Studies On Factors Affecting Haemagglutinating Character Of Rabbit Haemorrhagic Disease Virus

Owess G Affan, Hala M M El-Makaky and Sherif M Ibrahim
Veterinary Serum and Vaccine Research Institute, Abbasia, Cairo

ABSTRACT

Rabbit hemorrhagic disease (RHD) is a highly contagious, per acute and acute fatal viral disease of rabbits caused by a haemagglutinating, enveloped virus, with a single stranded RNA genome, belonging to Lago virus of the Caliciviridae. While HA and HI tests are gold standards for detection of RHD virus (RHDV) in liver tissues used for preparation of the inactivated vaccine, disappearance of this phenomenon at chronic stages of the disease directed this study to thoroughly investigate some factors which could affect HA titer in different steps of vaccine preparation. In addition, reverse transcription and polymerase chain reaction (RT-PCR) was used as a sensitive tool to detect the presence of RHD viral RNA in livers collected from rabbits at chronic stage of infection and, at the same time, negative in HA test. Experimental infection of susceptible rabbits with the locally isolated RHDV GIZA-2006 strain with a $10^{4.65}$ LD_{50}/ml titer and a haemagglutinating (HA) titer equal to 10^{14} HA units, was carried out. Liver samples were collected at different stages of the disease, 20 hours to 6 days post infection, as the clinical evolution of the disease can be peracute, acute, subacute or chronic. The haemagglutinating activity was measured for these liver samples using HA test. This study cleared that, increasing freezing and thawing more than 5 times decreased HA units (HAU) RHDV do not undergo illusion and can read HA test after 45 minutes and 24 hours after incubation and using plates with V button shaped wells to perform HA test gave more accurate results than those with U button shaped wells. While addition of antibiotic or antifungus had no effect on the obtained HAU, storage of the virus as liver suspension over 10 days in +4°C declined the obtained HAU. In addition, Using RT-PCR, a 530 base pair fragment of the RHDV Vp2 gene was detected in HA negative liver tissues collected at chronic stage of infection could indicate detection of RHD virus like particles as mentioned before. The obtained results could help to improve RHDV vaccine in future.

INTRODUCTION

Rabbit hemorrhagic disease (RHD) is a highly contagious, highly fatal, peracute and acute viral disease of both wild and domestic rabbits. The disease is characterized by a short course 2-3 days, high levels of morbidity (100%) and mortality (80-90%), severe signs (dyspnoea, anorexia, depression, convulsions and epistaxis) with severe necrotizing hepatitis *(1,2)*. The disease is caused by rabbit haemorrhagic disease virus (RHDV), a member of Lagovirus of the *Caliciviridae* family *(3,4)*.

Caliciviridae infection is the major cause of the severe disease in the stocks of wild and farm rabbits that has occurred world wide during the last two decades *(5)*. The clinical evolution of the disease can be peracute, acute, subacute or chronic *(6)*. In acute form, the rabbits showed severe depression, anorexia, dyspnoea with foamy bleeding from nostrils up to suffocation. Some rabbits developed nervous manifestations in the form of severe convulsion, in coordination and paralysis (in few cases before death). Sometimes, the animals showed a back bend head position and crying before die. Most affected rabbits died within 36-48 hours and occasionally 72 hours from the onset of the disease *(7)*.

RHD viral RNA was persisted for at least 15 weeks after experimental infection of rabbits. Accumulation of RHDV RNA was found in liver and spleen, which are the major target organs of RHDV *(8)*. Haemagglutination test (HA) using human RBCs type "O" is the primary test for routine laboratory diagnosis of RVHD *(9)* and

micro HA and HI tests were used extensively owing to their sensitivity, specificity and simplicity for diagnosis RVHD. While viral RNA and antigens were detected in the liver of 4-5 week old rabbits that subcutaneously inoculated with RHDV at 36 hrs intervals PI using HA test, the RT-PCR showed that RHDV RNA was present in the liver as early as 18 hs PI. *(10)* indicating sensitivity of RT-PCR in viral RNA detection. In addition, RT-PCR has been employed successfully for investigative studies *(1)*.

