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HAEMAGGLUTINATION AND RT- PCR FOR FOLLOW UP OF RABBIT HEMORRHAGIC DISEASE VIRUS IN DIFFERENT TISSUES OF EXPERIMENTALLY INFECTED RABBITS

BY

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ABSTRACT

A total of 21 rabbits were inoculated I/N with local Egyptian strain of rabbit hemorrhagic disease virus (RHDV), Giza 2006 with a titer of $10^{4.65}$ LD_{50}/ml. One rabbit for each period was sacrificed at 0, 12, 18, 24, 36, 48, 72 ,96 and 138 hrs post-infection (PI), respectively from the inoculated rabbits. Tissue samples from liver, spleen, lung, kidney and thymus were collected. Haemagglutination (HA) test and reverse transcriptase- polymerase chain reaction (RT-PCR) were used for virus detection. The results revealed that by using HA test, the virus was detected in all tested organs beginning from 31 hr PI with highest titer in liver and spleen, then in lung, kidney and thymus, respectively. The RT-PCR was able to detect the virus as early as 18 hrs PI in liver and spleen, then lung at 24 hrs PI, whereas kidney and thymus were found to be positive started from 31 hr PI. Therefore, we could conclude that RT-PCR was more sensitive test for detection of RHDV than HA test. The heaviest concentration of RHDV was in liver and it is the best organ for detection of the virus.

Key Words: Rabbit Hemorrhagic Disease Virus, Haemagglutination (HA), Reverse transcriptase-Polymerase chain reaction (RT-PCR).

INTRODUCTION

Rabbit hemorrhagic disease (RHD) is a contagious, per-acute, acute and highly fatal disease of rabbits especially wild and domestic European rabbits (Oryctolagus cuniculus) **(Zheng Tao and Parkes,J.P. 2011).** It is causing high morbidity and mortality (up to 100%) in the adult rabbits **(Pu et al., 1985 and Cao et al., 1986),** where the infected rabbits die within 48-72 hours due to acute necrotizing hepatitis and haemorrhage **(Xu & Chen, 1989)**.

The first RHDV epidemic occurred in China 1984 (**Liu et al.,1984**) and since 1986,the disease has been reported in different European countries , leading to high economic losses **(Mitro & Krauss 1993 and Ohlinger et al.,1993)** then appeared in much of Asia , Europe and other parts of the northern hemisphere (**Morisse et al.,1991)**.

In Egypt, the RHD has been reported for the first time in 1991 in Sharkia governorate **(Ghanem and ismail, 1992)** then many outbreaks were recorded later on in other governorates.

RHD is caused by a virus, belonging to the Caliciviridae family (**Ohlinger et al., 1990 and Parra and Prieto, 1990)** and recently designated with European brown hare syndrome virus (EBHSV) as the type species of the genus Lagovirus (**Pringle, 1998 and V.van Regenmortel et al., 2000)** and all isolates recovered from different geographical locations characteristically show close homology in terms of antigenic determinants and belong to a single serotype **(Capucci et el.,1996**

Routine diagnosis of RHD is based on clinical signs and histopathological finding, as well as on a haemagglutination test (HA) using human RBCs type "O" **(Liu et al., 1984).** Also, the RHDV was detected by electron microscopy (**Smid et al.,1989;Park et al .,1992)**, ELISA **(Ohlinger et al., 1990 and Capucci et al., 1991)**, direct immune-fluorescence **(Rodak et al., 1990)** or peroxidase procedures **(Alexandrov et al., 1992 and Stoerckle-Berger et al., 1992)** in the liver , blood , spleen, lung , kidney, thymus, heart muscle, brain and intestine.

Recently, a highly sensitive reverse transcriptase–polymerase chain reaction (RT-PCR) test was established for RHDV-RNA detection in rabbit liver samples **(Guittre et al., 1995).**

So our work was planned to follow up rabbit hemorrhagic disease virus (RHDV) in different tissues of experimentally infected rabbits with RHDV using HA test and RT-PCR for RHDV detection.

