**Vet. Serum and Vaccine Research Institute,
Abbasia, Cairo.**

A COMBINED INACTIVATED FREEZE DRIED VACCINE FOR ROTA AND CORONA VIRUSES

(With 4 Tables and 2 Figures)

By

IMAN, K.A. KASSEM; THANAA, I. BAZ AND H. M. MOHAMED

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لقاح مخمد مجفف ضد فيروسى الروتا و الكورونا

ايمان كامل احمد قاسم ، ثناء ابراهيم باز ، حسين متولى محمد

تم تحضير لقاح مخمد ثنائى لكل من فيروسى الروتا و الكورونا على خلايا MDBK باستخدام بينرى ايثيلين امين كمادة مثبطة. تم تقييم اللقاح بدراسة اختبار الأمان فى الفئران و الأرنب الغينى و كذلك العجول ثم تم متابعة الأجسام المناعية المتكونة بعد التحصين. اثبت اللقاح المجهز كفاءته حيث ان الأجسام المناعية المتكونة لكل من فيروسى الروتا و الكورونا تكونت فى اليوم العاشر بعد التحصين و قد وصلت الى القمة فى اليوم الخامس و الثلاثون بعد التحصين و استمرت عالية حتى نهاية الشهر الثانى و تم قياس الأجسام المناعية عيارياً باستخدام اختبارى التعادل المصلى و الاليزا.

SUMMARY

A combined inactivated Bovine Rota and Corona viruses vaccine was prepared on Madin Darby Bovine Kidney (MDBK), by use of Binary Ethyleneimine (BEI), as an inactivant. Evaluation of the vaccine for its safety and potency was conducted through study in laboratory animals and calves. The prepared vaccine proved potent as the developed antibodies for Bovine Rota Virus (BRV) and Bovine Corona Virus (BCV) appeared as early as 10 days post vaccination (PV), and reached its peak at the 35th day PV and remained high till the end of the 2nd month. The developed specific antibodies were quantitively measured by both Serum Neutralization Test (SNT) and ELISA technique.

Key words: *Rota and Corona viruses - Inactivated caccines*

INTRODUCTION

Diarrhoeal diseases are considered as one of the most important causes of young calves. Therefore, death thus the first few weeks of life of newborn calves are the most critical period in which a considerable number of animals could be lost. Neonatal calf diarrhoea has a complex etiology among which are the Rota and Corona viruses (Radostits, 1991).

Rota viruses are ubiquitous viruses that produce an acute diarrhoea in the young age of many domestic and wild animals and human. The disease is primarily species specific although Rota viruses from different host species share common antigens and cross react in certain serologic assays and there is a recent evidence of zoonotic infection (Saif and Theil, 1990).

Rota viral diarrhoea results from infection and replication of the virus in villous enterocytes of the small intestine. Damaged cells are shed, resulting in villous shortening and replacement by immature cells that initially lack the enzymatic and adsorptive capabilities of mature cells. Unabsorbed nutrients and concurrent bacterial growth exert an osmotic effect resulting in malabsorption, loss of nutrients and diarrhoea (McNulty, 1983).

Human Rota virus can experimentally infect animals and induce diarrhoea and illness (Mebus et al., 1976). Conversely, animal Rota viruses can infect human as has been observed in vaccine trials with live attenuated animal Rota virus vaccine given to infants (Kapikian et al., 1986).

Studies throughout the world have established the important role of BRV, BCV as well as K99 producing enterotoxigenic E. coli in disease complex.

In Egypt, BRV was isolated for the first time by Shalaby et al. (1981). Since that time many studies were conducted to record the incidence of infection (Shalaby et al., 1987 and Iman et al., 1995).

An inactivated BRV vaccine was prepared and it produced an excellent immunity when used in calves (Iman et al., 1997). The present study aimed to produce a combined inactivated BRV and BCV vaccine. Its evaluation was conducted under laboratory conditions.

MATERIAL and METHODS

Materials:

1. **Calves:** Ten susceptible native breed calves, aged three months old, were used to study the safety and potency of the vaccine.
2. **Laboratory Animals:** Four adult guinea pigs and thirty adult mice were used to study the safety of the vaccine.

3. Viruses:

A. Bovine Rota Virus: Nebraska strain, was used for vaccine preparation, SNT and ELISA technique and was obtained from Virology Department, Faculty of Veterinary Medicine, Cairo University.

B. Bovine Corona Virus: Mebus strain, was used for vaccine preparation, SNT and ELISA technique and was also obtained from Virology Department, Faculty of Veterinary Medicine, Cairo University.

4. Antiserum: Both specific BRV and BCV antiserum were obtained from United States, Ames Iowa Laboratory and they were used to study purity of the vaccine.

5. Tissue culture: Madin Darby Bovine Kidney cell line (**Nagesha et al.,** 1985), was used for vaccine preparation and SNT.

