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PREPARATION AND EVALUATION OF AN INACTIVATED COMBINED VACCINE OF BOVINE ROTA, CORONA VIRUSES AND K99 ENTEROTOXIGENIC *ESCHERICHIA COLI*

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Abstract

A formalin inactivated alum gel adjuvated combined vaccine of bovine rotavirus (BRV), bovine coronavirus (BCV) and K99 enterotoxigenic *Escherichia coli* (ETEC) was prepared and evaluated. Ten susceptible calves were vaccinated intramuscularly twice 2 weeks apart using 2 ml/calf. The vaccine was tested for safety in calves and mice and for sterility. Sera of immunized calves showed high antibody levels (4-8 log2 for both BRV and BCV) using serum neutralization test. The ELISA antibody titres were also satisfactory high for the three vaccine components. Passive mouse protection test revealed 80-100% protection against K99 ETEC component. The microagglutination test was also used for evaluation of the potency of K99 ETEC and the titres obtained reached 320 and 1810 at 2 and 8 weeks postvaccination, respectively.

It is recommended to prepare the trivalent vaccine locally for control of diarrhoea in calves.

INTRODUCTION

The diarrhoeal disease syndrome has a complex etio-pathogenesis because various infectious agents, whether alone or in combination, are involved in its causation (Cilli and Castrucci, 1981, Tzipori, 1985, and Mohamed, 1995).

The role of bovine rotavirus (BRV), bovifne coronavirus virus (BCV), and enterotoxigenic *Escherichia coli* (ETEC) in diarrhoea of neonatal calves is well known with major impact through mortality (Morin *et al.*, 1978, Snodgrass *et al.*, 1986 and Mohamed, 1995).

Attempts were made to control the disease syndrome by vaccination of new born calves (Mebus *et al.*, 1973). However, these attempts were unsatisfactory.

Another approach was made by vaccination of cows to secrete antibodies in their colostrum and milk to protect their calves in the first days and weeks of age (Snodgrass *et al.*,1980; Van-Opdenbosch *et al.*, 1981, Castrucci *et al.*, 1984).

The purpose of the present work is to prepare and evaluate an inactivated combined vaccine for the first time in Egypt that would protect vaccinated calves agaist BRV, BCV and K99 ETEC infections.

MATERIALS AND METHODS

Viruses and Antisera

Nebraska strains of BRV and BCV and their antisera were kindly supplied by the National Veterinary Services Laboratories, Ames Iowa, USA.

Bacterium

E.coli K99 strain was obtained from the Animal Reproduction Research Institute, Giza, Egypt.

Cell culture

Monolayers screened MDBK cell cultures were grown and maintained as described by Chasey (1977) and Dea *et al.* (1980).

Animals

Eighty-five Swiss Albino mice, 21 days old, and ten susceptible calves, 6 months old, were used.

Vaccines

a. BRV alum gel inactivated vaccine was prepared according to Wassel (1996) using alum gel as adjuvant.

b. BCV alum gel inactivated vaccine through adaptation of BCV to MDBK cells and trypsin 20 µg/ml MEM as described by Dea *et al.* (1980) to obtain high titre of 7 $\log_{10}$ $TCID_{50}$/ml. After the 10th passage, the virus was inactivated by overnight (18 hours) incubation at 4°C with 0.5% formaldehyde, mixed with addition of alum gel (20% final concentration) (Dauvergene *et al.*, 1983). Prepared virus vaccines were stored at 4°C.

c. *E.coli* K_{99} (enterotoxigenic K_{99} strain), alum gel vaccine (whole cell bacterin) was prepared according to Myers (1980) and Acresi *et al.* (1982).

d. Combined inactivated vaccine composed of BRV, BCV and K_{99} ETEC prepared sep-

arately and inactivated with 0.5% formalin for 18 hours at 4°C were mixed equally together, and then, alum gel (20% final concentration) was added. The concentration of each antigen before inactivation was (BRV 6.5 $\log_{10}$ $TCID_{50}$/ml, BCV 7 $\log_{10}$ $TCID_{50}$/ml and, $6X10^{10}$ bacterial cells/ml of *E.coli* K_{99} as used by Mebus *et al.* (1973); Danieli *et al.* (1979), Dea *et al.* (1980), and Castrucci *et al.* (1984), respectively. The combined vaccine was given intramuscularly (I/M) at a dose of 2 ml/animal.

