EFFICACY OF A NEW ISOLATE RABBIT HEMORRHAGIC DISEASE VIRUS VACCINE

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ABSTRACT

The present study was carried out to prepare and evaluate an inactivated vaccine from new isolate rabbit hemorrhagic disease (RHD) virus that causes outbreaks in rabbit flocks vaccinated with commercially available vaccines. Sixty, 3 months old New Zealand rabbits were allotted into four groups (A, B, C, D). Groups A, B, C were vaccinated subcutaneously with the new isolate of RHD virus vaccine, Izovac Mevax and SVRI vaccines respectively. Group D was kept as unvaccinated control. All rabbits were challenged at 10 days post vaccination by intranasal inoculation. Rabbits in all groups were observed daily for clinical signs and mortalities. Hemagglutination inhibition titers, hematological, biochemical and histopathological changes were studied throughout the experimental period. Immunological response and resistance to challenge were highest in group A which was vaccinated with the new isolate RHD virus vaccine, meanwhile rabbits in this group maintained a good general health status as reflected by biochemical and hematological pictures. According to these findings, the new isolate RHD virus vaccine is strongly recommended to minimize economic losses that RHD virus outbreaks can produce in industrial rabbitries.

INTRODUCTION

Rabbit hemorrhagic disease (RHD) is an acute highly contagious disease caused by a Calicivirus. RHD was first reported in 1984 in the

People's Republic of China ***(Liu et al., 1984)***. Currently it is endemic in east Asia, Europe and Oceania. Outbreaks have also been reported in Cuba, Mexico, and parts of north Africa. The first record of the disease in Egypt was in 1992 ***(Ghanem and Ismail 1992, Salem and El-Balal 1992)***. Subsequently, RHD outbreaks were recorded in several parts of Egypt ***(El-Zanaty 1994, Azhar et al., 1995, El-Mongy 1998, Mostafa 2001, and Metwally and Madbouly 2005)***. An inactivated formalized either oil or aluminum hydroxide-based tissue vaccines prepared from liver suspensions of rabbits infected with RHD have been developed in several countries including Egypt ***(Gunenkov 1990; Abd El-Motaleb et al., 1998 and Daoud et al., 1998)***. However, outbreaks of RHD were recorded in rabbit flocks that were vaccinated with formalized vaccine. ***Metwally and Madbouly (2005)*** proved that the newly emerged RHD virus isolates were not closely related to classical vaccinal strains that used in Egypt and may be a variant strain.

The purpose of this study was to assess the efficacy of a vaccine prepared from the new isolate of RHD virus as well as various commercially available vaccines.

MATERIALS & METHODS

Rabbit hemorrhagic disease virus vaccines:

1) The use of new isolate of rabbit hemorrhagic disease virus in preparation of new vaccine:

Livers from RHD virus-infected rabbits were collected, homogenized and then diluted in 1:10 in phosphate buffered saline (PBS). Homogenates were centrifuged at low speed centrifugation 3000 rpm for 30 minutes. For virus semipurification, supernatant was ultracentrifuged at 30000 rpm for one hour at 4C through sucrose cushion. Pellet was resuspended in distilled water and was examined by negative staining electron microscopy (EM). Hemagglutination (HA)

property of the virus was checked using 1% human type O washed red blood cells at room temperature with slow elution. The HA titer was higher than 1:1280 ***(Metwally and Madbouly 2005).***

RHD virus-containing suspension is inoculated intranasally into 3 months old-susceptible rabbits. Inoculated rabbits were kept in separate units under strict hygienic measures with satisfactory health conditions. Livers were collected aseptically from freshly dead rabbits that die between 24 and 72 hours post inoculation. Livers were homogenized and diluted 1:10 in PBS. Tissue suspension was frozen and thawed three times and then clarified by low speed centrifugation at 3000 rpm for 30 minutes. Supernatant fluid was collected and assayed by HA. Virus suspension was tested for presence of viable bacteria, other viruses, fungi and mycoplasmas according to protocol used for testing seed virus. Part of this suspension was used for challenging rabbits later in the experiment. Virus-containing suspension is inactivated by 0.4% formalin at 37C for 48 hours ***(Daoud et al., 1998)*** then incorporated with sterile aluminum hydroxide gel adjuvant and virus concentration was adjusted to 1280 HA units. (1ml /dose /rabbit).