MATERIALS AND METHODS

Rabbit haemorrhagic disease virus (RHDV):

Local Egyptian strain of RHDV designated as Giza 2006 **(Salman, 2007)** with a titer of $10^{4.65}$ LD_{50}/ml had HE963222 as accession no. in Gene Bank. This virus was kindly supplied from Newcastle Disease Department, Veterinary Serum and Vaccine Research Institute (VSVRI), Abbasia, Cairo, Egypt. It used for inoculation of experimental rabbits and for preparation of hyper-immune serum.

Preparation of hyperimmune serum against RHDV:

It was prepared according to **Green and Manson, (1992)** as follows: Three to four months old, cross breed, 2 rabbits were primary immunized with 0.5 ml complete adjuvant inactivated vaccine of $10^{4.65}$ LD_{50}/ml deeply into each of the thigh muscle, then immunization took place biweekly for a further 4 weeks but with 0.5 ml incomplete adjuvant inactivated vaccine. Each rabbit then boosted twice, with one week interval, intranasally and intramuscularly with virulent RHDV of 2^{14} HAU in a dose of 2ml, 1ml per each route. After 2 weeks from last challenge, blood collected aseptically from the ear vein. The serum was separated, placed in a sterile screw capped glass and heated in a water bath at 56 ºC for 30 minutes to destroy the non specific neutralizing activity of the sera. The obtained serum then titrated against 8 HAU of RHDV and kept at -20ºC till used.

Experimental inoculation of rabbits with RHDV:

Twenty one rabbits free from RHDV antibodies were inoculated I/N with Local Egyptian strain of RHDV (Giza 2006)**.** From the inoculated rabbits, one was sacrificed at each period of 0, 12, 18, 24,36,48,72 and 96 hrs post-infection (PI), respectively. Tissue samples from liver, spleen, lung, kidney and thymus were collected. Part of each sample was taken in dry bottle containing phosphate buffer saline (PBS) for HA and RT-PCR. Samples from two normal rabbits were included as negative control.

Haemagglutination (HA) test:

Clarified supernatants of a 10% tissue homogenate of liver, kidney, spleen, thymus and lung were used in HA technique according to **Capucci et al., 1996** as follows: 25 µl of saline was dispensed into a series of wells in a V-shaped microtiter plate from 1-12 wells. 25 µl of suspension of each sample (1/10 dilution) was dispensed in the first well only, and then mix the content. 25 µl from the first well was transferred to the second well, mix the content and then 25 µl from the second well was transferred to the third well and so on to well

number11. The last well was leaved as control. 25 µl of human RBCs type O of 0.75 % suspension was added in all the wells including the last one. The plated was shacked well to mix the reagents and incubate at room temperature and the plate was examined every 15 minute up to one hour. According to **OIE manual (2010)** the end-point dilution of > 1/160 is considered to be positive.

Reverse transcriptase-polymerase chain reaction (RT-PCR):

Total RNA extraction of RHDV from organs tissue:

Viral RNA extraction using genomic RNA extraction kit from tissue (Bioer cat # BSB 07 M1) was done according to **Sambrook et al., (1989).** Briefly, 30mg of the tissues were homogenized and clarified by centrifugation at 3000 rpm/5 min. the supernatant of infected and control tissues were then heated at 65 °C for 2 hours. After brief cooling, the lysis buffer was added and incubated at room temperature for 5 min. Neutralization was then done and the whole content was transferred to the spin column provided with the kits and centrifuged for 30 sec. at 14,000 rpm .The flow through was discarded and 600µl of washing buffer was add and centrifuge as before. Washing step was repeated, then spin column was transferred to a sterile microcentrifuge tube and the RNA was eluted with 50 µl of elution buffer and incubated for 5 min and centrifuged as before .The extracted RNA was stored at -80°C till used.

One step RT/PCR amplification of RHDV VP60 gene:

The extracted RNAs from infected and control tissues were used in amplification of the conservative portion of the VP60 gene using specific primers according to **Guittre et al., (1996).** The conservative region of the capsid gene was amplified by BioRT one-step RT-PCR done according to manufacture instruction. The reaction was done in a final volume of 25 µl **as follow**: 14.5 µl of RNase free water, 2.5 µl of 10X RT-PCR buffer, 4 µl of dNTPs mix, 1 µl of sense primer, 1 µl of antisense primer, 0.5 µl of AMV reverse transcriptase, 0.5 µl of Taq polymerase, 1 µl of RNase inhibitors and 6 µl of template RNA. The PCR had a step for revere transcription at 50°C for 30 min followed by an initial cycle of 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 50°C for 60 s, 72°C for 45s and a final elongation step of 72°C for 10 min.

