BENHA VETERINARY MEDICAL JOURNAL, VOL. 26, NO. 2:71-83, JUNE 2014

EGYPTIAN NON-HEMAGGLUTINATING ISOLATES OF RABBIT HEMORRHAGIC DISEASE VIRUS CAN CHANGE TO VARIABLE HA PROFILE

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ABSTRACT

Rabbit Hemorrhagic Disease Virus (*RHDV*) is highly contagious and fatal disease. Many researches about circulating *RHDV* isolates in the different Egyptian governorates showed the existence of HA negative as well as HA positive isolates. For the first time we report the presence of *RHDV* isolates with variable HA profile in Egypt as many HA negative isolates changed into positive after passage on susceptible rabbits that were confirmed by RT/PCR technique for the variable region (C-E) of VP60 gene. In conclusion, HAT is not reliable in diagnosis or typing of *RHDV* field isolates as there is no correlation between the HA activity and genetic typing of *RHDV* either classical or variant strains. However, for tracking the genetic evolution of Egyptian isolates, further investigations at molecular level will be needed depending on sequence and phylogenetic analysis of highly variable region of VP60 gene.

Keywords: RHDV, HA properities, Rabbit, Egyptian isolates

(BVMJ-26(2):71-83, 2014)

1. INTRODUCTION

Rabbit Hemorrhagic Disease (RHD) is highly contagious to both wild and domestic rabbits. Characteristic pathological lesions are found in the liver, trachea and lungs of infected rabbits and the animals die within 12 to 72 hours due to severe hepatitis and hemorrhages (Gregg et al., 1991 and Fuchs and Weissenbock 1992).

RHD was first reported in China in 1984 (Liu et al., 1984) and rapidly became endemic in most parts of the world. Other viruses in this group include the European brown hare syndrome virus (EBHSV), a highly pathogenic virus of hares, and rabbit calicivirus (RCV). RCV is a nonpathogenic variant reported in Italy that might be protective against the pathogenic virus (Capucci et al., 1996).

RHDV is an icosahedral, non-enveloped, positive-sense, single-stranded RNA virus within the genus *Lagovirus*, family *Caliciviridae* (Meyers et al., 1991a and Ohlinger et al., 1990). RHDV virions are small sized between 35-40 nm of diameter (Valicek et al., 1990). The viral genome is composed of approximately 7.4 kb and a subgenomic RNA of approximately 2.2 kb and both encoding for VP60 (Meyers et al., 1991b). *RHDV* capsid consists of a shell (S) domain which is buried and comprises the N-terminal connected by a hinge to the protruding (P) domain that encompasses the

C-terminal region and is exposed on the surface. The P domain can be subdivided into P1 (stem of arch) and P2 sub-domains (top of arch) (Neill,. 1992). Six distinct regions (A to F) can be distinguished in VP60; regions C and E are located in the exposed P2 sub-domain at the most exposed region of the capsid, displaying the greatest genetic variation (Neill,. 1992, Capucci et al., 1998, Schirrmeier et al., 1999).This variation is probably, at least in part, due to selection pressure because host antibodies recognize and target regions located in this sub-domain (Barcena et al., 2004, Capucci et al., 1995, Martinez et al., 1998). In order to avoid this recognition and the inherent selective pressure, these regions tend to evolve faster which results in an increase of the genetic variability and hence, of the antigenic variation (Esteves et al., 2008, Kinnear, and Linde 2009).

There is a single serotype with tow subtype, *RHDV* classical and a pathogenic antigenic variant strains, named *RHDVa*, were also reported in many parts of the world (Le Gall-Reculé et al., 2003). The *RHDVa* strains are characterized by molecular and antigenic differences with respect to the previous *RHDV* strains.

