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SOME STUDIES ON THE IMMUNITY OF RODENTS VACCINATED WITH COMBINED ROTA VIRUS (RV), CORONA VIRUS (CV) AND K99 *E.COLI* VACCINE AND ITS ROLE IN EPIZOOTIOLOGY OF THE DISEASE

SHAFAY, S.M.[1], K.A. IMAN[2], M.S. WASSEL[1], AND S. M. ZEIDAN[2]

1 Central Laboratory for Evaluation of Veterinary Biologics, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

2 Veterinary Serum and Vaccine Research Instititute, Agricultural Reasrch Centre, Ministry of Agriculture, Giza, Egypt.

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Abstract

In this study, a combined vaccine of rota virus, corona virus and enterotoxigenic K99 *E.. coli* (ETEC) was evaluated against safety, sterility and potency in pregnant mice, as well as, in their infants. The antibody level was measured in dams and their infants, using serum neutralization test for rota, corona viruses, microagglutination test and mouse protection test for K99 *E..coli* .A challenge test was conducted in vaccinated mice, as well as, in their infants using virulent strain of rota, corona viruses and K99 *E.. coli* strain and in control group. Trials for reisolation of these challengin strain were done and confirmed by using indirect fluorescent antibogdy technique (IFA) for rota and corona viruses and specific media and biochemical test for K99 *E..coli* strain. The vaccine was safe and potent for dams mice and their infants as the level of antibody titres, by using microagglutination test, was 430 geometric mean "GM" for dams mice and 355 GM for in fant mice, where the protective level was 80 GM. The mouse protection test in dams mice reached 100% and in infant mice was 80% . The serum neutralization titre (SNT) was 2.1$\log_{10}$ SN titre for dams and 1.2 $\log_{10}$ titre for infants for rota virus and 2.1$\log_{10}$ SN titre dams, 1.5 $\log_{10}$ SN titre for corona virus, where the protective level was 0.6 $\log_{10}$ SN titre for rota and corona visuses. The vaccine protected infant mice against challenge with virulent strains and study emphathized the transmission of passive immunity from vaccinated dams mice to their infant mice .It was clear that, mice play an important role in transmission of infection through their faeces to contaminate animal rations, as well as, human food .

INTRODUCTION

Diarrhoea due to diseases is considered one of the most important causes of deaths, specially during the early few weeks of life in which a considerable number of animals could be lost. Rota and corona viruses being the most dominant causative agent, in combination with enterotoxigenic *Escherichia coli* strain (Snodgrass *et al.,* 1986).

Animal rota virus can infect humans as has been observed in vaccine trials with the attenuated rota virus vaccine griven to infants (Kapikian *et al .,* 1976). Conversely, human rota virus strains can experimentally infect animals and induce diarrhoea illness (Mebus *et al .,* 1976). Genetic and antigenic relatedness of human and animal strains of antigenically distinct rota viruses were studied by Eiden *et al .* (1986).

Two approaches have been used to protect newborn calves from rota viral infection ; one is direct vaccination of newborn calves to elicit active immunity (Twiehaus *et al .,* 1975), and the other, is to immunize pregnant dams to provide passive immunity to their suckling calves via immune colostrum and milk (Snodgrass *et al.,* 1982 and Castruccil *et al.,* 1984) .

The aim of the present study was to vaccinate pregnant mice with combined inactivated rota, corona and enterotoxigenic K99 *E. coli* vaccine, monitoring the pasive immunity in their infant mice, and studying the role of rodents in transmission of the infection of rota, corona and *E. Coli* to human and animals.

MATERIALS AND METHODS

Materials

Microorganisms

A- Viruses

a. Rota virus : Nebraska strain of rota virus (RV) was used for vaccine preparation and serum neutralization. The virus was kindly supplied from Blue Tongue and Rinderpest Like Diseases Dept., Vet. Serum and Vaccine Research Institute, Abbasia, Cairo, Egypt.

b. Corona virus : Mebus strain of corona virus (CV) was used for vaccine preparation and serum neutralization test. The virus was obtained from Virology Dept.

Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

B- Bacterium

Enterotoxigenic *Escherichia* coil K99 strain (ETEC), was kindly supplied from Animal Reproduction Research Institute, Giza, Egypt.

Tissue culture

Madin Darby Bovine Kidney (MDBK) cell line (Nagesha *et al.*, 1985) was used for vaccine preparation and serum neutralization.

Mice

Two-hundred adult pregnant Albino mice of 50 g weight and their infants were used in this experiment.