Amplified product analysis by agarose gel electrophoresis:

This was done according to **Sambrook et al. (1989).**

RESULT

The study revealed that no clinical signs were appeared on the experimentally infected rabbits till 24 hrs PI. After 24 hrs PI, the experimentally infected rabbits showed anorexia with dullness, depression **(Fig 1)**, inability to raise their heads above(dropped head), nervous signs including jumping in the air, tremors, convulsions, paddling movement of limbs, opithotonus**,** and sharp cry and respiratory distress as dyspnea, shallow and rapid respiration, ataxia. Control rabbits showed no clinical signs till the end of the experiment.

Some infected rabbits were spontaneously died during the experiment period. Most of the dead rabbits were in a good body condition. RHDV start to induce visible PM lesions at 24 hours PI in form of enlarged and congested liver**,** spleen, kidney **(Fig 2)** and thymus. Pneumonia , oedema and petecheal to ecchymotic hemorrhages of lung **(Fig 2)**. The blood was found clotted in the main blood vessels.

RHDV could not be detected in all organs by HA test till 24 hours PI as according to **OIE manual (2010)** in which it was reported that agglutination at an end-point dilution of >1/160 is considered to be positive ($> 2^4$). Then it could be detected in all organs starting from 31 hour PI in a spontaneously dead rabbit in different titers. After that time, it was noticed that all examined organs of sacrificed and spontaneously dead rabbits gave +ve results in HA test till 71 hour PI where the thymus gave -ve results from 72 till 96hour PI **(Table 1)**.

RHDV was detected by RT-PCR as early as 18 hours PI in liver **(Fig 3)** and spleen**(Fig 4)** of experimentally infected rabbit and could not be detected in lung, kidney and thymus of the same rabbit. At 24 hours PI the virus could be detected in liver and spleen in addition to lung of sacrificed rabbit and –ve results were obtained with kidney and thymus. At 31, 32, 33 and 34 hours PI, 4 rabbits were died spontaneously. The organs of rabbits that died at 31and 34 hours PI were tested and all gave +ve results. The virus was continued to be present till 96 hours PI. In the negative control samples no amplification was detected **(Table 1)**.

DISCUSSION

In this study, The characteristic clinical picture of RHD, in experimental infected rabbits including sudden death without clinical signs were observed, that finding agree with that recorded by **Liu et al., (1984); An et al., (1988); Smid et al., (1989); Xu and Chen (1989); Du (1990); Nowotny et al., (1990); Salem and El-Ballal (1992); Chasey et al. (1994); El-Zanaty (1994), Salman, (1999) and Abd El-Ghaffar et al., (2000)**. Anorexia with dullness and depression were observed. That result as mentioned by **Liu et al., (1984); Gregg et al. (1989); Smid et al. (1989); Du (1990); Glavits et al.,(1991); Salem and El-Ballal, (1992) ; El-Zanaty (1994) and Kimura et al., (2001).** Also nervous signs including tremors, convulsions, opithotonus, and sharp cry before death were seen as observed by **El-Zanaty, (1994); Ramiro-Ibanez et al., (1999); Salman, (1999) and Abd El-Ghaffar et al., (2000)**.

The recorded PM findings in our experiment showed that most of the dead rabbits were in a good bodily condition. Similar finding was recorded by **Guning and Proud, (1994).** This finding is due to very short and rapid course of the disease. Marked enlargement of liver (hepatomegaly), friable and may be congested with dark red in color in some cases or pale in others, similar findings were observed by **Guning and Proud, (1994) and Abd El-Ghaffar et al., (2000)**. The spleen was severely enlarged (splenomegaly), similar findings were recorded by **Gregg et al., (1989)**; **Soltysiak and Michalska, (1989); Du, (1990); El-Zanaty, (1994) and Kimura et al., (2001).** The blood was found clotted in the main blood vessels as aortic artery and caudal vein. This agrees with **Ramiro-Ibanez et al., (1999)** who found severe thrombotic obstruction in the principal blood vessels, most frequently in pulmonary, suprahepatic and aortic branches and **Abd El-Ghaffar et al., (2000)** who found that large blood vessels contained clotted blood in rabbits affected with RHD.

