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DEVELOPMENT AND PRODUCTION OF A NOVEL BIVALENT INACTIVATED RABBIT HAEMORRHAGIC DISEASE VIRUS (*RHDV*) VACCINE

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ABSTRACT

During 2018 and 2019 a new variant RHDV2 emerged in some Egyptian governorates with high mortality rates especially in suckling rabbits. Classical RHDV vaccines showed low cross protection against RHDV2, revealing the need for a new vaccination strategy to face the current RVHD outbreaks. In this study, a novel bivalent inactivated RHDV vaccine including RHDVa and RHDV2. 300 rabbits were randomly divided into 3 groups; group 1 was vaccinated with the prepared bivalent inactivated RHDV vaccine once, group 2 was vaccinated twice 14 days apart, and group 3 was kept as negative control. Clinical signs and mortality were monitored after challenge. The antibody response against RHDVa and RHDV2 was measured by Haemagglutination inhibition test. No clinical signs, mortality or adverse reactions were observed in the vaccinated groups revealing the safety and potency of the prepared vaccine post challenge. All animals from the vaccinated groups showed clear seroconversion (Mean titers for RHDV HI antibodies higher than protective HI titer for RHDVa and RHDV2) beginning from 7DPV. These results suggest that the prepared bivalent vaccine was proved to be sterile, safe, and potent protecting rabbits against 2 viruses (RHDVa and RHDV2) in one shot.

Key words: RHDVa, RHDV-2, vaccine, production, bivalent, Rabbit.

INTRODUCTION

Rabbit Haemorrhgic Disease (RHD) is an acute febrile highly fatal infectious disease causing heavy losses among rabbits (Cao et al., 1986). The disease was first reported in China in 1984 (Liu et al., 1984). In Egypt, the RHD has been reported for the first time in 1991 in Sharkia governorate (Ghanem and Ismail, 1992). In france during 2010, a new lagovirus genotype related to RHDV was emerged and found to be new RHDV variant designated RHDV2 which is geneticaly and antigenically different from classic RHDV (Le Gall-Reculé et al 2013). In Egypt at mid 2018 and continued 2019 , severe mortalities were recorded in vaccinated rabbit flocks. Samples were confirmed to be positive for RHDV from different Governorates. Genotyping of these recent isolates was done by sequencing and phylogenetic analysis of (C to E) region of highly variable region of VP60 capsid gene which revealed clustering of all Egyptian isolates with RHDV2 Genogroup I.2 (GI.2) strains with high homology up to 98.4% and was genetically distinct from classical and variant RHDV with divergence around 23%. This is the first report to identify the arrival of RHDV2 (GI.2) in Egypt (unpublished data). RHD is characterised by high morbidity and high mortality of 70–90% for RHDV/RHDVa and 5–70% for RHDV2. In rabbits younger than 4–6 weeks, the RHDV/RHDVa infection course is subclinical, but when the causative agent is RHDV2, clinical signs and mortality are observed even in young animals from 15 to 20 days of age (Puggioni et al., 2013). Control policy of RHD mainly depends on vaccination using the appropriate vaccine. Sucessful control of RHD was easy during the last two decades due to the use of effective vaccine in addition to low antigenic variation of the field virus strains (Lavazza and Capucci, 2012) . In Egypt, an inactivated formalized RHDV vaccines have been developed using Egyptian classical strain (Daoud et al.1998) then using Egyptian variant RHDVa (Salman 2007). However,outbreaks of RHD with dramatic lethality were recorded in rabbit flocks that were vaccinated with commercial avilable vaccines prepared from classic or variant strains of RHDV (RHDV/RHDVa).Antigenically different RHDVfrom the classical was isolated and called RHDV2

(Dalton et al., 2012; Le Gall-Reculé et al., 2013). Cross protection was low between the classical strains and RHDV2 (Bárcena et al., 2015).
Objective: Classical RHDV vaccines showed low cross protection against RHDV2 and does not prevent infection and losses of clinical disease (OIE 2019), Therefore the aim of the present work is to prepare a bivalent vaccine containing both antigenic types RHDVa and RHDV2 and assessment its efficacy and safety for controlling RHD outbreaks in Egypt.