Methods

Preparation of combined inactivated BRV, BCV and enterotoxigenic K99 *E. coli* vaccine

Combined inactivated BRV and BCV viruses were prepared and inactivated by binary ethyleneimine according to the method described by Iman *et al.*, (1997). Inactivation was done for each virus separately.

Inactivated enterotoxigenic *Escherichia coli* K99culture was prepared according to Dauvergene *et al.* (1983). Mixture of two parts of inactivated viruses with one part of inactivated culture of K99 *E. coli* strain, sterile alum gel was used as an adjuvant in concentration of 20% as the method described by Dauvergene *et al.* (1983).

Sterility test

The Sterility tests were conducted according to US Code of Federal Regulations (1987), 9 CFR, 113.26,113.27,113.30 and 113.55

Vaccination

Pregnant mice were vaccinated intraperitoneally (I/P) with 0.1 ml of combined vaccine with 2 doses one week apart.

Sampling

1. Blood samples were taken from tail vein from the vaccinated dams and slaughtered infant mice of 10 days after their birth, and the collected sera were kept at-20°C for serological assays.

2. faecal swabs were taken from vaccinated challenge, infected and control mice.

Safetytest

The test was conducted according to the method described by Iman *et al.* (1997) by using 30 adult mice.

Serum Neutralization Test

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

Microagglutination test

The used technique was that of Collins *et al* . (1988).

Passive mouse protection test

The technique was used as described by Cameron and Fuls (1970).

Indirect fluorescent antibody technique

Slides from faecal samples and internal organs were collected post-challenge from vaccinated and control dams and their infants. The slides were fixed with absolute acetone for 20 minutes and kept for examination according to the method describede by Zeidan (1990) .

Challenqe test

a. Mice were challenged orally by administration of 10^6 $TCID_{50}$ / ml of rota virus and 10^6 $TCID_{50}$ / ml of corona virus according to Vonderfecht *et al* . (1984).

b. Mice were challenged orally with 10 ID_{50} of virulent enterotoxigenic K99 *E.coli* strain according to Camguilhem and Milon (1990).

Trials of viral reisolation

Trials of Viral reisolation from faeces and internal organs collected post-challenge from vaccinated and control dams mice and their infants 10 days after birth were conducted according to the method described by Vonderfecht *et al.* (1984).

Trials of bacterial reisolation

Trials of bacterial reisolation was conducted on intestine and faeces collected from vaccinated and control dams and their infants post-challenge with enterotoxigenic K99 *E. coli* virulent strain according to the method described by Youssef (1999).

RESULTS

The neutralization test SNT in vaccinated mice and in the their infants represented in Table 1 showed that in infants, the titres reached 1.2 and 1.5 $\log_{10}$ SNT for rota and corona viruses, respectively 10 day after birth, and the titre 2.1 $\log_{10}$ SNTfor Rota and Corona viruses on the 21 st day post-vaccination for dams mice. The development of antibody titres of k99 *E. coli* in vaccinated adult mice and their infants represented in tables was proved by microagglutination test and mouse protection test (Tables 2 and 3).

The challenge with virulent strains of RV, CV and virulent enterotoxigenic K99 *E. coli* strain in vaccinated and control mice and their infants are represented in tables 4 and 5, repectively. The manifestations are varied from poorly formed fecal pellets to liquid and mild bleeding around anus observed in rota challenged control infants.

The trials of rota and corona viruses reisolation are represented in Table 6. Ressults of reisolation of virulent enterotoxigenic K99 *E. coli* strain are recorded in Table 5. In control challenged mice and their infants, the virus was reisolatd from first day post-challenge (PC) till 10 days, with a peak of reisolation on the first day PC. The highest rate of reisolation was obtained from fecal content of the intestine.

The indirect immunofluorescent antibody technique on samples collected from vaccinated, vaccinated challenged and control challenged mice and their infants are repesented in Table 7 and illustrations 1and 2. The results proved that the rota and corona antigens are highly detected in intestinal wall and intestinal content in the control infected group.

DISCUSSION

Rota, corona viruses and enterotoxigenic *Escherichia coli* K99 strain are the major causes of diarrhoea in young animals, as well as, human being causing highly economic losses in farm animals. Many trials have been conducted to vaccinate pregnant dams (Snodgrass and Wells, 1978), and to vaccinate calves (Iman *et al.*, 1977),and these vaccines were safe and potent when used to protect calves.