RHDV is still representing a threat in the rabbit production farms in spite of vaccination programs. *RHDV* was first reported in Egypt in Sharkia Province, in1991 (Ghanem, and Ismail 1992) then reported by others (Salem and EI-Ballal 1992, EI-Mongy 1998). Many studies have been performed for diagnosis of the disease using different techniques either Hemagglutination test, Hemagglutination Inhibition Test or using molecular tools as western immune blot and PCR technique (EI-Mongy 1998, Ibrahim et al., 1999, Salman 1999, Salman et al., 2008, Fahmy et al., 2010, Ahmed et al., 2011). Many other studies reported the presence of non-hemagglutinating *RHDV* isolates (Salman 1999, Salman et al., 2008, El-Sissi and Gafer 2008). Moreover, Giza2006 vaccine strain was genetically characterized as an *RHDV* variant by sequence analysis of full length VP60 gene (Ibrahim et al., 2012).

Till now there is no full detailed study concerning the antigenic revolution of *RHDV* in the Egyptian field. The aim of this study is to tracking the existence of non hemagglutinating *RHDV* in Egypt and the differences between hemagglutinating and non-hemagglutinating *RHDV* isolates in Egypt.

2. MATERIALS AND METHODS

2.1. RHDV Samples:

Liver samples were collected from freshly dead rabbits in sterile glass ware from different suspected RHDV outbreaks in different localities in Egypt as shown in table (1) and stored at -20º C.

2.2. Hemagglutination test (HAT)

A liver fragment was mechanically homogenized in 10-20% (W/V) saline solution pH 7.2, clarified by centrifugation at 5000 g for 10 minutes.

Erythrocytes suspension: RBCs human type (O) was collected using equal volume of Alsever's solution as anticoagulant with overnight incubation. Erythrocytes were washed in PBS pH (6.5) centrifugation at 500 g for 10 minutes. Erythrocytes sediment was re-suspended at 0.75% in PBS for micro-technique of HA.

A twofold dilution of the clarified supernatant of a 10% and 20% tissue homogenate of liver is incubated with an

Table 1: Data of original collected samples from field with hemagglitonation results

Sample code No.	Government	Date	Total No. of adult rabbits	Mortality rate in adults	Total No. of young rabbits	Mortality rate in young	HA slide test	HA plate test	
								At 22ºC	At 4ºC
R1	Giza	Jan 1997	112	100%	153	0%	+ve	+ve	+ve
R2	Kafr El-Shiehk	May 2000	63	59%	237	0%	-ve	-ve	-ve
R4	Kalubia	May 2000	175	12.5%	275	10.9%	-ve	-ve	-ve
R18	Kalubia	March 2002	222	9%	308	25.9%	-ve	-ve	-ve
R49	Kalubia	Feb 2005	90	15.5%	180	0%	-ve	-ve	-ve
R59	Giza	April 2006	151	42%	25	4%	+ve	+ve	+ve
R2010	Giza	Feb 2010	0	0	250 (1.5-2m)	88%	+ve	-	+ve
R2011	Kalubia	May 2011	0	0	500 (5 ws)	100%	-	-ve	+ve
R2012	Kalubia	May 2012	80	0	500 (1-2 m)	70%	-	-	-ve

equal volume of washed human RBCs type "0" in a sealed V-bottom micro-titer plate at the recommended temperature 4°C (Salman et al., 2010 and OIE, 2010).

2.3. Sample preparation for virus isolation

A liver fragment was mechanically homogenized in 10 % (W/V) saline solution pH 7.2, Filtrated through cheesecloth and clarified by centrifugation at 5000 g for 15 minutes. Antibiotic mixture and antifungal solution were added at concentration of 100 IU penicillin-G-sodium, 100µg streptomycin and 100 µg clotrimazol per ml of homogenate supernatant then incubated at room temperature for 1 hour then examined for bacterial and fungal contamination using nutrient agar.

2.4. RHDV isolation on rabbits:

Blood Twenty eight cross breed and Bosket susceptible rabbits 3 months adults (1.5-2 KG) and 1 month age (0.5 KG) were obtained for virus isolation.

Each rabbit was inoculated with 1-2 ml of 10% liver homogenate by the intra muscular route. The rabbits were observed for one week (Ferreira et al., 2004). The mortality pattern was recorded with the HA activity of each strain in table (3).