MATERIAL AND METHODS

Quality control
The prepared vaccine was subjected to sterility and safety following standard international protocols of British Pharmacopoeia Veterinary (2005).
Challenge test: according to OIE, (2018) .
Rabbit Haemorrhagic Disease Viruses (RHDV):
RHDVa: Local Egyptian strain of RHDV designated as Giza/2006 (Salman, 2007) and RHDV2: Local Egyptian strain of RHDV2 designated as Mahala2019/VSVRI with Accession Number MK736667.
Haemagglutination (HA) test according to Capucci et al., (1996).
Haemagglutination inhibition (HI) test : according to Peshev and Christova, 2003.
Preparation of inactivated RHDV suspension: according to OIE, (2018).

RESULTS AND DISCUSSION

In Egypt, this variant (RHDVa) was emerged and isolated at 2006 (Salman 2007). Emergence of variant RHDV2 was reported in some Egyptian governorates with high mortality rates especially in suckling rabbits in Egypt during 2018 and early 2019. Ten isolates were consistently found and all were RHDV2 after sequence and submission to Gen Bank with the following accession numbers: Benha 2018 VSVRI (MK991768), M.Ghamr 2019 VSVRI (MK991769), Menofia 2019/VSVRI (MN007210), Kal2018 VSVRI (MN007207), Daqah2019 VSVRI (MN007208), K.Sheikh2018 VSVRI (MN007209), Sempel2019 VSVRI (MN007211), Alex2019 VSVRI (MN007212), Dosoq2018 VSVRI (MK991770) and Mahala2019 VSVRI (MK736667) (Unpublished Data). There is no cross protection immunity between RHDVa and RHDV2 (Bárcena et al., 2015).An inactivated vaccine against RHDV-2 was evaluated with its simultaneous administration with a classical RHDV vaccine. The results suggested that the simultaneous administration of classical RHD vaccine did not interfere with inactivated RHDV2 vaccine (Montbrau et al, 2016). Also the use of combined vaccination with both antigenic types (RHDVa & RHDV2) was highly advisable as mentioned in OIE (2018).
Evaluation of the vaccine was based on Humoral response was assessed by HI test. Estimated mean specific RHDV HI antibodies were recorded and shown in Table (1). All rabbits were seronagative before vaccination. Huang (1991) reported that immunity to RHD after vaccination is rapidly developed and persisted for more than 6 months. And OIE (2018) reported that vaccinated animals quickly produce solid protective immunity against RHD infection within 7-10 days. In this study, the specific RHDV HI antibodies began to be detected from the 1st WPV in agreement with Popovic (1990) .The vaccine induced fast immunity Montbrau et al (2016) who reported that the administration of inactivated RHDVa and inactivated variant RHDV2 vaccine simultaneously induced immunity within 7 days. In this study mean titers for RHDV HI antibodies increased gradually in the two vaccinated groups reaching $2^{10.7}$, $2^{10.3}$ in group(1) for RHDVa & RHDV2 respectively, while in group (2) they were $2^{10.85}$, $2^{10.3}$ for RHDVa & RHDV2 respectively at 3 $^{rd.}$ WPV, then quickly produced strong humoral immune response against RHDVa and RHDV2 as represented by elevated and sustained HI titers peak (11,11,12,11) log_2 at 6th WPV then decreased but still high at 12,16,20 and 24 WPV.
Following challenge test, the prepared bivalent inactivated RHDV vaccine gave protection of vaccinated rabbits against challenge with virulent RHDVa and RHDV2 (10^3 LD_{50}/ml) after observation period from 1 WPV and this agree with Montbrau et al (2016) who found that RHDV2 vaccine was protecting rabbits against challenge with virulent RHDV-2 strain seven days after vaccination. Protection percent was 70% when challenged with RHDV2 and it was 80% to RHDVa at 1 WPV. These results supported the onset of immunity to establish from 7 DPV. This protection

increased to 90% to RHDV2 and full protection (100%) was achieved for RHDVa at 2 WPV while full protection was gained for both viruses (100%) at 3 WPV and continued till the end of the experiment in vaccinated groups (Table .2).