Vaccine evaluation

The monovalent, as well as, the multivalent prepared vaccines were evaluated according to the following:

a. Purity test

In accordance with the United States Code of Federal Regulations (CFR) (1987), testing 9CFR 113.26, 113.27, 113.30 and 113.55.

b. Safety test

It was according to 9 CFR (1987), testing 113.41 (Calves and mice were used in this study).

c. Potency tests

1. Against BRV and BCV components as measured by seroconversion

i. Serum neutralization test (SNT)

This was carried out using the MDBK cell culture method according to Dauvergene *et al.* (1983); Castrucci *et al.* (1984) and Wassel (1996).

ii. Enzyme Linked Immuno Sorbent Assay (ELISA)

Antigen preparation against BRV and BCV and the test technique were described by Eman *et al.* (1995) and Mohamed (1995).

2. Against K_{99} ETEC

i. Microagglutination Test

Antigen preparation and the test techniques were according to Collins *et al.*

(1988), and geometric mean titres were calculated according to Max (1977).

ii. Enzyme Linked Immuno Sorbent Assay (ELISA)

Antigen preparation and test technique used were as described by Mettias *et al.* (1994) and Mohamed (1995).

iii. Passive mouse protection test

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

RESULTS

The preliminary studies of inactivated alum gel adjuvated combined and monovalent BRV, BCV and K_{99} ETEC vaccines gave satisfactory results in sterility and safety tests of 10 x dose in calves and mice.

The results of SNT are shown in Table 1. The antibody response of vaccinated calves peaked to 8 $\log_2$ and 16 $\log_2$ on the 8th week post vaccination (PV) with two doses 2 weeks apart against both viral components in the combined trivalent (BRV, BCV and K_{99} ETEC) and monovalent vaccines, respectively.

The serum levels of antibody titres, as measured by ELISA, are shown in Table 2. With the combined trivalent vaccine, they peaked to 6087 for BRV, 5433 for BCV and 8472 for K_{99} ETEC on the 8th week PV versus 6368, 5610 and 8933, respectively, for the monovalent vaccines of the three components in the combined vaccine.

Results of the passive mouse protection test with serum of vaccinated calves as given in Table 3. For the component K_{99} ETEC in the combined trivalent and monovalent vaccines results showed good protection (80-100%), 2-8 weeks PV compared to serum from non-vaccinated calves.

Agglutination antibody titres to K_{99} ETEC in calf sera vaccinated with combined trivalent and monovalent vaccines of K_{99} ETEC as shown in Table 4 peaked to 1810 and 2560 by the 8th week PV.

Generally, the results of seroconversion of all monovalent vaccines in the present study nearly matched those of the combined trivalent vaccine (Tables1, 2, 3 and 4).

Table 1. Immune response of calves vaccinated with combined trivalent and monovalent inactivated alum gel vaccines of BRV, BCV and K_{99} ETEC as measured by SNT.

Type of vaccine	Mean of serum neutralization tites* post-vaccination						
	Weeks Post-vaccination						
	0		2		5	6	8
BRV Combined vaccine	0.0		2.0		4.0	8.0	8.0
BRV Monovalent vaccine	0.0		2.0		4.0	8.0	16.0
BCV Combined vaccine	0.0	1st vaccination	2.0	2nd vaccination	4.0	8.0	8.0
BCV Monovalent vaccine	0.0		2.0		4.0	8.0	16.0
Non Vaccinated control calves	0.0		0.0		0.0	0.0	0.0

Titres expreseed as the reciprocal of $\log_2$ serum dilution

DISCUSSION

Rota and corona viruses and enterotoxigenic strain of *E.coli* seem to be the most commonly involved pathogens in the etiology of diarrhoea of young farm animals that cause serious economic losses threatning animal production in Egypt. As there is no locally prepared vaccine against rota and corona viruses and K99 ETEC in Egypt, trial was done to prepare and evaluate a formalin-inactivated, alum gel adjuvated trivalent vaccine containing BRV, BCV and K_{99} ETEC.

The satisfactory high antibody titres given by vaccinated calves in the present study might be attributed to the enhancing effect of adjuvant. Denis *et al.* (1988) emphasized the importance of adjuvant in increasing the neutralizing antibody in sera of BRV-vaccinated animals.

Our results indicated that, inactivated BRV and BCV are efficacious when combined with K_{99} ETEC. The results indicated that the combined vaccine can be prepared uniformly from individual lots of inactivated BRV, BCV and K_{99} ETEC vaccines.

Protective neutralizing titres were obtained already 5 weeks post-vaccination of calves with 2 vaccine doese (4 $\log_2$ for both BRV and BCV components), which are considered as good response (Van Opdenbosch *et al.*, 1981; Dauvergene *et al.*, 1983; Castrucci *et al.*, 1984; Snodgrass *et al.*, 1986 and Peters, 1993).

