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Research Article

Comparative Study Between the Immune Response of Different RHDV Vaccines Used in Rabbit Farms in Egypt

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Abstract

Viral hemorrhagic disease (RHD) of rabbits is an acute disease with high morbidity and mortality rates which affects mainly adult rabbits. The results of this study revealed that vaccination of rabbits with single dose in two batches from each of locally produced or commercially imported vaccines reached the protective level by the first week post vaccination and still immunogenic until the 6th and 7th month post vaccination for the commercially imported and local vaccine respectively. By following up the immune response of rabbits vaccinated with single dose, it began to decrease from the 6th month until it reached its lowest titer at the 12th MPV, but boostered rabbits after six months of single dose gave a satisfactory protective antibody titer and lasted for the 15th and 16th MPV for commercial and local vaccine respectively. The results of the challenge test revealed that the challenge after three weeks post vaccination of single dose was 85% for the local and 80% for the imported vaccine while challenge three weeks post booster dose was 100% for both local and commercial vaccines.

Conclusion: vaccination program is very important for controlling of RHD in rabbits industry and its recommended to use the booster dose program with rabbits flocks and the locally produced RHD vaccine is recommended as its produced from locally isolated Egyptian isolates.

Keywords: *RHDV vaccine, Rabbit, Single dose vaccine, booster dose*

Introduction

Rabbit hemorrhagic disease (RHD) is a highly contagious and fatal disease for both wild and domestic rabbits [1,2] caused by rabbit hemorrhagic disease virus (RHDV) which is a member of caliciviridae and associated with illnesses and deaths up to 90 - 100% of rabbit population [3].

RHD was first reported in china 1984 [4] and within two years later in Europe [5] and rapidly became endemic in most parts of the world [6] and reported in over than 40 countries in Africa [7] RHDV was firstly recorded in Egypt in an acute highly fatal out breaks among rabbits during the Spring of 1991 in Sharkia Province [8] then spread to most of the governorates in Egypt with high morbidity and mortality in adult rabbits.

The incubation period of the disease ranges between one to three days and rabbits usually succumb within 12 to 36 hours. The infected rabbits show clinical manifestations descried mainly in acute onset with nervous and respiratory signs [9].

Gross pathological lesions are variable according to the severity of infection. It includes circulatory and degenerative disorders, liver necrosis and splenomegaly [10,11].

In response to this infection specific inactivated vaccines were developed giving full and rapid setup of protective immunity [12,13] mean while, the data of the efficacy of these types of vaccines and the protocols of vaccination require more studies.

In this study two types of inactivated vaccines of RHDV that is available in the Egyptian market were compared to study immune response in rabbit flocks. The first is the locally prepared in VSVRI and the second is the commercially imported vaccine using either single dose or booster dose vaccination assay followed by evaluation of the protection achieved against challenge with virulent (RHDV).

Materials and Methods

Animals

A total of 340 rabbits of two months old weighed 1.5 to 2 kg at least was tested for freedom from RHDV with no evidence of previous infection or vaccination kindly supplied from the central laboratory for evaluation of veterinary biologics and housed in isolated cages specified for rabbits to be used according to the experimental design.

Vaccines

Two batches from each type of the inactivated RHDV vaccines which are available in the Egyptian market for rabbit's industry were used. the first is locally prepared from local isolate of RHDV in VSVRI and the second is commercially imported of a RHDV 3116-AP strain, They were both bought from the Egyptian market.

Expermintal design

Rabbits used were divided into three groups

1st group of 160 rabbits was vaccinated with the two batches (locally prepared vaccines) 80 rabbits for each. 20 rabbits were challenged against the virulent strain of (RHDV) three weeks after the single dose. The remaining 60 rabbits of each batch group were subdivided after 6th month into two subgroups; a group of 40 rabbits take the booster dose of vaccine and the second (20 rabbits) were left for tracking of the immune response induced by single dose vaccination assay. Three weeks after the booster does, 20 rabbits were challenged against the virulent (RHDV) and the remaining 20 rabbits were left for following up the immune response after booster dose vaccination.