The challenge resulted in 100% protection in vaccinated groups and this is identical with that recorded by Salman (1999) who showed that RHDV vaccine resulted in100% protection of rabbits. Also the challenge result agree with the result of Daoud et al (1998) who recorded that rabbits developed full protection against RHDV infection 3 weeks after single dose vaccination. The obtained results also agreed with those of Nowotny et al., (1993) who found that the adult rabbits with RHDV-antibody titers ranging from 2^6 to 2^{13} remained clinically healthy after inoculation with virulent RHDV and Simon et al (1993) who concluded that a titer> 20 HIU was protective.The mortality rate obtained in the unvaccinated (control) group of RHDVa was 100% and the mortality rate obtained in the unvaccinated (control) group of RHDV2 was ranged from 50-70% and these results were similar to pervious reported results by Le Gall-Reculé (2013) who obtained a mortality rate of 46% , Parra and Dalton (2013) who registered mortality rates of 50-55% 72 hours after challenge. These results agree with OIE (2018) which reported that RHD was characterised by high morbidity and a mortality of 70–90% for RHDV/RHDVa and 5–70% for RHDV2.

Table 1: Geometric mean of RHDV specific HI antibody titers (log2) in serum of vaccinated rabbit groups by prepared bivalent RHDV vaccine and unvaccinated rabbits

Time post vaccination	Geometric mean of RHDV specific HI antibody titers (log2)					
	Group (1)		Group (2)		Group (3)	
	Antigen used in HI test		Antigen used in HI test		Antigen used in HI test	
	RHDVa	RHDV2	**RHDVa**	RHDV2	**RHDVa**	RHDV2
0 Day	0	0	0	0	0	0
1st WPV	6	5.75	6.5	6	0	0
2nd WPV	8.9	9.4	8.5	9	0	0
3rd WPV	10.7	10.3	10.85	10.3	0	0
4th WPV	11.5	11	11.5	10.78	0	0
6th WPV	11	11	12	11	0	0
8th WPV	10.92	10.85	11.2	11	0	0
10th WPV	10.85	9.1	11	10.78	0	0
12th WPV	9.82	9	10.9	10.2	0	0
16th WPV	9.5	9	10.5	10	0	0
20th WPV	9	8.6	10	9.8	0	0
24th WPV	8.5	8.5	9	9.5	0	0

Protective HI antibody titer ≥ 20 HI units; RHDV= Rabbit Haemorrhagic Disease viruses; HI= Haemagglutinating inhibiting; WPV= Week Post Vaccination; Group (1) = Vaccinated; Group (2) = Vaccinated by booster dose. Group (3) = Un vaccinated control.

Table 2: Potency of bivalent inactivated RHDV vaccines to RHDVa and RHDV2

Groups	viruses used in challenge		Time of challenge			
			1WPV	**2WPV**	**3WPV**	**24WPV**
Group 1 vaccinated	RHDVa	SR/CR	8/10	10/10	10/10	10/10
		P%	*80*	*100*	*100*	*100*
	RHDV2	SR/CR	7/10	9/10	10/10	10/10
		P%	*70*	*90*	*100*	*100*
Group 2 vaccinated	RHDVa	SR/CR	8/10	10/10	10/10	10/10
		P%	*80*	*100*	*100*	*100*
	RHDV2	SR/CR	7/10	9/10	10/10	10/10
		P%	*70*	*90*	*100*	*100*
Group 3 *unvaccinated*	RHDVa	SR/CR	0/10	0/10	0/10	0/10
		MR	*0*	*0*	*0*	*0*
	RHDV2	SR/CR	3/10	4/10	5/10	4/10
		MR	*30*	*40*	*50*	*40*

SR/CR= Survived rabbits/ Challenged rabbits; *P%*=Protection percent WPV = Week Post Vaccination,*MR*=Mortality rate. Group (1) = Vaccinated, Group (2) = Vaccinated by booster dose, Group (3) = *Unvaccinated control*, RHDV= Rabbit Haemorrhagic Disease viruses

CONCLUSION

The prepared bivalent vaccine was proved to be sterile, safe and potent protecting rabbits against 2 viruses (RHDVa and RHDV2) in one shot consequently saving time and efforts of labor avoiding stress of rabbits during vaccination.

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