Similarly, the level of antibody titres, as measured by ELISA was satisfactory for all components of combined vaccine, which is in agreement with Mettias *et al.* (1994) and Mohamed (1995).

The result of passive immunization of mice with immune sera from vaccinted calves which were then challenged by lethal dose (10^7 CFU/ml) of virulent K_{99} ETEC indicated that the combined and monovalen of K_{99} ETEC gave good protection from the 2nd-the 8th week PV which agreed with Cameron and Fuls (1970).

Also, the microagglutination test for antibodies to K_{99} ETEC revealed high geometric mean titres (320-1810) from the 2nd-the 8th week PV with the combined vaccine which agreed with the findings of Collins *et al.* (1988). It could be concluded from all above mentioned results that the combined BRV, BCV and K_{99} ETEC can be prepared locally for commercial use to control diarrhoea in calves.

Table 2. Level of antibodies in calf sera following vaccination with trivalent and monovalent inactivated of combined alum gel vaccines of BRV, BCV and K99 ETEC as measured by ELISA.

Type of vaccine	Average ELISA titre*						
	Weeks Post - Vaccination						
	0		2		5	6	8
BRV Combined vaccine	<1000	1st vaccination	1130	2nd vaccination	1432	3750	6087
BRV Monovalent vaccine	<1000		1146		1758	4046	6368
BCV Combined vaccine	<1000		1016		1786	3768	5433
BCV Monovalent vaccine	<1000		1021		1914	4576	5610
K_{99} ETEC Combined vaccine	<1000		1135		2492	5383	8472
K_{99} ETEC Monovalent vaccine	<1000		1125		2946	5933	8933
Non vaccinated control calves	<1000		<1000		<1000	<1000	<100

* Average of two sera

Table 3. Mouse protection test for K_{99} ETEC with serum of calves vaccinated with combined trivalent vaccine (K_{99} ETEC, BRV and BCV compared with monovalent K_{99} ETEC vaccine.

Type of vaccine	No.of mice/ group	Percent of passive mouse protection post-vaccination																	
		Weeks Post Vaccination																	
		0				2				5			6			8			
		D	S	%		D	S	%		D	S	%	D	S	%	D	S	%	
K_{99} ETEC Combined vaccine	5	5	0	0		1	4	80		1	4	80	0	5	100	0	5	100	
K_{99} ETEC Monovalent vaccine	5	5	0	0	1st vaccination	1	4	80	2nd vaccination	1	4	80	0	5	100	0	5	100	
Non vaccinated positive control calves	5	5	0	0		5	0	0		5	0	0	5	0	0	5	0	0	

D : Dead within 24-72 hours.

S : Survival within 24-72 hours.

Table 4. Microagglutination of sera for K_{99} ETEC antibodies in calves vaccinated with combined trivalent vaccine of K_{99} ETEC, BRV and BCV as compared with monovalent K_{99} *E.coli* vaccine.

Type of vaccine used	Geometric mean titres post-vaccination							
	0 day		2 Weeks		5 Weeks		6 Weeks	8 Weeks
K_{99} ETEC Combined vaccine	first vaccination	20		320	second vaccination	905	1810	1810
K_{99} ETEC Monovalent vaccine		20		640		1280	1810	2560
Non vaccinated control calves		20		20		20	20	20

Titres expressed as the reciprocal of the dilution (Geometric mean).