The high antibody titres prduced by vaccination of pregnant mice and their infants in the present study protected them when challenged with the virulent strains of rota, corona and K_{99} enterotoxigenic *E. coli.* The antibody titres obtained in sera of dams mice and their infants (passive immunity) are considered of good protective level . These results agreed with Castrucci *et al.* (1984), Burki *et al* . (1986) and Snodgrass *et al.* (1986).

Our results showed that rats could contract rota, corona viruses and K99 *E. Coli* via an oral route, and the viruses and bacteria could be shed in faeces,and wherever the rats moved they could disseminate the diseases. This agreed with Eiden *et al.* (1986).

The absence of diarrhoea in protected infant mice is due to the increased level of passive antibodies in the colostrum and milk of dam mice, as well as, in the intestinal lumen of infant mice. This is in agreement with Snodgras *et al.* (1982).

From all the above mentioned results, it could be said that vaccintion of dams with inactivated combined vaccine of rota, corona and K99 *E.coli* could be used safely to protect their infants from diarrhoea.

Table 1. Active and passive immunity in mice against rota and corona viruses post vaccination with combined inactivated rota and corona viruses and *E.coli* vaccine.

Animal group	Antagonis virus	Mean log_{10} serum neutralizing antibody titre in time post vaccination									
		1st dose Days post vaccination					2nd dose				
		0	3rd	7th	10th		3rd	7th	10th	14th	21th
Dams	Rota	0	0.3	0.42	0.6		0.9	1.2	1.5	2.1	2.1
	Corona	0	0.3	0.36	0.72		0.9	1.2	1.7	1.9	2.1
Infant mice		They wasn't born yet					infants 10 days after birth				
	Rota						0.36	0.62	0.72	1.2	1.2
	Corona						0.42	0.72	0.9	1.2	1.5

N.B : Vaccination was done only on adult mice while the infants were born after one week from 2nd dose of vaccination.

Table 2. Results of microaglutination geometric mean (GM) antibody titres in pregnant mice (dams) vaccinated with combined vaccine of enterotoxigenic K99 *E. coli* strain (ETEC), rota and corona viruses and their suckling mice by using K99 *E. coli* antigen.

Days post vaccination	Number of tested sera of dams	GM antibody titre in sera of pregnant mice (dams)	Number of tested sera of infant mice	GM antibody titre in sera of infants mice (10 days after birth)
Pre-vaccination	30	10	-	-
1ry vaccination 7 days post vaccination	20	379.1	20	320
2ry vaccination 7 days post vaccination	20	430	20	355
Control	10	10	10	0

GM : Geometric Mean.

Table 3. Results of mouse protection test in serum samples of dams' mice vaccinated with combined vaccine of enterotoxigenic K99 *E. coli* strain ETEC rota and corona viruses and serum samples of their suckling mice using virulent *E. coli* strain .

Days post vaccination	No. of mice group	% of passive mouse protection in serum samples			No. of mice group	% of passive mouse protection in sera of suckling mice (10 days after birth)		
		D	S	% of protection		D	S	% of protection
Pre-vaccination	10	10	0	0	10	10	0	0
1ry vaccination 7 days post – vaccination	10	2	8	80	10	3	7	70
2ry vaccination 7 days post – vaccination	10	0	10	100	10	2	8	80
Non vaccinated Control dams & suckling mice	10	10	0	0	10	10	0	0

D: Dead within 24-96 hours.

Table 4. Percentage of Protection in vaccinated and control mice and their infants* post-challenge with rota and corona viruses.

Challenged group		No.	Inoculated virus	2nd DPC		4th DPC		7 DPC	
				D/N	P %	D/N	P %	D/N	P %
Vaccination	Adult	10	Rota	0/10	100	0/10	100	0/10	100
		10	Corona	0/10	100	0/10	100	0/10	100
	infants	15	Rota	0/15	100	0/15	100	0/15	100
		15	Corona	1/15	93.33	1/15	93.33	1/15	93.33
Control	Adult	10	Rota	8/10	20	9/10	10	9/10	10
		10	Corona	7/10	30	7/10	30	8/10	20
	infants	15	Rota	13/15	13.33	13/15	13.33	13/15	13.33
		15	Corona	14/15	6.66	15/15	0	15/15	0

No. : Mice numbers. D/N : Diseased/Normal.
DPC : Days Post-Challenge. P : Protection.
* Infant 10 days old.