While HA and HI tests are gold standards for detection of RHDV in liver tissues used for preparation of the inactivated vaccine, disappearance of this phenomenon at chronic stages of the disease directed this study to thoroughly investigate some factors which could affect HA titer in different steps of vaccine preparation. In addition, reverse transcription and polymerase chain reaction (RT-PCR) was used as a sensitive tool to detect the presence of RHD viral RNA in livers collected from rabbits at chronic stage of infection and, at the same time, negative in HA test. Of the factors investigated at this study are the number of freezing and thawing multiples, incubation period for reading HAT, the use of V or U-shape micro-haemagglutinating plate, addition of antibiotic and antifungal like gentamycine and nystatin and the duration of storage in +4°C.

MATERIAL AND METHODS

Rabbit haemorrhagic disease virus (RHDV)

Local Egyptian strain of RHDV designated as Giza-2006 *(11)* with a titer of $10^{4.65}$ LD_{50}/ml and of haemagglutination (HA) titer equal to 2^{14} HA unit was used for experimental infection of rabbits.

Experimental rabbits

Three to four months old, industrial hybrid rabbits with an average body weight of 2Kg were purchased from a conventional rabbitry. All rabbits were free from detectable RHDV antibodies. The rabbits were used for experimental infection with RHDV subcutaneously. Livers from freshly dead rabbits were collected at different intervals for 6 days post inoculation. Liver specimens were mechanically homogenized in 10% (w/v) PBS (pH 7.2), filtered through cheese cloth and, clarified by centrifugation at 5000 x g for 15 minutes before testing using rapid slide and plate HA test at +4°C then stored at-20^{0}C till used.

Antibiotics

Nystatin suspension (sigma, USA), was added at a concentration of 0.1ml/1ml liver homogenate. Gentamycin suspension (Egyptian Int. pharmaceutical Industries co. E.I.P.I.CO.) of 80mg/2ml concentration, was added as 0.5ml/4.5ml of liver homogenate.

Slide haemagglutination test

Liver specimens were tested according to the technique described elsewhere *(12)*. Briefly, one drop of washed 10% human type "O" from healthy volunteer RBCs was mixed with one drop of the liver extract on a glass slide. The drops were mixed and tilted several times. The presence and degree of agglutination was recorded within one minute.

Standard quantitative microhaemagglutination test

The test was carried out according to *(13)* as well as *(14)* . Briefly, two-fold dilutions of the liver extracts were incubated with an equal volume (50 µl) of washed 0.75% human RBCs type "O" in a sealed round-bottom micro-titer plate at two different temperatures. Lattice and button shapes were recorded. Reciprocal of the end dilution (last well) giving complete HA was considered the end titer.

Detection of RHDV genomic RNA in liver tissues

For cDNA synthesis and amplification of the 530 bp target RHDV Vp60 fragment from liver RNA extracts, total cellular RNA was extracted from liver tissues that collected from rabbits at chronic stage of infection and showed negative HA results, using SV total RNA isolation system (Promega, USA) following manufacturer procedure. To perform RT-PCR, the QIAGEN® One-Step RT-PCR kit (Qiagen, Germany) and a specific primer pair, designed using Oligo7™ software, were used [RHDV-DF (5'- TTg CCg ACA TTg ACC ATC gAA -3') and RHDV-DR

(5'- CCT gAC AAC AgA CGC gAA C -3')]. Briefly, 25 µl RT-PCR reactions were performed on ice in 0,2 ml nuclease-free PCR tubes with the following components added: 5 µl of 5x QIAGEN One-Step RT-PCR buffer (1x final concentration), 1 µl of 10 mM dNTPs mix (400 µM), 0,75 µl (0,6 µM final conc.) of 20 Pmole concentration specific forward and reverse primers (Bioneer, Germany), 1 µl QIAGEN® One-Step RT-PCR enzyme mix (Omniscript /Sensiscript reverse transcriptases and hotstart *Taq* polymerase), 1 µl viral RNA and nuclease-free deionized d.d. H_2O to 25 µl. The RT-PCR was carried out using a GeneAmp® PCR system 9700 thermal cycler (Applied Biosystems, USA). The cycling conditions were 30 minutes at 55°C for reverse transcription (1 cycle), 15 minutes at 95°C to activate the HotStart *Taq* DNA polymerase (1 cycle), 40 cycles each of 30 seconds at 95°C for denaturation of nucleic acid, 30 seconds at 58°C for primer annealing, 30 seconds at 72°C for chain extension, and one cycle at 72°C for 7 minutes for final extension. In parallel, two different positive controls (Vp60 cloned T-plasmid (Ibrahim et la., in preparation) and RNA extracted from positive control liver tissue that collected at acute stage of infection and showed positive HA result), were used in two different PCR and RT-PCR reactions, respectively, using the same primers to confirm the obtained results. The PCR amplicon size was determined by running 9 µl of the RT-PCR reaction in 1% Agarose gel stained with ethidium promide (0.5µg/ml) in parallel with a 100 bp GeneRuler™ 100 bp plus DNA ladder (Fermentas, Lithuania). Thereafter, gel was photographed using a starlight express MX516® 16-bit CCD camera under UV illumination and analyzed using gel documentation and analysis system supplied with an AAP-M5® software package.