2.5. Two step Reverse transcription Polymerase chain reaction (RT /PCR) test:

The total RNA extraction of fresh liver tissues was performed with the SV Total RNA Isolation system. (Promega) Cat. No # Z3100 according to the manufacturer's procedure.

The specific primers were designed for amplification of the highly variable area of RHDV genome from C region (6205- 6288) to E region (6334- 6605) by RT/PCR.

The primer set were designed using Primer-Blast software to amplify a product length 600 bp and their positions , numbered according to the RHDV complete genome sequence **(Meyers et al., 2000)** with accession no. NC_001543, were as follows:

Upstream primer (6106-6125 bp):5' CCT GGA GGG TTT TCT ACG TG 3' and the downstream primer (6688- 6706 bp):5' AGA CGA CAG ACG CGA ACA T 3'. It was synthesized by Bio Basic Inc.

Reverse transcriptase (RT) reaction was done for cDNA synthesis by incubating 2 µg of viral RNA and 100 pmol of the downstream primer with nuclease free water at 65°C for 5 minutes. Then the mixture was cooled on ice for 5 minutes. The reaction was performed as following: 5 µl of 5X reaction buffer, 1.25 µl (500µmol) of PCR Nucleotide mix (Promega)10 mmol,1 µl (40 U) of Recombinant RNAseine (rRNasine) Ribo-nuclease inhibitor (Promega):, 1µl (200 U) of Revert Aid M-MuLV (Fermentas) and Nuclease free water up to 25 µl. The thermal cycler program used was at 42°C for 60 minutes then 95°C for 5 minutes.

Polymerase chain reaction (PCR) The reaction was performed as following: 25 µl of 2X Dream Taq Green PCR Master Mix (Fermentas), (100 pmol) of upstream primer, (100 pmol) of downstream primer, 8 µl of template DNA and water, nuclease-free up to 50 µl.

The amplification reactions were performed using thermal cycler Perkin Elmer Gene Amp. PCR system 9700. The thermal cycler was adjusted to 1 cycle at 94°C for 1 minute, then 25 cycles at 94 °C for 1 minute, 56 °C for 1 minute, 72°C for 1 minute followed by 1 cycle at 94 °C for 10 minutes.

The amplified product was analyzed by electrophoresis using 1% agarose gel and visualized by ultra violet transilluminator after staining the gel with ethidium bromide stain (Fisher). The product size was measured using 1Kb DNA Ladder (Gibco).

3. RESULTS

3.1. Virus isolation and passage in rabbits:

The data of original RHDV samples from field is summarized in table (1) with their hemagglutination results and these observations were noted: As these samples are covering a period of 1997 to 2012 the mortality rate at 1997 was 100% at adult age, these rates were descending till 0% from 2010 and upward. The mortality rate in young age at 1997 was 0%, but it was ascending gradually till reaching 100% at 2011.

3.2. Hemagglutination test for RHDV samples after passage on susceptible rabbits

The objective is to check the HAT profile of these isolates after each passage. This test was done using two different concentrations (10 % and 20 %) of liver homogenate at 4ºC after two passages on susceptible rabbits. The HAT of original samples and 1st passage of (R1, R2, R4, R18 and R49) were previously done (Salman et al., 2008), sample R2010 (Noah. 2012) and sample R2011 (El-Nahas. 2011). All the results of original samples and passages are summarized in table (2).

The results showed that the selected samples with HAT negative profile at both original and 1st passage have changed into positive HAT after the 2nd passage as (R2, R4, R18 and R49). R1, R2010 and R2011 isolates showed positive HA profile and persisted to be positive even after 2 passages. R2012 showed negative HA profile and persisted to be negative or of low HA capacity till 2nd passage.