2) Izovac Mevax:

Each dose (1 ml) of vaccine contains purified and inactivated RHD virus 1: 2048 HA adsorbed to aluminum hydroxide. This vaccine was obtained from IZO S.P.A. Italy through Mostafa Ghannam Co., Egypt. (Dose 1ml / rabbit).

3) Rabbit hemorrhagic disease virus vaccine:

RHD virus vaccine was obtained from Veterinary Serum and Vaccine Research Institute (SVRI), Abbasiya, Cairo, Egypt) (Dose 0.5 ml / rabbit).

Animal groups and experimental design:

Sixty, 3 months old New Zealand rabbits were used in this study. Rabbits were tested before start of experiment and proved RHD virus seronegative by Hemagglutination inhibition test (HI). Rabbits were allotted into four groups (A, B, C, D) and housed in independent cage units. Feed and water were offered *ad libitum*. Groups A, B, and C were vaccinated subcutaneously with the new isolate RHD virus vaccine, Izovac Mevax, and SVRI vaccines respectively. The fourth group (D) was kept as unvaccinated control group. All rabbits in all groups were challenged at 10 days post vaccination by intranasal inoculation. The challenge dose consisted of 1 ml of liver homogenate containing RHD virus with HA titer more than 1: 1280. All rabbits were daily observed for clinical signs and mortalities for 15 days and blood samples were collected at intervals up to 45 days post vaccination.

Serological testing:

Individual blood samples were collected from ear vein of rabbits before vaccination, 4 days post vaccination and then weekly until 45 days post vaccination. Sera were separated and tested by HI according to Office of International Epizootics ***(OIE) (2000).***

Hematological and biochemical analysis:

Blood samples were collected from ear vein at 24, 48, and 72 hours post inoculation, sera were separated and biochemical procedures were done by colourimetric methods using commercial kits to determine SGOT, SGPT (Randox Laboratories, USA) Creatinine and Urea (Human biochemical diagnostica, Germany) Total protein and albumin (ELITECH Diagnostics, France) Serum globulin was determined by difference.

Prothrombin time:

Citrated blood (100 µl sod. citrate 3.8%, added to 1 ml blood) at 24, 48, 72 hours post inoculation to assess prothrombin time according to commercial kits obtained from BioMed Diagnostic, Egypt.

Histopathological examination:

Tissue specimens were collected from liver, Kidneys, Lung, and spleen of freshly dead rabbits that was challenged with RHD virus. Tissue samples were fixed in 10% neutral buffered formalin. Processed by paraffin embedding technique, sectioned at 4-6 microns thickness and stained with (H&E) and examined microscopically ***(Bancroft et al., 1996).***

RESULTS

Clinical signs of vaccinated rabbits:

Vaccinated rabbits by different vaccines exhibited good health status. No abnormal local vaccine granulomas were observed, likewise no adverse systemic reaction developed.

Response to vaccination and challenge:

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 4, 10, 17, 24, 31, 38 and 45 days post vaccination. Higher HI titers were observed in rabbits vaccinated with the new isolate RHD virus vaccine (Table 1). Resistance to challenge virus was minimal in the unvaccinated control group, 80% of infected rabbits died 48-96 hours post inoculation. The observed clinical signs are depression, convulsions, dyspnea, and fever with cyanosis of lips and nostrils which was occasionally accompanied by frothy bloody nasal discharge (Fig.1). The most prominent lesions were hemorrhage of variable size scattered

through liver with visible reticular pattern. Lungs were edematous, congested, and hemorrhagic (Fig. 2). Dark red kidneys with bladder filled with discolored urine (Fig.3). Stomach in some rabbits had engorged blood vessels and necrotic spots (Fig.4). Complete protection was recorded in rabbits vaccinated with the new isolate RHD virus vaccine (Table 2). Results of serum biochemical parameters showed drastic increase in values at 72 hours post inoculation in diseased rabbits that exhibited signs of RHD (Table 3).