Table 5. Immunizing efficacy of combined vaccine (K99) *E. coli*, Rota virus and Corona virus) by chal viulent enterotoxigenic K99 *E.coli* strain.

Groups of challenged mice	No. of challenged mice	D/N 2nd day post challenge	Protection %	D/N 3rd day post challenge	Protection %	D/N 4th day post challenge	Protection %	Overall mean protection %	Mean disease time (hours)	Lesion score			Organism reisolation
										Weak	Moderate	Severe	
Adult vaccinated mice	20	0/20	100	1/20	95	1/20	95	97.5	96	1	-	-	-
Adult control mice	10	8/10	20	9/10	10	9/10	10	15	48	-	-	9	+
Suckling mice from vaccinated dams *	20	0/20	100	2/20	90	2/20	90	95	72	2	-	-	-
Control suckling mice	10	9/10	10	10/10	0	10/10	0	10	48	-	-	10	+

D/N : Diseased / Normal.

* Mice of 10 days after birth.

Table 6. Reisolation of Rota and Corona viruses from vaccinated challenged, control challengred dams and their infants mice.

Time / day	Isolated virus	Vaccinated Challenged							Control Challenged						
		Dams mice			Infants			VRA/D	Dams mice			Infants			VRA/D
		F.	In.	O.M.	F.	In.	O.M.		F.	In.	O.M.	F.	In.	O.M.	
1	Rota	0/10	1/5	0/5	0/10	2/5	0/5	3/40	5/10	5/5	0/5	3/5	5/5	0/5	23/40
3		0/10	0/5	0/5	0/10	0/5	0/5	0/40	5/10	3/5	0/5	3/5	4/5	0/5	18/40
7		0/10	0/5	0/5	0/10	0/5	0/5	0/40	1/10	3/5	0/5	4/5	2/5	0/5	10/40
10		0/10	0/5	0/5	0/10	0/5	0/5	0/40	0/10	2/5	0/5	0/5	0/5	0/5	2/40
VRA/S		0/40	1/20	0/20	0/40	2/20	0/20		11/40	13/20	0/20	10/20	11/20	0/20	
1	Corona	0/10	2/5	0/5	0/10	0/5	0/5	2/40	6/10	5/5	1/5	6/10	4/5	2/5	24/40
3		0/10	0/5	0/5	0/10	0/5	0/5	0/40	4/10	4/5	1/5	6/10	5/5	0/5	20/40
7		0/10	0/5	0/5	0/10	0/5	0/5	0/40	3/10	4/5	0/5	4/10	4/5	0/5	15/40
10		0/10	0/5	0/5	0/10	0/5	0/5	0/40	4/10	3/5	0/5	5/10	2/5	0/5	14/40
VRA/S		0/40	2/20	0/20	0/40	0/20	0/20		17/40	16/20	2/20	21/40	15/20	2/20	

F. : Faeces. In. : Intestine O.M. : Organ MiXture.

VRA/D : Virus Reisolation Average / Day VRA/D : Virus Reisolation Average /Sample.

Table 7. Detection of Rota and Corona virus antigen in internal organs, intestine and faeces of vaccinated challenged and control challenged mice by indirect immunofluorescent antibody techique.

Animal Group	Detected Antigen	Intestine	Feaces
Vaccinated challenged Adult mice	Rota	-	-
	Corona	-	-
Infant mice	Rota	-	-
	Corona	-	-
Control challenged Adult mice	Rota	+++	++
	Corona	++	++
Control infected Infants mice	Rota	++	++
	Corona	++	++

- Negative for antigen detection.
+ Positive for antigen detection when using specific antibodies.

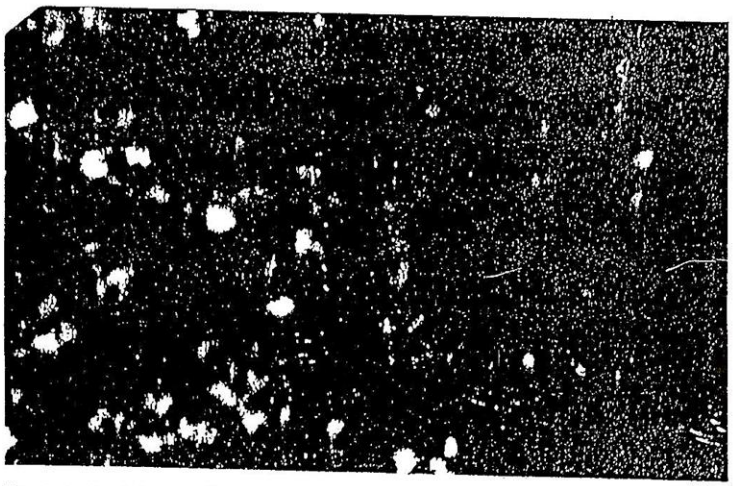

Fig. 1. Indirect immunofluorescent technique (IFA) detecting rota virus in intestinal content of control infected mice (X10).