Table (2): A summary of hemagglutination activity of RHDV different isolates and after first and second passages

Sample No.	Original	1st passage	2nd passage		Result
			10%	20%	
R1	14 HAU	13 HAU	8 HAU	8 HAU	positive
R2	-ve	-ve	13 HAU	14 HAU	variable
R4	-ve	-ve	15 HAU	15 HAU	variable
R18	-ve	13 HAU	4 HAU	6 HAU	variable
R49	-ve	-ve	13 HAU	14 HAU	variable
R2010	12 HAU	12 HAU	10 HAU	11 HAU	positive
R2011	7 HAU	NR	12 HAU	13 HAU	positive
R2012	3HAU	4 HAU	3 HAU	4 HAU	Negative

HAU: hemagglutinating units expressed as Log 2. R: not recrded, -ve: negative result 4 HAU (1/160) (**OIE. 2010**)

Table (3): A summary of hemagglutination activity of RHDV different isolates and two passages in correlation to the mortality pattern

Sample	HA of original sample	1st passage		2nd passage	
		DPI	HA	DPI	HA
R1	+ve	2nd	+ve	2nd	+ve
R2	-ve	5th	-ve	2nd	+ve
R4	-ve	5th	-ve	2nd	+ve
R18	-ve	5th	+ve	5th	+ve
R49	-ve	2nd	+ve	2nd	+ve
R2010	+ve	2nd	+ve	2nd	+ve
R2011	+ve	3rd	+ve	6th	+ve
R2012	-ve	6th	-ve	12th	-ve

DPI: days post inoculation

R18 and R2011 original samples were subjected for bad storage condition that may lead to decrease the virus titer leading to prolonged course of the disease.

The correlation between HA activity and mortality pattern table (4) showed that all studied HA negative isolates resulted in mortalities during the subacute/ chronic stage of the disease as no one showed mortality during the acute stage. The HA positive isolates mostly showed mortalities during acute stage and to lesser extent (only 2 samples) showed mortality during the subacute stage.

3.3. RT-PCR assay for RHDV isolates

A liver The purified total cellular RNA extracted from fresh liver samples of RHDV isolates after first and second passage on susceptible rabbits was used for cDNA synthesis by reverse transcriptase (RT step) followed by PCR for amplification of 600 bp fragment of VP60 structural gene using specific primers flanking regions (C-E) in VP60. These regions are known as highly variable region and it represent the P2 subdomain which is embedded in P1 subdomain in the C-terminal of VP60 gene.

Electrophoresis of the amplified products on 1% agarose gel Photo (1) revealed the presence of specific product at the correct size (600 bp) according to the standard marker. The negative control Lane (8) showed no amplification of any product.

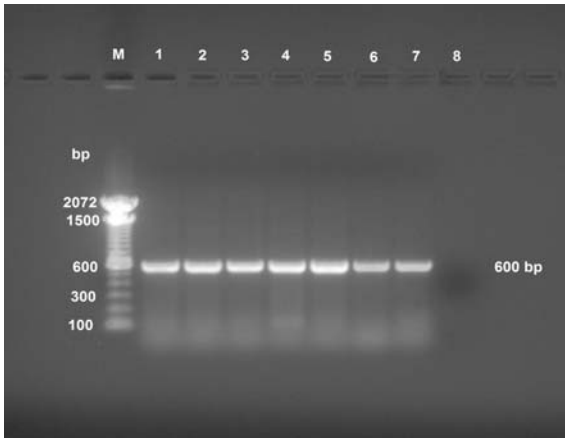

Photo (1): RT/PCR products of 600 bp fragment of VP60 gene using 1% agarose stained with ethidium bromide electrophoresis Lane M: 1Kb DNA ladder marker, Lane 1: Giza 97 (R1), Lane 2: KS 2000 (R2), Lane 3: Kal 2000 (R4), Lane 4: Kal 2005 (R49), Lane 5: Giza 2010 (R2010), Lane 6: Kal 2011 (R2011), Lane 7: Kal 2012 (R2012) and Lane 8: negative control (normal liver).

4. DISCUSSION

The Hemagglutination test using type O human RBCs were the first test to be used for routine laboratory diagnosis of RHD (Du. 1990) as most of RHDV strains have hemagglutination activity. Liver samples were collected from different suspected RHDV outbreaks in different localities in Egypt and HAT was performed at the recommended temperature 4°C (OIE, 2010).