RESULTS AND DISCUSSION

Samples from liver extract showed strong positive slide HA activity against human RBCs type "O" at intervals of 20 to 46 hrs post inoculation (PI), but samples collected 49 hrs to 6 days post inoculation gave -ve slide HA activity. Plate HA activity showed +ve results till 91 hrs PI (Table 1). These variations in HA activity of RHDV in samples of liver extract collected at different intervals after infection may be explained by the stage of the disease at which the samples were collected (per acute, acute, sub-acute or chronic). The loss of haemagglutinating activity of RHDV was associated with the chronic stage of RHDV ***(15)***. Samples agglutinated human type "O" RBCs with variable HA titers ranging from 12 HAU/50µl at 20 hrs PI to 4 HAU/50ul at 89 hrs PI, Table (1). Results were considered positive when having an agglutinating end point dilution of >1/16 (24 HAU) as mentioned in ***(13)***.

The effect of multiple freezing and thawing and period of storage at -20°C on the result of HA test for RHDV have been reported (Table 2).The HA titers were variable. At 2nd time of freezing and thawing, it was 12 HAU/50ml from 20 to 34 hrs PI, 13 HAU/50ul from 37 to 42 hrs PI and then declined to reach 6 HAU/50ul at 89 hrs PI. At the 4th round of freezing and thawing, HA titers ranged from 12 HAU/50UL to 10 HAU/50ul and reached 4 HAU/50ul at 120 hrs PI. At the 5th round of freezing and thawing HA titers elevated to 16 to 14 HAU/50ul in samples of liver extract collected from 20 to 40 hrs PI. These results could be explained by the nature of the virus as an intra-cellular replicating virus, where freezing and thawing of liver homogenates leads to rupture of hepatocytes and virus release leading to elevation of HA titers. When the freezing and thawing increased than 5 times the HA activity declined. These results agree with those of ***(1)*** who found that repeated freezing and thawing could scientifically reduce the agglutinating titers of RHDV. Table (3) revealed that reading of HA test after different times of incubation 45 minutes, 24 hours and 90 hours PI. After 45 minutes, HA reached 15 HAU/50µl at 27 hrs PI and decreased till reached minimum volume at 89 hrs PI. At 24 hours, HA results run in parallel while at 96 hrs PI, HA reached 14 HAU/50µl at 23 hr PI and 7 HAU/50µl at 49 hrs PI after that the samples undergo haemolysis. These results indicated that the RHDV do not undergo illusion and can read HA test or HI test after 24 hours. These results agree with ***(16)*** who proved that RHDV non-eluted from human "O" type.

Table 1. Haemagglutinating activity test (HAT) for liver extracts from rabbits inoculated with RVHD virus at different intervals 1st thawing

Samples of liver extract (hours P.I.)	HAT of liver samples	
	Slide	Plate at 4°C* (HAU)
20	+ve	12
22	+ve	12
23	+ve	12
27	+ve	12
29	+ve	12
31	+ve	12
34	+ve	12
37	+ve	12
40	+ve	12
42	+ve	12
46	+ve	12
49	+ve	12
52	-ve	12
65	-ve	10
89	-ve	9
91	-ve	4
120	-ve	2
137	-ve	4
6 days	-ve	Zero

HAT: Haemagglutinating test
-ve: negative
* Haemagglutination titers expressed as $\log_2$