Our results revealed that RHDV could not be detected in the tested organs when examined by HA till 24 hours PI and this agree with finding of **Shien et al., (2000) and Hasanin, (2007).**

At 31 hours PI, RHDV was detected in all examined organs with titre ranged from (2^5 - 2^6) in spontaneously dead rabbit and at 36 hours PI in sacrificed rabbit that in agreement with **Shien et al., (2000) and Hasanin, (2007)** who found that the first detection of the virus by HA was at 36 hours PI in sacrificed experimentally infected rabbit.

The highest HA titer (2^{13}) was found in liver of rabbit at 80 hours PI followed by spleen (2^{11}) then lung, kidney and thymus with a titer of 2^8. This was because the liver is the target organ of RHDV, and so it considered the organ of highest virus concentration. Our result agreed with that of **Zhao et al., 1988 and Abd El-Mottelib, 1993** that proved that liver contain the highest concentration of the virus. Also, **Nowotny et al. (1990)** detected large quantities of RHDV in the bile. This also agree with **Galemetti et al., (1998)** who found that RHDV replication occurred almost immediately after inoculation and liver appeared to be the main site of replication. These results are in concurrence with **Salman, (1999)** who found that the highest virus concentration was in liver followed by kidney, spleen and lung when infect rabbits intranasal. Also, in agreement with **Abd El -Motelib (1993)** who found the HA titres of various tissues of rabbits which had dead of experimental infection were in the following order liver>spleen>lung.

Our results for RT-PCR confirmed that RT-PCR is the most sensitive method for detection of RHDV where the virus was detected as early as 18 hours PI in liver and spleen sacrificed rabbits and this came in a full agreement with **Guittre et al., (1996)** who found accumulation of RHDV RNA was found in liver and spleen, which are the major target organs of the virus.

In lung, RHDV RNA was detected as early as 24 hours PI in sacrificed rabbits and not before. This result is in a full agreement to results of **Shien et al., (2000).** Our negative results for virus detection by RT-PCR in lung of experimentally infected rabbits at first 18 hours PI disagree with the results of **Guittre et al., (1996)** where they could detect RHDV RNA in lung even at 0 hour PI.

When kidney of experimentally infected rabbits were tested by RT-PCR for detection of RHDV RNA, it was noticed that, RHDV RNA was detected late at 36 hour PI in sacrificed rabbits and not before and this result matched that of **Guittre et al., (1996)** who could detect RHDV RNA in kidney at 36 hours PI in one of 2 killed experimentally infected rabbits. While our results disagree with that of **Shien et al., (2000)** who could detect RHDV RNA in kidney as early as 26 hours PI.

RHDV RNA could not be detected in thymus gland of sacrificed experimentally infected rabbits till 24 hours PI and started to be positive at 36 hr PI agree with results of **Guittre et al., (1996) and Shien et al., (2000)** where RHDV was detected in thymus as early as 36 and 30 hour PI. In spontaneously dead experimentally infected rabbits, RHDV RNA was

detected in thymus gland of all rabbits starting at 31 hours PI. This result could not match result of **Guittre et al., (1996)** who also detected RHDV RNA in spontaneously dead rabbits but at 48 hours PI.

From this study, it could be concluded that,

1- RT-PCR was the more sensitive test for detection of RHDV than HA test.

2- It was proved that the heaviest concentration of RHDV was in liver and it is the best organ for detection of the virus.

Figures:

1- Experimentally infected rabbits with RHDV (Giza 2006 strain) showed ataxia, severe dullness, depression and reluctance to move.

2- Experimentally infected rabbit showed petecheal to ecchymotic haemorrhages in Lungs, enlarged friable and pale in colour liver with Petecheal haemorrhages were scattered all over the liver. Sever congested, dark bluish in colour, enlarged and friable spleen. congested and enlarged kidneys.