Histopathological findings:

Liver showed marked congestion in hepatic sinusoids, necrobiotic changes in hepatocytes as focal or diffuse coagulative necrosis. Lungs showed congestion of blood capillaries, thrombosis, and edematous fluid in some alveolar spaces. Spleen was congested, and hemorrhagic with marked hemosiderosis. Kidneys were also congested, with hemorrhage in addition to tubular damage and necrobiosis of tubular epithelium.

Table (1): Serological response after vaccination and challenge with RHD virus by HI test (mean $\log_2$ HI antibody titer).

	HI titers at days post vaccination						
	4 days	**10 days***	**17 days**	**24 days**	**31 days**	**38 days**	**45 days**
(A) New isolate RHD vaccine	5	7	10	11	11	11	11
(B) Izovac Mevax Vaccine	4	6	8	8	8	8	8
(C) SVRI Vaccine	4	6	8	8	8	8	8
(D) Unvaccinated control group	Negative	Negative	5	5	5	5	5

*** Sera were collected before challenge infection.**

Table (2): Results of rabbit groups challenge.

Group	**Type of Vaccine**	**Morbidity**	**Mortality**	**Protection Percent**	**HA activity***
A	New isolate RHD virus	2/15	0/15	100%	Not applicable
B	Izovac Mevax	5/15	3/15	80%	Positive
C	SVRI	6/15	5/15	66.66%	Positive
D	Unvaccinated control	15/15	12/15	20%	Positive

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

Fig. (1): Rabbit with cyanosis of lips and nostrils with frothy bloody nasal discharge

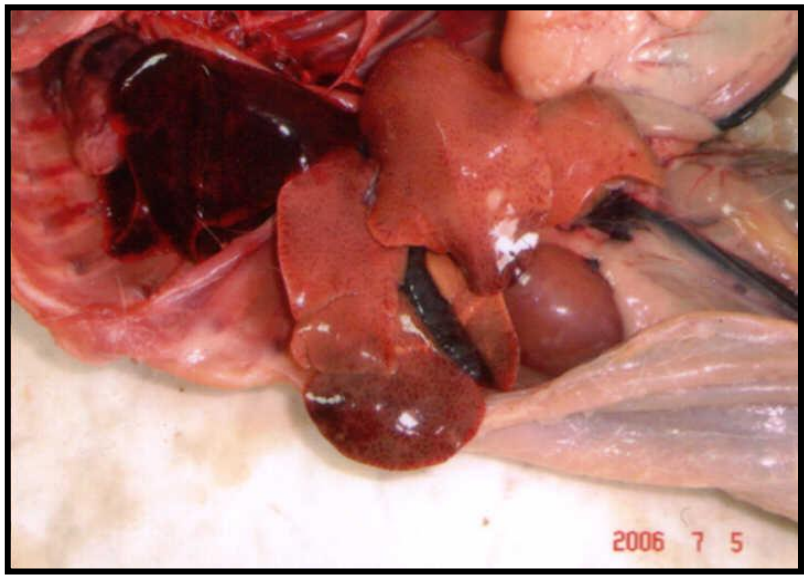

Fig. (2): Rabbit liver and lungs. Liver with focal and lobular necrosis and hemorrhage, lungs with congestion and hemorrhagic areas