Methods:

1. Preparation of combined inactivated BRV and BCV vaccine:

For vaccine preparation, each of BRV or BCV was passaged in MDBK cell line roller bottles tissue culture. When the cytopathic effect (CPE), reached 75%, the bottles were collected and preserved at -70°C till used.

At time of usage bottles were thawed and freezed for two times. The Supernatant viral fluids were then collected by centrifugation and the titre of the virus was calculated according to **Reed and Muench (1938)**. The titres were 10^8 and 10^7 for BRV and BCV, respectively. Viral fluid for each virus was tested separately to be free from other viruses by using their specific antiserum.

Inactivation was conducted by using Binary Ethyleneimine according to the method described by Iman et al. (1997). Inactivation was done for each virus separately.

Equal amounts of both inactivated BRV and BCV fluids were polled together in a sterile flask. Gelatin was added with a concentration of 0.5% for preparation of vaccine fluid.

Vaccinal fluid was distributed in 2 ml volume sterile neutral glass ampoules, sent for freeze drying and then finally preserved in -20°C till use.

2. ELISA technique:

ELISA microplates were coated with either BRV or BCV antigen with 1:50 dilution in carbonate-bicarbonate buffer and pH 9.6. The procedure was completed as described by Folken et al. (1980). The titre was calculated according to Synder et al. (1984).

3. Serum Neutralization Test:

The test was conducted according to the method described by Robson et al. (1960).

4. Trials of viral isolation :

Trials of viral isolation from swabs and buffy coat samples collected from vaccinated and control calves was attempted on MDBK cell line and according to the method described by Zeidan (1986).

EXPERIMENT and RESULTS

Experiment "1" (Purity Tests):

A. Purity from other viruses:

The test was conducted to confirm the purity of the vaccine from other viral agent and it was achieved by mixing equal amount (0.2 ml) of each viral fluid with its known specific hyperimmune serum. The mixed samples were incubated for 2 hours at 37°C. Each mixture was inoculated into 4 test tubes containing confluent layer of MDBK and examined daily for 10 days.

Result: No cytopathic effect was produced post inoculation (PI) of the virus antiserum mixture into MDBK culture cell line.

B. Purity from bacteria:

Samples from reconstituted freeze lyophilized inactivated combined BRV and BCV vaccines were inoculated into 4 tubes containing thioglycolate media to prove its freedom from bacteria.

Result: Neither aerobic nor anaerobic bacterial growth in the inoculated thioglycolate media and it remained clear for 10 days post inoculation.

Experiment "2" (Safety Tests):

A. Safety test in laboratory animals:

The test was conducted by using thirty adult mice and four guinea pigs. Ten mice were inoculated intracranially (I/C), with 0.02 ml of the vaccine that was reconstituted by using normal saline as diluent (2ml). Other 10 mice were inoculated intraperitoneally (I/P), with 0.2 ml, while the rest of the mice were kept as a control.

Two guinea pigs were inoculated I/P with 0.5 ml of the vaccine. While the other two guinea pigs were kept as a control.

All the laboratory animals were kept under observation for 10 days.

Result: All inoculated mice and guinea pigs as well as the controls were in good condition till the end of the observation period.

B. Safety test in calves:

The test was conducted by inoculation of three calves with 10 times of the vaccinal dose (20 ml). Another uninoculated three were kept as

control. Daily rectal temperature and clinical observations were recorded for 21 days. Buffy coat and swabs were collected on days 0, 3, 7, 10, 14 and 21 for trial of viral isolation.

Result: Both inoculated and control calves showed no signs of illness as well as no viral isolation was recorded from all the samples collected through the experiment.

Experiment "3" (Potency):

Potency test was conducted on 6 male susceptible calves. Three calves were vaccinated intramuscularly with two doses, three weeks apart; each doses contained 2 ml of the reconstituted vaccine. The rest of calves were unvaccinated and kept as a control. Serum samples were collected from vaccinated and control groups just before vaccination then after 3, 7, 10, 14, 21, 28, 35 and 60 days.

Result: The immune response towards vaccination with the combined inactivated BRV and BCV vaccine was evaluated by SNT and ELISA technique and the results are tabulated in tables (1 and 2) and illustrated in Fig. (1 and 2).

DISCUSSION

Neonatal calf diarrhoea has a complex etiology, Bovine Rota and Corona viruses appear to be of major importance and are considered the most etiologic agent of calf diarrhoea throughout the world. Moreover, Rota viruses have been demonstrated to be a major cause of neonatal diarrhoea in most species of animals and humans.

In Egypt, incrimination of BRV and BCV in mild and severe diarrhoeal cases were recorded by Iman et al. (1995); Ikram et al. (1990) and Shalaby et al. (1981). Clinical signs range from mild to severe diarrhoea resulting in depression, dehydration and death. the disease severity is influenced by infection with other entero-pathogens as well as environmental factors.

The present study aimed for production of a combined inactivated Rota and Corona viruses vaccine. The prepared vaccine was proved to be free from other heterogenous viral agent as it was completely neutralized by its specific antibodies. Cultivation of the vaccinal fluid on thioglycolate proved its purity from aerobic and anaerobic bacteria.