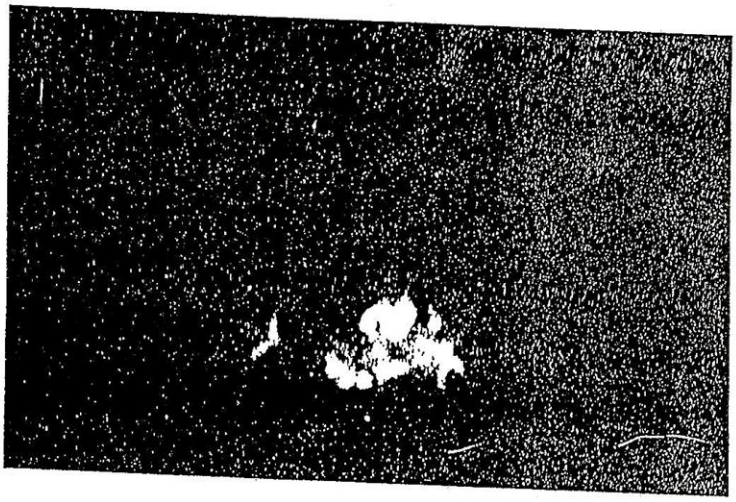

Fig. .2. Indirect immunofluorescent technique detecting corona virus in intestinal content of control infected mice (X10).

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بعض الدراسات المناعية فى القوارض المحصنة بلقاح مركب من فيروس الروتا والكورنا مع التهاب القولون المعدى ودورها فى نشر المرض

سامى محمد شافعى [١]، ايمان كامل احمد [٢]،
محمد سعيد واصل [١]، سيد محمد زيدان [٢]،

١- المعمل المركزى للرقابة على المستحضرات الحيوية البيطرية -مركز البحوث الزراعية - وزارة الزراعة - الجيزة - مصر.
٢- معهد بحوث الامصال واللقاحات البيطرية - مركز البحوث الزراعية -وزارة الزراعة - الجيزة - مصر .

فى هذه الدراسة تم تقييم لقاح جامع للروتا والكورنا والايشريشيا كولاى فى فئران حوامل من حيث النقاوة والسلامة وقياس المستوى المناعى فى الامهات وكذلك فى النتاج باستخدام الطرق السيرولوجية المختلفة مثل اختبار التعادل فى السيرم واختيار التلازن الدقيق. وتم عمل اختبار للفئران المحصنة والضابطة ونتاجها بالعترات الضارية للروتا والكورونا والايشريشيا كولاى ك ٩٩. وتم اعادة عزل العترات المختلفة من الضوابط مع التعرف على عترات الروتا والكورنا باستخدام اختبار الفلورسنت المشع الغير مباشر والتعرف على ميكروب الايشريشيا كولاى باختبار الكيمياء الحيوية. وأعطى النتاج مقاومة لاختبار التحدى ومستوى المناعة ٢,١ لوج ١٠ بالنسبة لفيروس الروتا (أمهات) ، ١,٢ لوج ١٠ (نتاج) ، ٢,١ لوج ١٠ بالنسبة لفيروس الكورنا (امهات)، ١,٥ لوج ١٠ (نتاج) وذلك باختبار التعادل فى السيرم. وعند استخدام اختبار التلازن الدقيق بالنسبة لميكروب ايشريشيا كولاى اعطى متوسط مناعى هندسى لامهات ٤٣٠ وللنتاج ٣٥٥ وبالنسبة لاختبار التحدي فى الفئران فأعطت مستوى حماية للأمهات ١٠٠ ٪ وللنتاج ٨٠٪ وكان اللقاح آمن فى اختبار السلامة.

ويتضح مما سبق انتقال المناعة من الام الى النتاج بمستوى حماية مرتفع بما يعزى كفاءة هذا اللقاح الحماية من مسببات الاسهال فى الفئران الصغيرة كما اكدت الدراسة ان الفئران لها دور فى انتقال العدوى لمزارع العجول ولغذاء الانسان اذا تعرض للتلوث بتلك العترات.