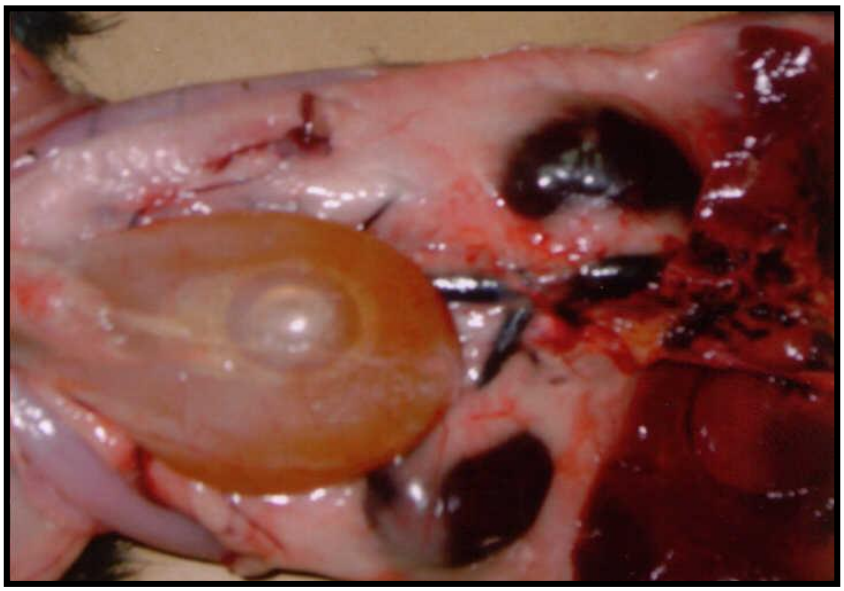

Fig. (3): Rabbit kidneys with severe congestion, Urinary bladder filled with discolored urine.

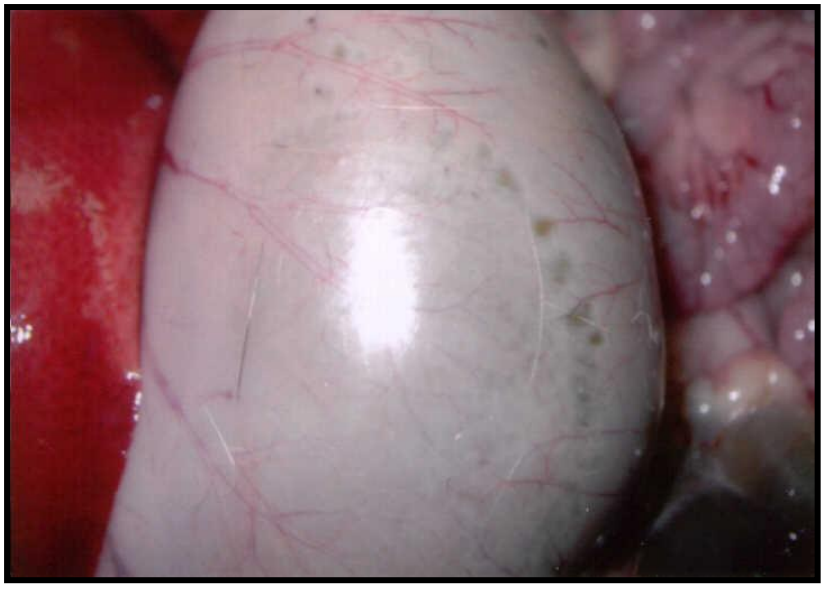

Fig. (4): Rabbit stomach with engorged peripheral blood vessels and necrotic spots.

DISCUSSION

Rabbit hemorrhagic disease is a major viral disease threatening rabbit populations. It is responsible for high economic losses in rabbitries due to high mortality rates in adult rabbits or young rabbits older than 40 days. In all countries where RHD is endemic, indirect control of the disease is achieved by vaccination using killed vaccines prepared from clarified liver suspensions of infected rabbits and subsequently inactivated and adjuvanted. The methods used for inactivation (formaldehyde, beta propiolactone, or other substances) and the adjuvants used (incomplete, mineral oil or aluminum hydroxide) according to the protocol used by different manufacturers ***(OIE, 2000).***

In the present investigation, trials for preparation of inactivated vaccine from new isolate of RHD virus vaccines ***(Metwally and Madbouly 2005)*** was carried out in order to control this serious disease. Different RHD virus vaccines induced variable serological responses, meanwhile new isolate RHD virus vaccine induced the highest HI titers starting from 4^{th} day post vaccination till end of the experimental period. This may be attributed to the high immunogenicity of this local new isolate RHD virus and its homologous relationship to the initial vaccinal strain.