3- Detection of PCR-amplified RHDV cDNA in liver samples of experimentally infected rabbits: Lane (M): DNA marker from 100-1000 base pair - Lane (1): Negative control organ sample - Lane (2): Liver sample of rabbit at 18 hour PI - Lane (3): liver sample of rabbit at 24 hours PI - Lane (4): liver sample of rabbit at 31 hour PI - Lane (5): Liver sample of rabbit at 36 hour PI - Lane (6): Liver sample of at 42 hour PI - Lane (7): Liver sample of rabbit at 12 hour PI (negative) - Lane (8): Liver sample of rabbit at 48 hour PI - Lane (9): Liver sample of rabbit at 72 hour PI - Lane (10): Liver sample of rabbit at 80 hour PI - Lane (11): Liver sample of rabbit at 96 hour PI - Lane (12): Liver sample of rabbit at 138 hours PI (negative).

4- Detection of PCR-amplified RHDV cDNA in spleen samples of experimentally infected rabbits: Lane (M): DNA marker from 100-1000 base pair - Lane (1): spleen sample of rabbit at 18 hours PI - Lane (2): spleen sample of rabbit at 24 hour PI - Lane (3): spleen sample of rabbit at 31 hours PI - Lane (4): spleen sample of rabbit at 36 hour PI - Lane (5): spleen sample of rabbit at 39 hour PI - Lane (6): spleen sample of rabbit at 42 hour PI- Lane (7): spleen sample of rabbit at 48 hour PI - Lane (8): spleen sample of rabbit at 63 hour PI - Lane (9): spleen sample of rabbit at 72 hour PI - Lane (10): spleen sample of rabbit at 80 hour PI - Lane (11): Spleen sample of rabbit at 96 hour PI- Lane (12): spleen sample of at 136 hours PI.


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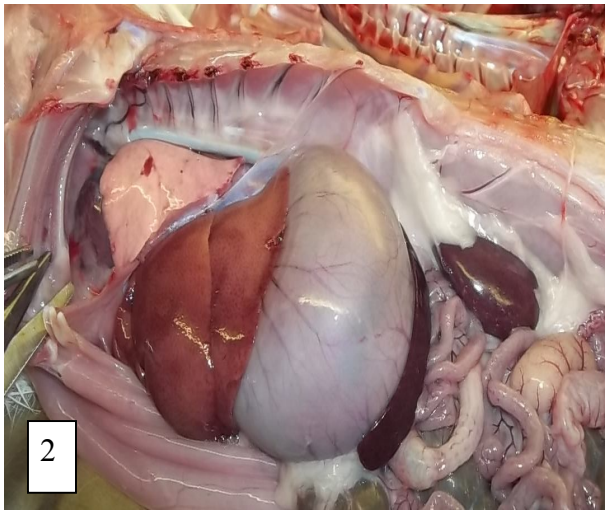
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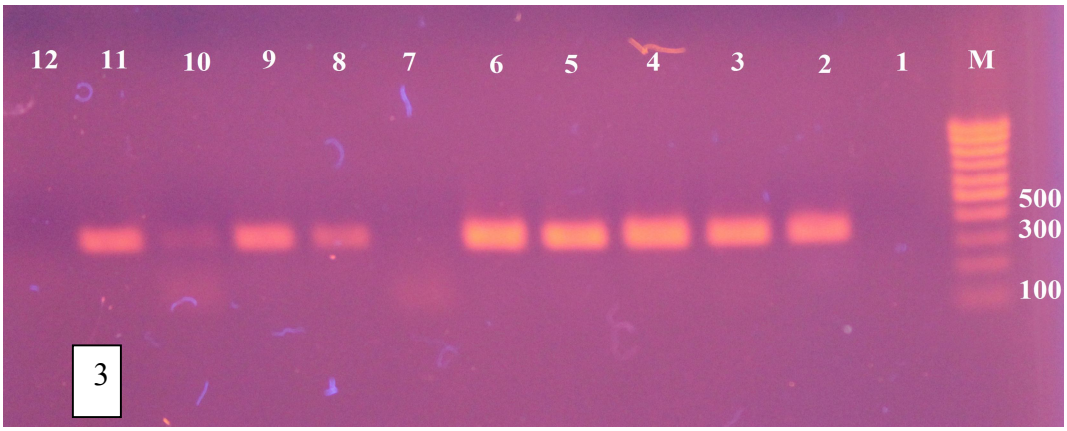
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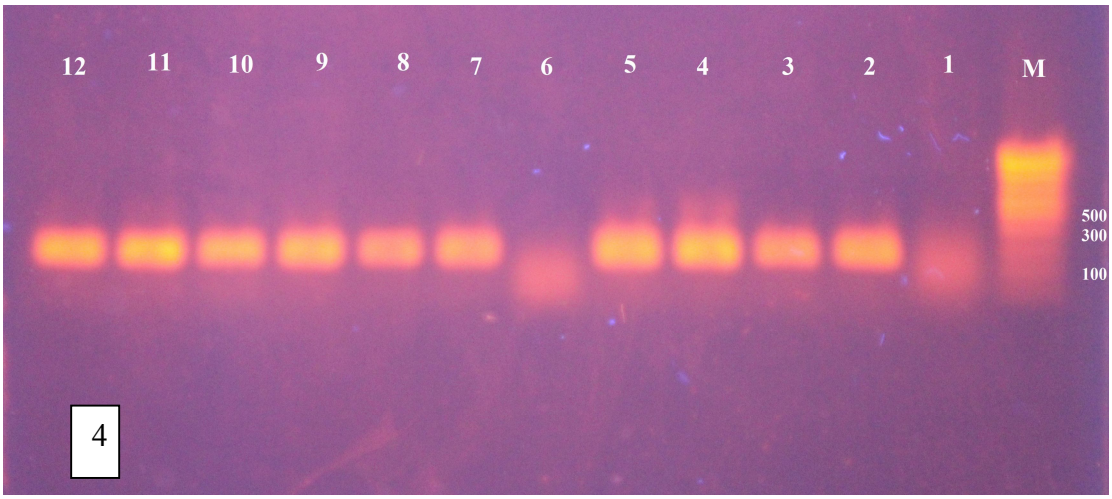
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Table (1): Comparison between results of HA and RT-PCR for virus identification in experimentally infected rabbits:

Sampling time	Death	organ	HA	RT-PCR
0 hr	Sacrificed	Liver	-	-
		Spleen	-	-
		Lung	-	-
		Kidney	-	-
		Thymus	-	-
12 hr	Sacrificed	Liver	-	-
		Spleen	-	-
		Lung	-	-
		Kidney	-	-
		Thymus	-	-
18 hr	Sacrificed	Liver	-	+
		Spleen	-	+
		Lung	-	-
		Kidney	-	-
		Thymus	-	-
24 hr	Sacrificed	Liver	-	+
		Spleen	-	+
		Lung	-	+
		Kidney	-	-
		Thymus	-	-
31 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	+	+
32 hr	Spontaneously dead	Liver	+	N.D
		Spleen	+	N.D
		Lung	+	N.D
		Kidney	+	N.D
		thymus	+	N.D

hr = Hour ND = not done

Table (1) (Continued): Comparison between results of HA, FAT and RT-PCR for virus identification in experimentally infected rabbits

Sampling time	Death	organ	HA	RT-PCR
33 hr	Spontaneously dead	Liver	+	N.D
		Spleen	+	N.D
		Lung	+	N.D
		Kidney	+	N.D
		Thymus	+	N.D
34 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	+	+
36 hr	Sacrificed	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	+	+
39 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	+	+
42 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	+	+
48 hr	Sacrificed	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		thymus	+	+

hr = Hour ND = not done

Table (1) (Continued): Comparison between results of HA, FAT and RT-PCR for virus identification in experimentally infected rabbits

Sampling time	Death	organ	HA	RT-PCR
54 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	+	+
63 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	+	+
65 hr	Spontaneously dead	Liver	+	N.D
		Spleen	+	N.D
		Lung	+	N.D
		Kidney	+	N.D
		Thymus	+	N.D
70 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	+	+
71 hr	Spontaneously dead	Liver	+	N.D
		Spleen	+	N.D
		Lung	+	N.D
		Kidney	+	N.D
		Thymus	+	N.D
72 hr	Sacrificed	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		thymus	-	+

hr = Hour ND = not done

Table (1) (Continued): Comparison between results of HA, FAT and RT-PCR for virus identification in experimentally infected rabbits