Animal inoculation proved the safety of the vaccine as the inoculated guinea pigs and mice remained in a good condition all over the experiment which considered another prove for the purity of the vaccine from any

pathogenic agent. Moreover, vaccinated calves showed neither clinical signs nor an elevation in temperature during the observation period as well as no shedding of the virus. These results agreed with those obtained by Mebus et al. (1973); Twiehaus et al. (1975) and Saif et al. (1984)

Post vaccinal developed antibodies were recorded for BRV and BCV by SNT and ELISA technique. Serum neutralizing antibodies were detected for both viruses at 10th days PV with a mean $\log_{10}$ serum neutralizing antibody titres of 0.5 and 0.7 for BRV and BCV, respectively. These results agree with those obtained by ELISA technique since the mean ELISA titres were increased to 1399 and 1166, respectively. The developed specific antibodies were increased and reached its peak at the 35th day PV and remained high till the end of the 2nd month PV.

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Table 1 : BRV immune response in calves post vaccination with combined inactivated BRV and BCV vaccine by SNT.

Days post vaccination	Log_{10} Serum Neutralizing Antibody Titre				
	Animal Number			Mean Titre	SD
	1	2	3		
Zero	-	-	-	-	-
3	-	-	-	-	-
7	-	-	-	-	-
10	0.6	0.6	0.3	0.5	± 0.17
14	0.9	0.9	0.6	0.8	± 0.14
21	1.2	1.2	0.9	1.0	± 0.14
28	1.5	1.5	1.2	1.4	± 0.17
35	1.8	1.8	1.5	1.7	± 0.17
60	1.8	1.8	1.5	1.7	± 0.17

Table 2: BCV immune response in calves post vaccination with combined inactivated BRV and BCV vaccine by SNT.

Days post vaccination	Log_{10} Serum Neutralizing Antibody Titre				
	Animal Number			Mean Titre	SD
	1	2	3		
Zero	-	-	-	-	-
3	-	-	-	-	-
7	-	-	-	-	-
10	0.6	0.9	0.6	0.7	± 0.17
14	0.9	1.2	0.9	1.0	± 0.14
21	1.2	1.5	1.2	1.3	± 0.14
28	1.5	1.8	1.5	1.6	± 0.17
35	1.8	2.1	1.8	1.9	± 0.17
60	1.8	2.1	1.8	1.9	± 0.17

BRV: Bovine Rota Virus. SD: Standard Deviation.

BCV: Bovine Corona Virus.

- : No antibodies could be detected.

Control calves: No serum neutralizing antibodies could be detected allover the experiment.

Table 3: ELISA antibody titre against BRV in calves post vaccination with combined inactivated BRV and BCV vaccine*.

Days post vaccination	ELISA antibody titre			
	Animal Number			Mean Titre
	1	2	3	
Zero	209	210	142	187
3	408	460	378	415
7	650	690	508	616
10	1450	1490	1257	1399
14	3020	3388	2693	3033
21	4281	4460	3988	4243
28	5637	5830	4980	5482
35	7792	7850	6110	7250
60	7800	7990	6190	7326

Table 4: ELISA antibody titre against BCV in calves post vaccination with combined inactivated BRV and BCV vaccine*.

Days post vaccination	ELISA antibody titre			
	Animal Number			Mean Titre
	1	2	3	
Zero	110	231	148	148
3	326	450	367	381
7	639	727	650	672
10	1130	1250	1110	1166
14	3450	4150	3320	3640
21	4162	5120	4100	4460
28	5130	6124	5003	5086
35	7800	7900	7010	7570
60	7850	7890	7030	7590

BRV: Bovine Rota Virus. BCV: Bovine Corona Virus.

*: Mean ELISA antibody titre in control calves were <400 allover the experiment.

Fig. (1) : BRV and BCV immune response in calves post vaccination with combined inactivated BRV and BCV vaccine by SNT

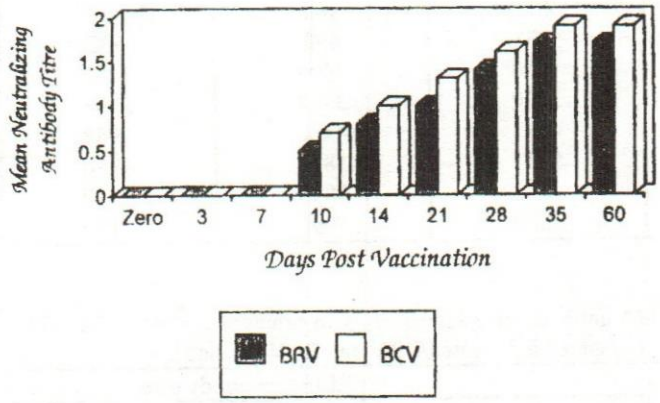

Fig. (2) : ELISA antibody titre against BRV and BCV in calves post vaccination with combined inactivated BRV and BCV vaccine.