2nd group of 160 rabbits vaccinated with two batches (commercially imported vaccine) 80 rabbits each. 20 rabbits were challenged against the virulent strain of RHDV three weeks after the single dose. The remaining 60 rabbits of each batch group were subdivided after 6th month into two subgroups; a group of 40 rabbits take the booster dose of vaccine and the second was left for tracking of the immune response induced by single dose vaccination assay. three weeks after the booster does, 20 rabbits were challenged against the virulent (RHDV) and the remaining 20 rabbits left for following up the immune response after booster dose vaccination.

3rd group kept as negative control

A group of 20 rabbits of negative control rabbits were challenged with the virulent RHDV 10 rabbits challenged three weeks after single dose and 10 rabbits challenged three weeks after booster dose vaccination.

Serum sampling

Blood sampling were collected individually from all vaccinated and negative non vaccinated rabbits. from each rabbit group in relation to the vaccination assay. Sera of individual rabbits were collected and subjected to inactivation process by heating in water bath at 56ºC for 15 minutes then kept at -20ºC tell examined with hemagglutination inhibition test (HI).

Challenge strain

RHDV (local isolate Giza 2006) with titer of 2^{10}- 2^{12}HA unit was kindly supplied from the Reference Strains Bank in central laboratory for evaluation of veterinary biologics (CLEVB). It was used for challenge test in a titer of 1000LD50 and in (HI) test.

Control sera

Positive control hyper immune serum of (RHDV) and negative control. was kindly supplied from Reference Strains Bank (CLEVB). Also, negative control sera were prepared from sera of negative control rabbits. to be used as positive and negative control in serological tests.

Erythrocyte suspension

Human type (O) red blood cells were collected on 3.8% sodium citrate solution as anticoagulant from a healthy volunteer then the packed erythrocytes were suspended in sterile saline in a final concentration of 1% for both HA and HI tests.

Haemaglutination technique

A twofold serial dilution of RHDV was incubated with an equal amount of 1% washed human type (O) RBCs in a round bottom micro titer plate at 4º C to determine the HA unit that used in HI test. [14].

Haemagglutination inhibition technique

HI test was carried out according to [15] by using 8 HA unit of RHDV and human RBCs type (O) of concentration 75% was kept at 25ºC for 30 - 60 min to settle down to estimate the level of specific RHDV antibodies in the sera of vaccinated rabbits, compared with the positive and negative control sera.

Challenge test

Twenty rabbits from each group of rabbits vaccinated with two batches of either local or commercial vaccine were challenged with a virulent isolate of RHDV with titer of $10^{4.6}$ LD_{50}/ml. All rabbits were observed for motilities and clinical signs which later were sacrificed to apply post mortem examination.

Results

Regarded to monitoring the antibody titers of the vaccinated rabbits with single dose of either local or commercially imported vaccine along the period of one year table 1 reveals that the immune response reached the protective level ≥ 7 $\log_2$ HI unit at the 3rd WPV (week post vaccination) and reached its peak at the 4th WPV and still immunogenic until it started to decline at the 6th MPV (month post vaccination) reaching its lowest titer by the 12th MPV.

The results of follow up the antibody titers of the boostered rabbits either by local or commercial vaccine along the period of 18 month as shown in table 2 reveal that the immune response become satisfactory at the 7th MPV and remain in a protective level ≥ 7 $\log_2$ HI unit until the end of the experiment by the 18th WPV.

	Single dose				
	Local		Imported		Control
	Batch 1	Batch 2	Batch 1	Batch 2	
1st WPV	6.75	6	6.5	6	0
2nd WPV	6.75	6.25	6.75	6.5	0
3rdWPV	8.25	8	7.75	7	0
4th WPV	8.5	8.25	8	7.5	0
2ndMPV	8.5	8.5	8.25	7.5	0
3rd MPV	8.25	8.25	8	7.5	0
4th MPV	7.75	7.5	7	7.25	0
5th MPV	7	6.75	6.5	7	0
6th MPV	6.25	6	5.75	6.25	0
7th MPV	5.75	6	5.25	6.5	0
8th MPV	5.25	5.5	5	6	0
9th MPV	5.25	5.25	5	5.75	0
10th MPV	5	5.25	5	5.5	0
11thMPV	4.5	5	4.75	5	0
12thMPV	4.5	5	4	4.5	0