Table (4) it was clear that HA value in V-shape plate was beginning with 12 HAU/50 µl from 20-27 hrs PI and reached its maximum 14 HAU/50 µl at 29 hrs PI, slight decline 10 HAU/50µl at 46 hrs PI till reached its minimum value 2 HAU/50 µl at 6 days PI. When using U-shape microtitre plate, it was noticed that HA value was beginning with 10 HAU/50 µl at 20 hrs PI and elevated till reached its maximum value 12 from 22 hrs-31 hrs PI. The HA value gradually declined 10-29 HAU/50 µl from 37-46 hrs PI and continue to reach its minimum value zero at 127 hrs PI. From the previous results indicated that using V-shape microtitre plate in HA test is preferred as it gave high and accurate results.

Table (5) comparing the HA value of liver extract when storage at 4°C for 9, 19 days and one month, it was clear that the HA value of liver extract stored at +4°C for 9 days was 12 HAU/50µl from 20-27 hrs PI and reached its maximum value 14 HAU/50 µl at 29 hrs PI, slight decline 10 HAU/50 µl at 46 hrs PI till reached its minimum value 4 HAU/50 µl at 6 days post infection. When liver extract stored for 19 days at +4°C, it was found that HA value was 7-8 HAU/50 µl from 20-29 hrs PI and reached its maximum value 12 HAU/50 µl at 31 hrs PI, gradual decreased till reached its minimum value zero at 120 hrs PI. While, liver extract stored for one month at 4°C the highest value was 8 HAU/50 µl at 31 hrs PI till reached zero at 89 hrs PI. It was clear that the highest value of HA was obtained when stored for 9 days at +4°C. These results were in disagreement with *(17)* who mentioned that the RHDV survived at least 225 days in an organ suspension kept at 4°C.

Reverse transcriptase polymerase chain reaction (RT-PCR) was used at this study, as a sensitive tool, to detect genomic RNA of rabbit hemorrhagic disease virus (RHDV) liver tissues. RHDV genomic RNA was detected in the examined liver tissue 7 days post infection (Fig. 1) giving an evidence for the presence of the virus like particles in liver tissues, at chronic stage of infection, even with negative HA results. Persistence of viral RNA after experimental infection of rabbits was proven before *(8)* and Presence of RHDV particles with diameters of 25–27 nm in Liver homogenates of rabbits with the protracted disease displaying no haemagglutinating activity, was mentioned *(15)*. The detected particles were assumed to be core-like particles (CLPs) arised from a truncated RHDV genome or defective expression, hence lacking the ability to agglutinate human RBCs. RT-PCR detection in liver samples could indicate the presence of viral RNA but not the complete virus particle. In addition, disappearance of the haemagglutinating activity in chronic stages of infection, indicates presence of incomplete viral particles that are lacking the HA activity present in the mature virion.

From over all results we can concluded that haemagglutinating test gave +ve results in slide till 49 hours post infection while in plate gave +ve results till 91 hrs. Increasing number

of freezing and thawing more than five times decreased the HA titers of HRDV. Storage of virus as liver suspension in +4°C not exceed more than 10 days after that HA titers of HRDV begins to decline and these is undesirable in vaccine preparation. Using of V shape micro-titer plate in HA test is preferred as it gave accurate results. Addition of antibiotics as gentamycin and nystatin not affect HA titers of virus, as it used as bactericidal and fungistatic. And finally, detection of RHDV in liver homogenates at late stages of experimental infection not a prove for the presence of complete virions.

Table 2. Plate (micro) haemagglutination technique (HAT) for liver extracts of rabbits inoculated with RVHD virus and collected at different intervals after storage at -20°C

Haemagglutination titers expressed as log_2					
Samples of liver extracts hours PI	**4th month storage at -20°C (2nd thawing)**	**5th month storage at -20°C (4th thawing)**	**6th month storage at -20°C (5th thawing**	**7th month storage at -20°C (6th thawing)**	**9th month after storage at -20°C (7th thawing)**
20	12 *	12	16	10	6
22	12	12	13	11	11
23	12	12	13	11	12
27	12	12	15	11	1
29	12	12	12	15	12
31	12	12	13	13	11
34	12	12	12	12	7
37	13	12	14	12	7
40	13	10	11	11	9
42	13	10	12	12	5
46	13	12	9	9	6
49	10	10	9	8	3
52	9	-	9	6	2
65	7	6	4	Zero	2
89	5	6	5	Zero	Zero
91	6	5	3	3	Zero
120	2	4	4	Zero	Zero
137	3	7	4	Zero	Zero
6 days PI	Zero	Zero	5	Zero	Zero

