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PREPARATION AND EVALUATION OF BIVALENT INACTIVATED VACCINE AGAINST RABBIT HAEMORRHAGIC DISEASE VIRUS AND CLOSTRIDIAL ENTEROTOXAEMIA IN RABBITS.

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SUMMARY

A combined bivalent inactivated Vaccine against rabbit haemorrhagic disease and Rabbit enterotoxaemia was prepared using aluminum hydroxide gel as an adjuvant . Efficacy of this vaccine comparing with monovalent ones against both disease was tested in 3 different groups of mature rabbit and a control group . Evaluation of humoral immune respons using the hemagglutination inhibition test for RHDV and serum neutralization test for *C. perfringens* type A toxoid was carried out , morover a challenge test for RHDV was done . The prepared vaccine induced excellent and highly protective titer against both diseases. No mutal interference or compitition occured and the vaccine could be used safely to protect rabbits against both diseases .

INTRODUCTION

Rabbit can play a significant role in solving the problem of meat shortage in many parts of the world , due to their high potential for reproduction , rapid growth rate and short generation interval (Rashwan and Marai , 2000)

Enteritis in rabbits is the major cause of economic losses in the commercial rabbitories . *C. perfringens* must be considered as one of causative agents of diarrhoeal disease camplex in rabbit (Bernal et al., 1987and El. Gad et al ., 1994) .C.perfringens type A is the most isolated type that induced enterotoxaemia and bloat in rabbits (Diab et al,. 2003) .Recently weaned rabbits are most susceptible as young rabbits do not digest and absorb starch as efficiently as adults which is required for *C.perfringens* type A toxin production (De bles and Gidenne, 1998) enterotoxaemia

in adult pet rabbits is not associated with high carbohydrate diet but usually follows a disruption of the gut flora by antibiotic, other pathogen , toxins or stress (Carman and Evans, 1984).

Drug and antibiotic therapy have proved to be expensive and of a little lasting value (Ellis et al., 1991) thus control by active immunization is of considerable importance (Kenndey et al., 1977and Diab etal., 2003).

Rabbit haemorrhagic disease is an acut febrile highly fatal infections disease attacking rabbit (Liu etal., 1984) were the 1st to record the disease in China, and based on the physical and morphological characterestics, the causitive virus was classified to the Calicivirus family (Capucci et al ., 1990).

Affected rabbits showed sever dullness depression, anorexia dyspnoea , fever and convulsions with bloody discharge from nostrils (Du,1990) but sudden death without any clinical signs was a common feature with high mortality rate reached to 90% ,and the 2 month old and adult rabbits were the mostly affected with the disease (Cao et al ., 1986).

In Egypt the 1st epidemiological investigation of the disease was reported by Ghanem and Ismail, 1992 in Sharkia province .

The wide prevalence of the disease in Egpyt elaborated the need of adequate control measures to prevent the disease (Salman ,1999), inactived tissue culture vaccine produced locally was used to control the disease and gave satisfactory results (Daoud et al., 1998) moreover, attempt had been done to combine another pathogen with RHDV to prepare a combined vaccine against rabbit pasteurllosis and RHDV(Daoud et al ., 1998).

The present study has been conducted in the same direction to prepare a combined vaccine against RHDV and rabbit enterotoxaemia to be used as a single vaccination aming to save time, reducing the over stress on rabbit resulted from repeated handling of the animals.

MATERIALS AND METHODS

Strain :

- C .perfringens strain

A local strain of *C.perfringens* type A isolated from rabbits suffering from enterotoxaemia was used

- Rabbit haemorrhagic disease virus (RHDV).

RHDV field isolate (Salman .,1999) routinely used for vaccine preparation with LD 50 of $10^{3.59}$ / ml ., the virus used also for estimation of humoral immune response against RHDV antibody as well as a virulent virus in challenge test .

Standard antitoxin :

Clostridium perfringens type A standard antitoxin was obtained from National Institute for Biological Standards and Control , United Kingdom.

It contain 270 alpha antitoxic Interntional Units per ml.

Toxin :

Dried alpha toxin of C .perfringens type A was prepared according to Dixon and Webb, (1958)

Vaccine preparation :

C .perfringens type A vaccine :

Vaccine was prepared from the toxigenic strain of *C .perfringens* type A according to the method described by (Ahmed , 1975) . Toxoid was clarified and concentrated by ultra filtration system . aluminum hydroxide gel was added in a percentage of 20% of toxoid volume as an adjuvant.

Rabbit haemorrhagic disease virus vaccine : (Daoud et al., 1998)

100ml of aluminum hydroxide gel inactivated RHD vaccine prepared locally (with titer of 9 $\log_2$ HA uints/0.5ml) was used.

Preparation of the combined inactivated enterotoxaemia and rabbit haemorrhagic disease virus vaccine:

100ml of formalin inactivated RHDV (with titer of 9 $\log_2$ HA uints/0.5ml) and 400ml of clarified concentrated *C. prefringens* type A toxoid were mixed togther and aluminum hydroxide gel was added in percentage of 20% of total volume as an adjuvant.

Test for purity and safety of the prepared Vaccines :-

The monovalent types as well as the combined prepared vaccines were tested for sterility and safety following standared international protocols described by British Veterinary Codex (1970). The vaccine was paked in bottles and stored in Refrigerator at 4-8°C.

***Expermental design:**

Vaccination of Rabbits :

Fourty boskat rabbits of 1.5-2 kg body weight were grouped and vaccinated as shown in table (1) .