The results presented in this study table (3) showed that R1 (Giza 1997), R2010 (Giza 2010) and R2011 (Kal 2011) isolates showed positive HA profile and persisted to be positive even after 2 passages in susceptible rabbits.

Surprisingly, many selected samples that showed HAT negative profile at both original liver suspension and after 1st passage have changed into positive HAT after the 2nd passage as R2 (KS 2000), R4 (Kal 2000), and R49 (Kal 2005) exceptR18 (Kal 2002) that changed into positive HAT after the 1st passage. So, these isolates are better to be called with variable HA profile agreeing with the results of German Hagenow strain in previous study that has variable HA profile as it showed no hemagglitonation activity either at ambient or at 4ºC, but after two additional passages in rabbits, the prolonged disease course (6 days) has disappeared and the hemagglutination activity as well as ELISA reactivity were regained (Schirrmeier et al., 1999).

In Egypt, a previous study demonstrated the existence of non-haemagglutinating RHDV in the Egyptian environment as when 25 samples from both HA positive and HA negative isolates were passaged on susceptible rabbits, the HA activity showed different patterns; 64% of samples kept their HA activity without change either the positive or negative isolates while 24% had changed from HA negative to positive. Also there were 12% changed from HA positive to negative (Salman et al., 2008).

Another pattern was found as R2012 (Kal 2012) table (3) showed negative HA profile and persisted to be negative or with low HA capacity till 2nd passage by using either 10% or even 20% liver homogenates showing similar results with many authors worldwide who reported the existence of HA negative strains as Italian Pv97 (Capucci et al., 1998) German Frankfurt (Schirrmeier et al., 1999), Spanish Asturias (Prieto et al., 2000), Chinese Whn-1 (Tian et al., 2007) and Polish BLA &OPO (Fitzner et al., 2012)either at different temperature profile or after several passages and they all confirmed being RHDV positive strains using other virological methods as Immuno electron microscopy, ELISA and western blot. Rainham strain in England was considered a variant strain as it failed to haemagglutinate in standard conditions at 37ºC (Chasey et al., 1995) but it was HA positive at 4ºC (Capucci et al., 1996). Then western blot and IEM of Rainham strain showed that it was antigenically and morphologically indistinguishable from other HA positive strains suggesting that it may differ by only a few amino acid changes in its capsid protein sequence (Capucci et al., 1996).

Also a previous study recorded 7 out of 22 Egyptian outbreaks were caused by variant strain of RHDV as they lacked hemagglutinating (HA) activity (El-Sissi and Gafer 2008).

Owing to the low level of sequence variation among RHDV isolates and the high

sensitivity of PCR, reverse transcription (RT)-PCR represents an ideal rapid diagnostic test for RHD (OIE, 2010).Also the application of the RT-PCR to the detection of RHDV-specific nucleic acid has been described by several authors (Gould et al., 1997 and Guittre et al., 1995).

The study confirmed whether these isolates were RHDV positive and checked the intact presence of highly variable regions (C and E) at P2 domain in the C-terminal part of VP60 by RT/PCR technique as a sensitive method for detection using specific primers flanking C- E regions of VP60 that produced 600 bp fragments.

The results showed all isolates either HA positive or negative or even variable HA profile were all positive for RHDV, moreover they all showed intact sharp band at size corresponding to 600 bp without any signs of degradation or truncation photo (5) agreeing with previous work of (Schirrmeier et al., 1999), (Salman et al., 2008), [36] as well as another work showed no degradation of VP60 protein or gene using RT/PCR technique (El-Sissi and Gafer 2008).

Many hypotheses tried to explain the differences between HA positive and HA negative strains. The first trial to explain depended on western blot. The results of HA positive samples showed intact VP60 polypeptide weighing approximately 60 KDa while HA negative samples showed a proteolytic degradation of VP60 that might be due to the autolytic process in the carcass or due to the pathogenic evolution of the disease. Also the IEM showed a high percentage of viral particles that were approximately 23-25 nm in diameter (Capucci et al., 1991).