* HAU: Haemagglutination units / 50 µl

PI: Post inoculation

Table 3. Plate (micro) haemagglutination technique (HAT) for liver extracts of rabbits inoculated with RVHD (5th thawing) reading at different time of incubation

Samples of liver extract hrs P.I.	Haemagglutination titers expressed as log_2		
	45 minutes	24 hours	96 hours
20	16 *	16	16
22	13	13.5	13
23	13	14	14
27	15	15	12
29	12	12	13
31	13	13	13
34	12	12	14
37	14	15	16
40	11	11	12
41	12	13	13
42	9	11	9
46	9	10	9
49	9	9	7
52	4	6	6 +hemolytic.
65	5	5	4 +hemolytic
89	3	4	4 +hemolytic
91	4	hemolytic	8 +hemolytic
137	4	4	4 +hemolytic
120	5	5	4 +hemolytic
6 days P.I.	6	7	3 +hemolytic
Control	Zero	Zero	zero

* HAU: haemagglutinating unit /50µl

P.I.: Post Inoculation

Table 4. Plate (micro) haemagglutination technique (HAT) for liver extracts of rabbits inoculated with RVHD virus and collected at different intervals, storage in +4°C using V and U shape plate.

Samples of liver extracts hrs P.I.	Haemagglutination titers as log_2 at +4°C	
	Using V shape plate	Using U shape plate
20	12	10
22	12	12
23	12	12
27	12	12
29	14	12
31	12	12
34	10	8
37	11	10
40	10	9
41	-	-
42	10	9
46	10	9
49	7	7
52	7	8
65	6	4
89	2	3
91	4	5
120	4	5
137	6	Zero
6 days P.I.	2	3

* HAU: haemagglutinating unit /50µl

P.I.: Post Inoculation

Table 5. Plate (micro) haemagglutination technique (HAT) for liver extracts of rabbits inoculated with RVHD virus and collected at different intervals, after storage in 4°C for 9 , 19 days and one month.

Samples of liver extracts hrs P.I.	Haemagglutination titers expressed as log_2		
	9 days	19 days	Month
20	12	7	6
22	12	8	7
23	12	7	5
27	12	7	6
29	14	8	6
31	12	12	8
34	10	8	5
37	11	7	5
40	10	6	4
41	-	-	-
42	10	7	5
46	10	6	4
49	7	6	4
52	7	6	5
65	6	5	3
89	4	1	Zero
91	4	4	2
120	4	Zero	Zero
137	6	1	Zero
6 days	2	1	Zero
Control	zero	Zero	Zero

* HAU: haemagglutinating unit /50µl

P.I.: Post Inoculation

Table 6. Plate (micro) Haemagglutination technique (HAT) for liver extracts of rabbits inoculated with RVHD virus and collected at different intervals, after adding different antibiotics

Samples of liver extracts hrs P.I.	Haemagglutination titers expressed as log_2 at 4°C	
	Gentamycin 0.5 cm /4.5 cm virus	Nystatin 0.1 ml/1 cm virus
2	14	13
22	12	12
23	13	12
27	12	12
29	12	12
31	14	13
34	11	11
37	11	11
40	11	11
42	11	11
46	11	11
49	8	9
52	6	5
65	7	2
89	2	1
91	3	4
120	5	Zero
137	2	2
6 days P.I.	4	2

