; Zwingelstein, F ; Laurent, S ; DeBoissesson, C ;Portejoie, Y and Rasschaerts, D (2003): Phylogenetic analysisun of rabbit hemorrhagic disease virus in France between 1993 and 2000and the characterization of rabbit hemorrhagic disease virus antigenic variants Arch. Virol. 148: 65- 81.

22.Marchandeau S , LE Gall-Recule G , Bertagnoli S , Aubineau J , Botti G and Lavazza A (2005): Serological characterization of antigenic variants. Arch. Virol. 144: 719-735.

23.Metwally, A. Y and Madbouly, H M (2005): Study on a new isolate of rabbit heamorrhagic disease virus. Bani-suef Vet. Med. J., 15(2): 283-243.Vet. Bulletin, 55: 53-54.

24.Office of International Epizootics (OIE) Terrestrial Manual (2008): Rabbit viral hemorrhagic disease.. Chapter 2.6.2 pp 947-961 .

25.Vende, P, Le Gall, G and Rasschaert, D (1995): An alternative method for direct sequencing of PCR products for epidemiological studies performed by nucleic sequence comparison. Application of rabbit haemorrhagic disease virus. Vet. Res. 26: 174-179.

26.Rosell,J M and Badiola ,J J (1989): RHDV, Epidemiology and Control. Cuniculture (Paris), 89:21-26

27.Pu, B Q , Quian, N H and Cui, S (1985): Micro HA and HI tests for the detection of

antibody titres to so-called 'haemorrhagic pneumonia' in rabbits [in Chinese]. Chinese Journal of Veterinary Medicine 11, 16-17, Abstract in Veterinary Bulletin (1987), 57, p. 110, Abstr. 809..

28. ***Calvete, C , Estrada, R , Villafuerte, R , Osacar, J J and Lucientes, J (2002):*** Epidemiology of viral haemorrhagic disease and myxomatosis in a free-living population of wild rabbits. Veterinary Record 150, 776-782.

29. ***Lenghaus, C, Westbury, H, Collins, B, Ratnamohan, N and Morrissy, C (1994):*** Overview of the RHD project in Australia. In 'Rabbit Haemorrhagic Disease: Issues in Assessment for Biological Control'. (Eds. R. K. Munro and R. T. Williams.) pp. 104-129. (Bureau of Resource Sciences, Australian Government Publishing Service: Canberra.)

30. ***Elghaffar, S K A , Aly, M , Fatma, A , Moustafa, A and Mahmoud, A Z (2000):*** Pathological studies on the rabbit viral hemorrhagic disease (RVHD) with special reference to the use of vitamins A, E and C as prophylaxis. Assiut Veterinary Medical Journal 43, 251-274.

31. ***Mizoguchi, T , Itou, K , and Sakurai, M (2003):*** Haemagglutination and antigenic comparison of Rabbit haemorrhagic disease virus. Journal of Veterinary Medical Science 65, 95-97.

32. ***Collins, B J , White, J R , Lenghaus, C , Boyd, V , and Westbury, H A (1995):*** A competition ELISA for the detection of antibodies to rabbit haemorrhagic disease virus. Veterinary Microbiology 43, 85-96.

33. ***Rodak, L, Smid, B and Valicek, L (1991):*** Application of control measures against viral haemorrhagic disease of rabbits in the Czech and Slovak Federal Republic. Revue scientifique et technique de l'Office International des Epizooties 10, 513-524.

34. ***Dedek, J and Frölich, K (1997):*** Serological survey for calicivirus antibody among free-living red foxes in Mecklenburg-Vorpommern, Germany [in German]. Tierärztliche Umschau 52, 149-152.

35. ***Capucci, L , Nardin, A , and Lavazza, A (1997):*** Seroconversion in an industrial unit of rabbits infected with a non-pathogenic rabbit haemorrhagic disease-like virus. Veterinary Record 140, 647-650.

36. ***Cooke, B D , Robinson, A J , Merchant, J C, Nardin, A and Capucci, L (2000).*** Use of ELISAs in field studies of rabbit haemorrhagic disease (RHD) in Australia. Epidemiology & Infection 124, 563-576.

37. ***Metwally, A Y ; Ismail, M M ; Hegazy, A M and Soliman, H A (2006):*** Efficacy of anew isolate rabbit haemorrhagic disease virus vaccine. Kafrelsheikh Vet. Med. J. Vol. 4,no. 2:45-58.

الملخص العربي

عزل وتصنيف العترات الحقلية المصرية لفيروس المرض النزفي الارنبي من ٢٠٠٥-٢٠١٠

عبد النبى يونس متولى طاحون* ، **عبد الستار عرفه محمد**** ، **عبد الحليم محمد حجازي*** – **عيد جمعة شحاتة حسين***** ، **سعاد عبد العزيز عبد الونيس** ****

معهد بحوث صحة الحيوان معمل فرعى كفر الشيخ*- المعمل القومي للرقابة البيطرية على الإنتاج الداجني**
معهد بحوث صحة الحيوان معمل فرعى دمنهور***

أجرى هذا العمل لدراسة بعض التقاط الوبائية لمرض الفيروس النزفي في قطعان الأرانب المحصنة في الفترة من ٢٠٠٥-٢٠١٠. تم دراسة عدد ٢٥ مزرعة مشتبه إصابتها بـــ الفيروس النزفي وكانت كلها إيجابية للفيروس باستخدام اختبار التلازن الدموي والتلازن الدموي المثبط. تم عمل تصنيف لبعض العترات الحقلية المعزولة وكذلك التوصيف الجزئي لها. تم إجراء العدوى الصناعية ودراسة نسب الصد للتحصينات المتاحة محليا والمستوردة وكذلك التحصين المحضر معمليا من العترات الحقلية وكانت عياريه التحصينات المحلية والمستوردة تتراوح من $٢^{٧}$حتى $٢^{١٠}$ وكذلك مستوى الأجسام المناعية للصد تتراوح من ١/٨٠ وحتى ١/٦٤٠ في نفس الوقت كانت نسبة الحماية في التحصينات المحلية والمستوردة من ٧٠-٨٠% على الترتيب بينما التحصين المحضر معمليا من عترة حقلية أعطى حماية بنسبة ١٠٠% بعد ١٠ أيام.