Table 1: *Mean antibody titers of vaccinated rabbits (single dose) with local and commercial rabbit hemorrhagic disease virus vaccine using haemagglutination inhibition test.*

	Booster Dose				
	Local		Imported		Control
	Batch 1	Batch 2	Batch 1	Batch 2	
7th MPV	7.5	8.5	7.25	7.0	0
8th MPV	8.25	8	8.75	8.25	0
9th MPV	9.75	10	9.5	9.75	0
10th MPV	10.25	10.5	9.25	9. 5	0
11thMPV	10.25	10.5	9.5	10	0
12thMPV	10	10.5	9.5	9	0
13th MPV	9.75	9.5	9	8.75	0
14th MPV	9.25	9	8.25	8	0
15th MPV	8.25	8	7.25	7	0
16th MPV	7	7.25	6.25	6	0
17th MPV	6.25	6.5	5.75	5.5	0
18th MPV	6.25	6	5	5.25	0

Table 2: *Results of haemagglutination inhibition test on sera of rabbits vaccinated with local and commercial vaccines and a booster dose of vaccination.*

As shown in table 3 challenge test against virulent strain of RHDV among vaccination with either single or booster doses of vaccination with the locally prepared vaccine showing appearance of protective level ≥ 70% reaches 80% and 90% with average 85% for the two batches of the vaccine 3 weeks post single dose vaccination while was 100% post boostering for the two batches of vaccine under test.

	Single dose vaccination				Booster dose vaccination			
	Batch (1)	Batch (2)	Mean	control	Batch (1)	Batch (2)	Mean	Control
Number of tested rabbits	20	20	20	10	20	20	20	10
Mortalities	2	2	2	10	0	0	0	10
Symptoms	2	0	1	0	0	0	0	0
PM lesions	0	0	0	0	0	0	0	0
Total affected	4	2	3	10	0	0	0	10
Protection %	80%	90%	85%	0%	100%	100%	100%	0%

Table 3: *Results of challenge test against virulent rabbit hemorrhagic disease virus (RHDV) 3 weeks post single and booster dose vaccination with locally prepared vaccine.*

Results of challenge test against virulent strain of RHDV among vaccination with either single or double doses of the commercially imported vaccine with six month apart showing appearance of protective level ≥ 70% reaches to 75% and 85% with average 80% for the two batches of the vaccine 3 weeks post single dose vaccination while challenging 3 weeks post booster dose gave 100% protection for the two batches of vaccine under test.

	Single dose vaccination				Booster dose vaccination			
	Batch (1)	Batch (2)	Mean	control	Batch (1)	Batch (2)	Mean	Control
Number of tested rabbits	10	10	10	5	10	10	10	5
Mortalities	1	1	1	4	0	0	0	5
Symptoms	1	0	0.5	0	0	0	0	0
PM lesions	1	0	1	1	0	0	0	0
Total affected	3	1	2	0	0	0	0	0
Protection %	75%	85%	80%	0%	100%	100%	100%	0%

Table 4: *Results of challenge test against rabbit hemorrhagic disease virus (RHDV) three weeks post single and booster dose vaccination with commercial vaccine.*

Discussion

Rabbit viral hemorrhagic disease (RVHD) nowadays is endemic in most parts of Europe, Asia, in some African countries and in Australia. In Egypt, RHVD spreads all over most the of Egyptian governorates where it was recorded in many different localities by many authors. It is characterized by high rates of morbidity and mortality among rabbit flocks reaches up to 90% in adults causing high commercial and economical losses [16].

Inactivated vaccines against RHDV was developed by several countries and succeeded in the control of the disease. In Egypt, an inactivated RHDV vaccine with aluminum hydroxide gel adjuvant was developed giving a satisfactory level of protection among rabbits industry administered as a single shot vaccination [17] aiming to protect rabbits for long duration but it was observed that single dose vaccination of rabbits with inactivated vaccine is not enough to cover long period of immunity in rabbit flocks [18].