This theory was disproved by the results of many workers as the first found that VP60 was largely predominant in Pv97 HA negative strain (Capucci et al., 1998), a second study showed that the major polypeptide was of 55 KDa in both positive and negative HA Egyptian isolates (Salman et al., 2008). While another stated that the lack of HA is not a consequence of the capsid protein degradation as HA negative isolate exhibited the VP60of 60 KDa (El-Sissi and Gafer 2008).

Another hypothesis was explained that RHDV infected rabbits with protracted course of disease and negative HA activity at the standard condition is due to Core like particles (CLPs) that were detected in other samples with low HA titer. CLPs were characterized by smooth surface and smaller diameter (25-27 nm) while virus samples from rabbits with typical RHDV peracute course contained only virions with characteristic morphology (32- 40 nm) suggesting that the production of CLPs appeared to result from incomplete expression of viral genome rather than proteolytic digestion supposing if only the N-terminal half of the VP60 is expressed, CLPs lacking the biological activities of the C-terminal half of VP60 was formed (Granzow et al., 1996). Our RT/PCR results disagree with this theory as the highly variable regions (C and E) at P2 domain in the C-terminal part of VP60 did not show any signs of degradation or truncation at photo (5).

Also we disagree with this theory for two reasons. The first that HA negative isolates were performed at room temperature so these samples may show another HA profile as Rainham strain if HAT was performed at 4ºC (Capucci et al., 1996). The second that

CLP preparations were never absolutely free from complete RHDV and it was not possible to determine the infectivity of pure CLPs or to find any morphological evidence for transitional stages between intact particles and CLPs (Granzow et al., 1996).

Moreover, other authors disagreed this theory by a conclusion that VLP (T = 1) particles are produced if the N-terminal residues of the capsid are deleted or disordered (Laurent et al., 2002). Also another author disagreed with this hypothesis as they found that native viral particles and virus-like particles from the recombinant virus capsid protein specifically bound to synthetic A and H type 2 blood group oligosaccharides (Ruvoen-Clouet et al., 2000).

The third hypothesis identified degraded viral particles (small, smooth RHDV particles) in the liver of a rabbit with HA negative activity and considered it as a marker of the subacute /chronic form of RHD that usually evolves between 4 and 8 days post-infection and is followed either by the death of the rabbit or, more often, by its recovery suggesting that the genesis of the particle is due to a degradative process which is probably the consequence of physiological clearance of the RHDV-IgM immuno-complex formed in large amounts at the beginning of the humoral response (Barbieri et al., 1997). So it means the combination of the two former theories (Granzow et al., 1996 and Barbieri et al., 1997) claiming that CLP or smooth RHDV particles is responsible for the negative HA activity.

These theories was disproved by the results of many workers either who mentioned that investigations of electron microscopy and western blot showed that a possible predominance of CLP's was not the major reason for HA negative results (Schirrmeier et al., 1999) or the other study that isolated (whn-1) in China with HA negative activity at 25, 37 and 4ºC even after two passages in experimentally infected rabbits with morbidity rate and mortality rate 100% within 24–48 h DPI during the acute stage. Also the negatively stained particles of Whn-1 isolate were approximately 35 nm in diameter by electron microscope (Tian et al., 2007).

A recent explanation for VLP was presented by the 3D structure of the intact virion and core-like particles that confirmed the fact that the CLP was just produced from intact virion but with dissociated P domain at the jungle domain. They added the existence of CLP in all caliciviruses species not only RHDV (Hu et al., 2010).

So, OIE has recommended to perform HAT at 4ºC to avoid HA false negative thus this test has been replaced by virus detection ELISA (OIE, 2010).

The correlation between HA activity and mortality pattern table (4) showed that all studied HA negative isolates resulted in mortalities during the subacute except the HA positive R2012 (Kal 2012) extended to the chronic stage of the disease after the second passage at young age only but no HA negative showed mortality during the acute stage.