* HAU: haemagglutinating unit /50µl

P.I.: Post Inoculation

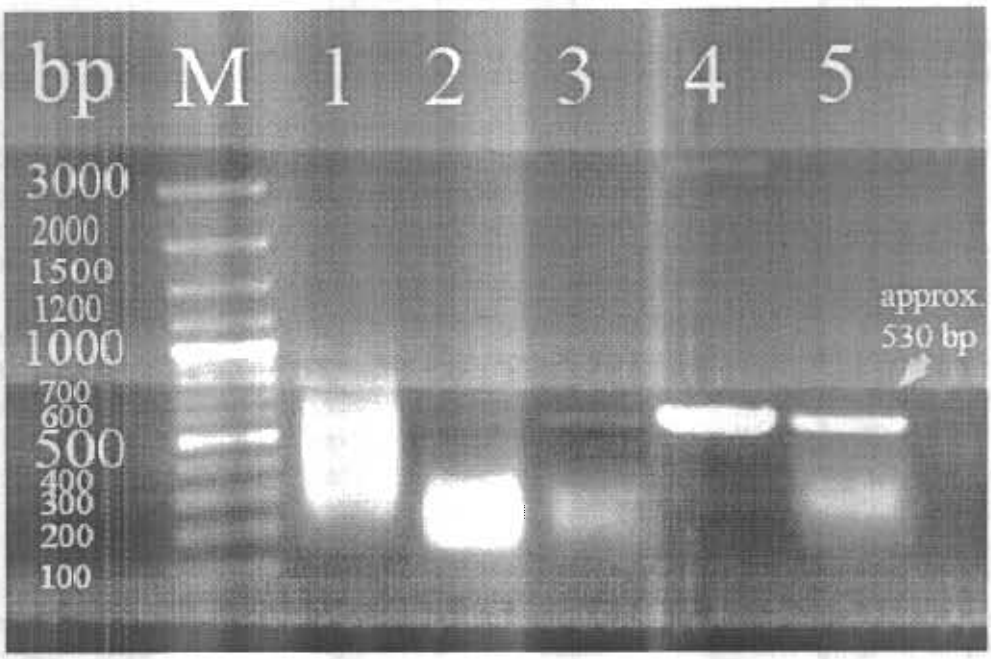

Fig.1 Detection of RHDV genomic RNA in liver tissues collected from rabbits at chronic stage of infection and showing negative HA results, using RT-PCR.

M = GeneRuler™ 100 bp plus DNA ladder (Fermentas, Lithuania). RT-PCR was performed on total cellular RNA extracted from liver tissues collected from rabbits at chronic stage of infection and showing negative HA results, lanes 1, 2, and 3 respectively. Two different positive controls (Vp60 cloned T-plasmid (lane 4) and RNA extracted from positive control liver tissue that collected at acute stage of infection and showed positive HA result (lane 5)) were used, in parallel, to confirm the obtained results. 1% agarose gel electrophoresis of the RT-PCR products revealed amplification of fragments of 530 bp approximate size in the three samples and positive cotrols, indicating detection of RHDV genome in the analyzed samples. Smear obtained at the 530 bp size in the sample in lane one could be due to using RNA template in high concentration in the RT-PCR.

REFERENCES

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

2.Peeters J E , Broes A and Charlier G (1990): First observations of viral haemorrhagic disease of rabbits (RHD) in Belgium. Annales de Med. Vet., 134 (8): 567-571.

3.Ohlinger V E , Haas B , Meyers G , Weiland F and Thiel H J (1990): Identification and characterization of the virus causing RHD. J. Virol., 64 (7): 3331-3336.

4.Parra F and Prieto M (1990): Purification and characterization of a calicivirus as causative agent of a lethal haemorrhagic disease in rabbits. J. of Virol., 64 (8): 4013-4015.

5.Ferreira P G ,Costa-E-Silva A , Oliveira M J , Monteiro E , Cunha E M and Aguas A P (2006): Severe leukopenia and liver biochemistry changes in adult rabbits after calicivirus infection. Res. Vet. Sci., 80 (2): 218-225.

6.Marcato P S , Benazzi C , Vecchi G ,; Galeotti M , Salda L dell Sarli G and Lucidi P (1991): Clinical and pathological features of viral haemorrhagic syndrome. Rev. Sci. Tech. off. Int. Epiz., 10 (2): 371-392.

7.El-Zanaty K (1994): Some investigation on rabbit viral haemorrhagic disease in Upper Egypt. Assuit Vet. Med. J., 30 (60): 293-305.

8.Gall A , Hoffmann B , Teifke J P Lang B

and Schirrmeier H (2006): Persistence of viral RNA in rabbits which overcome an experimental RHDV infection detected by a highly sensitive multiplex real-time RT-PCR. Veterinary Microbiology, No. of pages 16.