Sampling time	Death	organ	HA	RT-PCR
73 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	-	+
		Kidney	+	+
		Thymus	-	+
80 hr	Spontaneously dead	Liver	+	+
		Spleen	+	+
		Lung	+	+
		Kidney	+	+
		Thymus	-	+
96 hr	Sacrificed	Liver	+	N.D
		Spleen	-	N.D
		Lung	-	N.D
		Kidney	-	N.D
		Thymus	-	N.D

Sampling time	Death	organ	HA	RT-PCR
117 hr	Spontaneously dead	Liver	+	+
		Spleen	-	+
		Lung	-	+
		Kidney	-	+
		Thymus	-	+
136 hr	Spontaneously dead	Liver	+	N.D
		Spleen	-	N.D
		Lung	-	N.D
		Kidney	-	N.D
		Thymus	-	N.D
138 hr	Sacrificed (control negative)	Liver	-/-	-/-
		Spleen	-/-	-/-
		Lung	-/-	-/-
		Kidney	-/-	-/-
		thymus	-/-	-/-

hr = Hour ND = not done

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الملخص العربى

إختبار التلزن الدموي واختبار تفاعل البلمرة المتسلسل العكسي لتتبع فيروس النزف الدموي الأرنبي في الأنسجة المختلفة لأرانب مصابة تجريبيا

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قسم النيوكاسل معهد بحوث الأمصال واللقاحات البيطرية بالعباسية

في دراسة لتتبع فيروس مرض النزف الدموي الأرنبي في الأنسجة المختلفة للأرانب المحقونة معمليا بالفيروس، تم حقن عدد ٢١ أرنب قابل للعدوى (خالي من الأجسام المضادة الخاصة بفيروس مرض النزف الدموي الأرنبي) عن طريق فتحتي الأنف بعتره جيزة ٢٠٠٦ لفيروس مرض النزف الدموي الأرنبي و الذي كان تركيزه $10^{4,65}$ جرعة نصف مميتة لكل مل. تم ذبح أرنب من الأرانب المحقونة بعد الحقن مباشرة حيث ذبح أرنب عند الساعة ١٢ ثم ١٨، ٢٤، ٣٦، ٤٨ و ٧٢ و٩٦ و حتى ١٣٨ساعة بعد الحقن. أخذت عينات من خمسة أعضاء و هي الكبد، الطحال، الكلي، الرئة و الغدة التيموسية لكل الأرانب لتحديد وجود الفيروس.وبإجراء اختبار التلزن الدموي تم التعرف على وجود الفيروس في جميع الأعضاء المختبرة بعد ٣١ ساعة من العدوى في حالات النفوق التلقائي و ٣٦ ساعة في حالات الذبح حيث تم اكتشاف وجود الفيروس بنسبه عاليه في الكبد 2^{13} ثم الطحال 2^{11} ثم الرئة و الكلي و الغدة التيموسيه بتركيز 2^{8} .

و بإجراء اختبار تفاعل البلمرة المتسلسل المسبوق بعملية النسخ العكسي تبين انه الأكثر حساسية لتحديد وجود الفيروس حيث تم تحديد الفيروس في الكبد و الطحال مبكرا جدا بعد ١٨ ساعة من العدوى و في الرئة بعد ٢٤ ساعة ثم الكلي و الغدة التيموسية بعد ٣١ ساعة من العدوى.

و قد خلصت الدراسة الى ان اختبار تفاعل البلمرة المتسلسل المسبوق بعملية النسخ العكسي هو الأكثر حساسية لتحديد وجود الفيروس من اختبار التلزن الدموي و أن الكبد يحتوي على أعلى تركيز من فيروس مرض النزف الدموي الأرنبي وأنه أفضل الأعضاء التي تستخدم لعزل الفيروس.

الكلمات الدالة: فيروس مرض النزف الدموي الأرنبي ، اختبار التلزن الدموي، اختبار تفاعل البلمرة المتسلسل المسبوق بعملية النسخ العكسي.