These findings were supported by previous studies reported that HA positive samples had higher mortality rate up to 80% within 48-72 hrs, while HA negative samples had up to 60% only within 5-6 days post inoculation (Salman et al., 2008). This may be explained to be dependent upon the size of the initial viral inoculum as the acute

infection may be a result of a 'large' inoculum received from an acute-infected rabbit leading to rapid progress, usually before sufficient production of antibodies to control the infection. But when a smaller inoculum from a chronically infected rabbit would allow more time for the host immune system to respond before fatal liver damage regardless it's HA activity (White et al., 2002).

The present study showed also a correlation between the mortality rate and the age of RHDV affection and year of isolation. At 1997 mortality rate was 100% at adult age these rates were descending till 0% from 2010 and upward. Meanwhile the mortality rate in young age at 1997 was 0% but it was ascending gradually till reaching 100% at 2011. These data showed no correlation between the mortality rate and age of RHDV affection with the hemagglutination activity.

From all presented data, it is clear that until now there no satisfactory theory explaining the variable or negative HA profiles as they have no clear origin or specific mechanism. We only can suggest that HA negative isolates may recognize other cell receptor at the rabbits other than that recognized at the human RBCs type O.

So, the conclusion is that HAT test is not reliable in diagnosis or typing of RHDV field isolates as most of the researches did not include HAT in its protocols or search for it anymore and another preliminary diagnostic method should be designed.

The most important point is to find out an interpretation for the emergence of RHDV in young age (Kal 2011) (El-Nahas. 2011) and R2012 (Kal 2012) presented in this study, as one striking characteristic of the pathogenesis is the resistance of young rabbits less than 2 months of age to RHD (Xu et al., 1989). Also an outbreak in Spain was recorded to affect young rabbits less than30 days with mortality rate up to 20% and 50% in adult and young respectively (Dalton et al., 2012). This RHDV-N11 showed no agglutination of blood groups O or A, but did agglutinate blood groups B and AB, so they used multiple sequence alignment and phylogenetic analysis of VP60 gene that showed this isolate is genetically distant from classical and variant RHDV and it is more closely related to the so called RCV a pathogenic virus (Dalton et al., 2012).

Conclusion

There is no correlation between the HA activity and genetic typing of RHDV either classical or variant strains. Also many questions have risen about these isolates types and whether it depends on period or geographical distribution in Egypt as well as the appearance of RHDV at young age. So, for real classification and tracking the genetic revolution of Egyptian isolates, further investigations at molecular level will be needed depending on sequence and phylogenetic analysis of highly variable region of VP60 gene.

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المعزولات المصرية السالبه لإختبار التلازن الدموي لفيروس النزف الأرنبي يمكن ان تتحول إلى متغيرة التلازن الدموي.

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الملخص العربي

مرض النزف الأرنبي الفيروسي واسع الأنتشار ويسبب ارتفاع عدد الوفيات.كما أظهرت الدراسات السابقة ان عترات فيروس النزف الأرنبي المنتشرة في المحافظات المصرية المختلفة سالبة وموجبة لإختبار التلازن الدموي. لأول مرة يتم الكشف عن وجود معزولات لفيروس النزف الأرنبي متغيرة لإختبار التلازن الدموي وذلك بعد تمريرها في أرانب قابلة للعدوى بهذا الفيروس. وقد تم التأكد من وجود الفيروس بعد التمرير باستخدام تفاعل النسخ العكسي/تفاعل البلمرة المتسلسل في المنطقة عالية التباين (C–E) للجين الخاص ببروتين 60. نستنتج من ذلك ان اختبار التلازن الدموي لا يمكن الأعتماد عليه في تشخيص أو تصنيف فيروس النزف الأرنبي سواء من النوع الأصلي أو النوع المتباين. لذلك فإنه من المطلوب تتبع التطور الجيني في المعزولات المحلية واجراء بحوث اضافية على المستوى الجزيئي عن طريق تحليل التتابع النيوكليوتيدي والفايلوجيني للمنطقة عالية التباين للجين الخاص ببروتين 60.

(مجلة بنها للعلوم الطبية البيطرية: عدد 26(2):71- 83, يونيو 2014)

Egyptian non-HA isolates of RHDV can change to variable HA profile