9.Capucci, L ; Scicluna, M T and Lavazza, A (1991): Diagnostic of viral haemorrhagic disease of rabbits and the European brown hare syndrome. Rev. Sci. Tech. Off. Inter. Epizoo., 10 (2): 347-370.

10.Shien H J , Shieh H K and Lee L H (2000): Characterization of rabbit haemorrhagic disease virus monitored by the polymerase chain reaction. Res. Vet. Sci., 68 (3): 255-9.

11.Salman, O G A (2007): Further studies on haemorrhagic viral disease in rabbits in Egypt. Ph.D. Vet. Thesis, Faculty Vet. Medicine, Cairo Univ.

12.Du, N X (1990): Rabbit haemorrhagic disease (RHD). A new disease and its viral etiology. Deutch Tierarztliche Wochenschrift, 97 (3): 114-116.

13.OIE (2008): Rabbit haemorrhagic disease. Manual of Diagnostic Tests and Vaccine for Terrestrial Animals, 2.6.2.: 947-961.

14.Capucci L , Chasey, Lavazza A and Westcot D (1996a): Preliminary characterization of a non-haemagglutinating strain of rabbit haemorrhagic disease virus from the United Kingdom. J. Vet. Med. [B], 43 (4): 245-250.

15.Granzow H , Weiland F , Strebelow H G , Liu C M and Schirrmeier H (1996): Persistence of viral RNA in rabbits which overcome an experimental RHDV infection detected by a highly sensitive multiplex real-time RT-PCR. Veterinary microbiology, No. of pages 16.

16.El-Mongy F A A (1998): Studies on viral haemorrhagic disease in rabbits. Ph.D. Thesis, Fac. Vet. Med., Zagazig Univ., Benha Branch, Moshtohor.

17.Smid B, Valicek L, Rojak L, Stepanek J and Jurak E (1991): Rabbit haemorrhagic disease; An investigation of some properties of the virus and evaluation of an inactivated vaccine Vet. Micro. Biol. 26 : 77-86.

المخلص العربى

دراسة العوامل المؤثرة على خاصية التلازن الدموي لفيروس مرض النزف الدموي في الأرانب

عويس جلال عفان - هالة محمد المتولي المكاكى - شريف محمود إبراهيم

معهد بحوث الأمصال واللقاحات البيطرية – العباسية - القاهرة

لذلك تمت دراسة هذه الخاصية وبعض العوامل التي مرض النزف الفيروسي في الأرانب مرض عالي الوبائية حاد وتحت الحاد ومزمن يتسبب بواسطة فيروس له غطاء أحادى الحمض الريبوزي النووي وله خاصية التلازن الدموي . حيث أن خاصية التلازن الدموي واختبار تثبيط التلازن الدموي هما المعيار الاساسى في تحديد الفيروس في أنسجة الكبد المستخدمة في تحضير اللقاح و اختفاء هذه الخاصية في الطور المزمن للمرض .

تؤثر فيه بالإضافة إلى استخدام اختبار النسخ العكسي / تفاعل البلمرة المتسلسل في معرفة وجود الفيروس في مستحلب كبد الأرانب المحقونة والتي أعطت نتائج سلبية في اختبار التلازن الدموي (في الطور المزمن للمرض) . تم حقن أرانب قابله للعدوى بفيروس النزف الارنبى عتره (٢٠٠٦ جيزة) وبعد ذلك تم تجميع عينات الكبد على فترات تطور المرض إبتداءاً من ٢٠ ساعة بعد الحقن ولمدة ٦ أيام.

أوضحت هذه الدراسة التالي: تعدد مرات الذوبان والتجمد أكثر من خمس مرات تقلل قوة تلازن الفيروس (HAU) وكذلك يفضل استخدام أطباق ذات قاعدة على شكل V أفضل من U حيث أنها تعطى نتيجة أفضل ، بينما استخدام مضادات الحيوية ومضادات الفطريات لا يؤثر على هذه الخاصية . تخزين الفيروس كمستحلب الكبد في ثلاجة +٤° م أكثر من عشرة أيام يقلل من قوه تلازن الفيروس. نتيجة اختبار البلمرة أوضح وجود أجزاء من الفيروس في كبد الأرانب المحقونة بة والمعطى نتيجة سلبية في اختبار قرة التلازن.