So the objective of this study was to evaluate the immune response achieved by single dose of vaccines either the locally produced from the local isolates of RHDV in Egypt or by the commercially imported vaccine that is nowadays present in the Egyptian field. Following up and monitoring of the vaccination programs post single dose of vaccination using haemagglutination inhibition test directing us and the rabbit breeders to the best time for booster vaccination and this is confirmed by the challenge test using virulent local isolate of RHDV.

Regarding the monitoring of antibody titer using HI test indicated that the immunity in adult rabbits induced with single dose either in the locally produced or the commercially imported vaccines lasted protective for at least 6th month up to the 7th month post single dose of vaccination, and this is confirmed by [19] then gradually declined as shown in table 1.This may indicate that rabbits vaccinated with locally produced or commercially imported vaccines may have a protective level of antibodies against RHDV up to the 6th month post single vaccination for the commercial vaccine or up to the 7th month for locally produced vaccine post single vaccination, The same finding were observed by who added a booster dose of vaccination will be more beneficial. A satisfactory protective antibody titer were obtained in rabbits received booster dose either from local or commercial imported RHDV vaccine reached up to the 15th and 16th month in commercial and locally produced vaccines respectively ,Thus it is necessary in breeding rabbits to be vaccinated twice a year as recommended by [20]. As shown in table 1 both groups vaccinated with local and imported vaccines had RHDV specific antibodies by haemagglutination inhibition test at the first week post vaccination in the two batches from each type of vaccine which was 6.75 and 6 log2 for the tested local vaccine and 6.5 and 6 for the two batches of the tested imported vaccine respectively. Antibody titers induced by the two vaccine types began to rise to reach the highest titer in the first month post vaccination as said by [21] although the results reveals that the RHDV antibodies were higher in the locally prepared vaccine than the imported one as reported by [22,23] than that of the commercial imported one. The two vaccines remain immunogenic until 6th month then the antibody titer began to decline as measured by HI test. This results disagreed with [24] who said that the rabbits still immunized until the 12th month post single dose vaccination but with the oil emulsified vaccine.

A group of rabbits were boostered at the 6th month with second dose of the vaccine. Following up of the antibody titers for both groups of single and booster vaccinated rabbits showed that the single dose vaccinated rabbits showed decrease in the antibody titers began from the 6th month until reached to the lowest titer at the 12th month post vaccination (Table 1) with 4.5 , 5 log 2 for local vaccine and 4 , 4.5 log2 for imported vaccine. Mean while the boostered Table (2) rabbits at 6th month showed an increase of the antibody titers from 6thmonth post vaccination with 7.5 ,8.5 log2 and 7.25 , 7.0 log2 for the local and commercial vaccine post boostering respectively reaching a second beak of immunity at the 10th month from the primary vaccination with 10.25, 10.5 log2 for locally produced vaccine and 9.5 at 9th , 9.75at 10th log2 for the commercial vaccine. These results fully agreed with [18].

After challenge test, all of the remaining rabbits were sacrificed to examine the post mortem picture to calculate the protection percent. The results of challenge test of rabbits vaccinated with either single or booster dose of RHDV vaccine for local and imported vaccines as shown in Table (3) against virulent local strain were in agreement with [25,26] who reported that booster vaccination serve a 100% protection in the vaccinated rabbit flocks.

Finally, from the previous mentioned data of sero-evaluation, challenge and protection assay we concluded that. The locally produced monovalent inactivated RHDV adjuvanted with aluminium hydroxide gel induces better immune response than that the imported one in challenge against local virulent isolates of the virus.

All the tested vaccines showed better immune response by boostering of the vaccinated rabbits with a booster dose of vaccine after the 6th month from the first dose.

So, the findings recommended the use of locally prepared RHDV vaccine will be better under the Egyptian circumstances and booster dose vaccination is needed after six months from the primary vaccination to achieve full protection against RHDV in Egypt.

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