Resistance to challenge infection was 100% in rabbits that were vaccinated with new isolate RHD virus vaccine in comparison with 80% and 66.6% in rabbits vaccinated with Izovac Mevax and SVRI vaccines respectively. This result prove that the new isolate RHD virus vaccine isolated from recent outbreaks is not closely related to classical vaccinal strains and there is no complete cross protection between this new isolate and the currently used vaccines. These results are generally in agreement with that recorded by ***Capucci et al., (1998) and Schirrmeier et al., (1999)*** who characterized antigenic variant strain of RHD virus in Italy and Germany. These variants were considered to be a distinct subtype

designated (RHDva). ***Moss et al., (2002)*** carried out molecular epidemiology investigation of RHD virus in Britain and suggested that a greater variability between RHD virus strains exists. ***Moreover Le Gall et al., (2003)*** isolated and characterized two new variants of RHD virus in France.

Hematological features of RHD virus infection represented by prolonged prothrombin time (13.6 minutes). This may be due to decrease in factors V, V11 and X with high levels of soluble fibrin as well as reduction of thrombocyte number. Similar results were reported by ***Plassiart et al., (1992).*** Enzymatic study of liver and kidney in rabbits challenged with RHD virus showed higher values. This could be due to vascular changes as hemorrhages, congestion, thrombosis and degenerative changes in liver and kidneys. This result coincide with that obtained by ***Nowotny et al., (1993)*** and ***Zhang and Chen (1993).*** Total protein and albumin of infected rabbits had somewhat lower figures in comparison with normal rabbits although globulin was slightly elevated. Such elevation may be due to necrobiotic changes in hepatic cells with periportal coagulative necrosis.

From these findings, it could be concluded that the inactivated vaccine prepared from new isolate RHD virus is safe, potent, and of superior efficacy in controlling RHD virus disease and must be included in vaccination programs to prevent economic losses that a RHD virus outbreak can produce in industrial rabbitries.

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كفاءة تحصين العترة الجديدة من فيروس المرض النزفي في الأرانب

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** **قسم الدواجن والأسماك – كلية الطب البيطري – جامعة كفر الشيخ**

فى هذه الدراسة تم تحضير وتقييم لقاح ميت من عترة جديدة محلية من فيروس المرض النزفي للأرانب. هذه العترة تم عزلها من قطعان مصابة كانت محصنة بالتحصينات التقليدية وتم إجراء تجربة على عدد 60 أرنب نيوزيلاندي في عمر 3 شهور وزعت على أربعة مجموعات كل مجموعة تكونت من 15 أرنبا. المجموعة الأولى حُصنت باللقاح المحضر من العترة الجديدة والثانية بلقاح شركة آيزو الإيطالية والثالثة بلقاح معهد الأمصال واللقاحات البيطرية بالعباسية. وتركت المجموعة الرابعة بدون تحصين لتكون المجموعة الضابطة. وتم بعد ذلك إجراء عدوى إصطناعية لإختبار مدى صد هذه الأرانب للعدوى بالفيروس. تم بعد ذلك ملاحظة الأعراض الإكلينيكية والوفيات على الأرانب وأجريت دراسات على الدم ودراسات كيميائية وباثولوجية وسيرولوجية على كل المجموعات بعد التحصين وبعد العدوى الإصطناعية.

وقد لوحظ وجود رد فعل مناعي قياسي ومستوى عالي من الحماية (100%) فى المجموعة المحصنة باللقاح المحضر من العترة الحقلية الجديدة من فيروس مرض الأرانب النزفي, كذلك كانت الحالة الصحية لهذه المجموعة جيدة جدا كما هو واضح من نتائج قراءات القياسات الكيميائية فى الدم وغيرها. وطبقا لهذه النتائج فإنه ينصح باستخدام اللقاح المحضر من العترة الحقلية الجديدة حتى يمكن تقليل الخسائر الناجمة عن وباء فيروس الأرانب النزفي في تجمعات الأرانب.