Table (1):Groups of rabbits vaccinated with different prepared vaccines

Animal group	No.of rabbits	Type of vaccine	Dose *		Route of administration
			1st	2nd	
I	10	Rabbit clostridial enterotoxaemia	2ml	2ml	S/C
II	10	Rabbit haemorrhagic disease monovalent vaccine	0.5ml	0.5ml	S/C
III	10	Combined vaccine of vaccine I and vaccine II	2.5ml	2.5ml	S/C
IV	10	Control	-	-	-

Interval period between two doses were three weeks .

Blood samples were collected from ear vein 2 weeks post secondery vaccination for both vaccines . The samples were taken every 2 weeks for RHDV vaccine and every month for *C. perfringens* type A vaccine Sera were separated and stored at -20°C till used.

Serological test :

I- For RHDV

Haemagglutination inhibition (HI) test .

HI was performed according to the method described by (Pu et al ., 1985) using 4HA unit of RHDV and human erythrocytes group (0).

II For *C. perfringens* type A

Serum neutralization test:

It was carried out according to the standered method described by European pharmacopiea 2001 , the titre was expressed as international unit/ml (Iu/ml).

Challenge test for RHDV.

Five animals from groups II,III and IV .Were chosen randomely and subjected for challenge test 3 weeks post poostring using $1000LD_{50}$ of virulent virus .The animal were observed for 15 days post challenege to detect any clinical signs or deaths .

RESULT AND DISCUSSION

Rabbit enterotoxaemia caused by toxigenic Clostridium perfringens type A has been diagnosed in Egypt (Diab et al., 2003) Vaccination against the disease with the type specific toxoid or whole culture is the only preventive measure available , because of the acuteness of the disease.

Occasionally because of the high resistance of Rabbit haemorrhagic disease virus to enviromental exposure and its wide distribution , hygenic measures alone is often insufficient and vaccina-

tion is essiential for pevention and control of disease (Thiaboult ., 1990).

It is now desirable to have a combined vaccines given as one shot , which protect against several pathogens where efforts , funds and time could be save (Awaad,2004) , moreover the combined vaccines will protect the host from stress factors on application of the repeated single vaccination on several occasions according to the vaccination programe . The present study deals with the preparation and experimental vaccination of different groups of rabbits with a combined bivalent inactivated vaccine against rabbit enterotoxaemia caused by toxigenic *Clastridium perfringens* type A and rabbit hemorrhagic viral disease.

Obtained results revealed that the prepared vaccine is completely sterile free from any bacterial and fungel contamination as recommended by the British Veterinary Codex (1970). Moreover the prepared vaccine was found to be safe and vaccinated rabbits did n't show any symptoms of the discases or adverse reaction even with double the dose of the prepared vaccine . Dealing with humoral immune response of rabbit clostridial enterotoxaemia table (2) it was clear the antibody titre of group I (vaccinated with rabbit Clostridial enterotoxaemia Bloat monovalent vaccine) and group III(vaccinated with the combined rabbit Closteridial enterotoxaemia bloat and rabbit haemorrhagic virus vaccine) were closely similar and stable up to the 2nd month post vaccination (1.5 IU/ ml) then decrease gradually till 5th month. However the antibody titres in both groups (I and III) were more than the minimum protective level allover the period of the experiment (5 month), where the minimum protective level for *C. perfrjngons* type A as stated by Tytell et al., 1947 , Weipers et al , 1964 and Diab et al, 2003 was 0.1 IU / ml .

Concerning the humoral immune response of RHDV as illustrated in table (3) it could be noticed that both of the vaccinated groups (gr II) and (gr III) showed approximitaly the same serological patteren and non significant differences could be detected with a starting titre of 8.9 log 2 HI and 8.6 log 2 were recorded two weeks post boostringfor both groups in order ending with 7.6 log 2 and 7.5 log 2 HI antibody titres 20 weeks post vaccination for both groups respectevely , the obtained results revealed neither competition nor interfernce between the two antigens this results comes parellel with that of (Daoud et al., 1998) who used a combined vaccine against RHDV and rabbit pasteurllosis. Obtained resultses also clarified that the obtained titres is fully protective for both groups as a titre of >20 HIU was concedered protective against RHDV (Simon et al ., 1993) moreover this results is fully assured after challenge test using the virulent RHDV(Table 4)

From the aforementioned discused results it could be concluded that a combination of both rabbit

Table (2):Antibody titer in sera of rabbits vaccinated with *C. perfringens* type A alone or with rabbit haemorrhagic disease virus .

Period post 2nd vaccination	Antitoxin titer in sera of vaccinated rabbits (Iu / ml)	
	***C. perfringens* type A** vaccine	***C. perfringes* type A** with rabbit heamorrhagic disease virus vaccine .
2 weeks	1.5	1.5
6 weeks	1.5	1.5
10 weeks	1.0	0.9
14 weeks	0.7	0.7
18 weeks	0.5	0.5

Table (3): Mean haemagglutination inhibition antibody titer of RHDV either using RHDV vaccine alone or combined with Rabbit enterotoxaemia vaccine.

Animal group	Weeks post vaccination									
	2	4	6	8	10	12	14	16	18	20
Group II	8.9	8.9	8.7	8.7	8.3	8.3	8	8	8	7.6
Group III	8.6	8.6	8.6	8.3	8	8	7.8	7.6	7.6	7.5
Group IV	0	0	0	0	0	0	0	0	0	0

Group II vaccinated with the monovalent RHDV vaccine

Group III vaccinated with the combined rabbit enterotoxaemia and RHDV vaccine Group IV non vaccinated control .

enterotoxaemia and RHDV antigens in a bivalent inactivated vaccine revealed neither competition nor mutual interference between the two antigens moreover the vaccine offered a good protective immunity against the used antigens and could be used safely for protection against both diseases.

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