REFERENCES

1. Acres, S.D., A.J. Forman and R.A. Kapitany. 1982. Antigen extinction profile in pregnant cows using a K_{99} containing whole cell bacterin to induce passive protection against enterotoxigenic colibacillosis of calves. Am. J. Vet. Res., 433 (4): 576-575.
2. Cameron, C.M. and W.J.P. Fuls. 1970. Immune response to dead and live E. coli vaccines and colostral transfer of immunity to calves and lambs. Onderstepoort J. Vet. Res., 37 (3): 157-164.
3. Castrucci, C., F. Frigeri, Mm. Ferrari, V. Cilli, F. Caleffi, V. Aldrovandi and A. Nigrelli. 1984. The efficacy of colostrum from cows vaccinated with rotavirus in protectin calves to experimentally induced rotavirus infection. Comp. Immun. Microbiol. Infect. Dis., 7 (1): 11-18.
4. Chasey, D. 1977. Different particles types in tissue cultures and intestinal epithelium infected with rotavirus. J. Gen. Virol., 37: 442-451.
5. Cilli, V. and G. Castrucci. 1981. Viral diarrhoea of young animals. Comp. Immun. Microbiol. Infect. Dis., 4 (5): 229-242.
6. Collins, N.F., T. Halbur. W.H. Schwenck. P. Hoogeveen, R.L. Pierce, R.W. Behan and D. Pankratz. 1988. Duration of immunity and efficacy of an oil emulsion E.coli bacterin in cattle. Am. J. Vet. Res, 49 (5): 674-677.
7. Danieli, Y., I., Komitzer, K. Tamarin, S. Ram, and Y.Haber. 1979. Prevention of neonatal diarrhoea in calves by vaccintion of their dams with an enteropathogenic *E.coli* vaccine, a field study. Refuah Vet., 36 (4): 125-130.
8. Dauvergene, M., J. Laaporte. G. Reymoud, P. Soulebot. A. Brun and J.Espinasse. 1983. Vaccination of dams with a combined rota virus, corona virus vaccine to protect new born calves against diarrhoea. Proc. 4th Int. Symp. Neonatal Diarrhoea, VIDO, Saskatchewan, Canada, pp. 424-432.
9. Dea, S., R.S. Roy, and M.E. Begin. 1980. Bovine corona virus isolation and cultivation in continuous cell line. Am. J. Vet. Res., 41: 30-38.
10. Denis, A., M. Guylaine, E.N. Youssif, S.R. Raymond, and H.J. Jean. 1988. Immune response of pregnant heifers and cows to bovine rota virus inoculation and passive protection to rota infection in newborn calves fed colostral antibodies or colostral lymphocytes. Am. J. Vet. Res., 49 (7): 71-79.

11. Eman, K., M.S. Wassel. S. Zeidan and E. El-Ibiary. 1995. A serologic survey of bovine rotavirus infection in Egypt. Assiut Vet. Med. J., 334 (67): 26-45.

12. Max, B.J. 1977. A simple method for recording and analyzing serological data. Avian Dis., 22 (2): 362-365.

13. Mebus, C.A., R.G. White. E.P. Bass, and M.J. Twiehaus. 1973. Immunity to calf diarrhoea viruss. J. Am. Vet. Med. Assoc., 163: 880-883.

14. Mettias, K.N., A.A. El-Rashidy, M.M. El-Garhi. N.N. Hussien, E.R. Hassan, S.M. Saleh and N.A. El-Danaf. 1994. Studies on the protection of bovine neonates against diarrhoea by vaccination with trivalent vaccine (rota, corona, K99). Estimation of K99 antibodies in vaccinated dams and their neonates. Zag. Vet. J., 22 (3): 137-142.

15. Mohamed, F.G. 1995. Bacteriological and immunological studies on neonatal calf diarrhoea with special reference to vaccination of pregnant cows and buffaloes with monovalent K_{99} vaccine and a combined rota virus, corona virus, K_{99} vaccine. Thesis, Ph. D. Fac. Vet. Med., Cairo Univ.

16. Morin, M., S. Kariviere and R. Lallier. 1978. Neonatal calf diarrhoea, pathology and microbiology of spontaneous cases in dairy herds and incidence of enteropathogens implicated as etiological agents. Proc. 2nd Int. Symp. Neonatal Diarrhoea, pp. 347-367.

17. Myers, L.L. 1980. Passive protection of calves against experimentally induced and naturally occurring enteric colibacillosis. Am. J. Vet. Res., 41 (12): 1952-1956.

18. Peters, A.R. 1993. Enteric vaccines for farm animals and horses. Vaccines for Veterinary Application, Chapter (3); pp. 59-82. Butterwoth, Heimmann Ltd. Linacre House, Jordan Hill, Oxford, Great Britain.

19. Snodgrass, D.R. K.J. Fahey. P.W. Wells. I., Campbell, and A. Whitelaw. 1980. A passive immunity in calf rotaviruss infections maternal vaccination increases and prolongs immunoglobulin G1 antibody secretion in milk. Infect. Immun., 28:344-349.

20. Snodgrass, D.R. H.R. Terzolo, and D. Sherwood. 1986. Aetiology of diarrhoea in young calves. Vet. Rec., 119:31-34.

21. Tzipori, S. 1985. The relative importance of enteric pathogens affecting neonates of domestic animals. Adv. Vet. Sci. Comp. Med., 29: 192-206.

22. United States Code of Federal Regulations. 1987. Animal and animal products 9, 1987. Published by the USA Office of the Federal Register, National Archives and Records Administration.

23. Van Opdenbosch, E., G. Welleman. R. Strobbe and D.L. De Brubander. 1981. Evolution des anticorps anti-rota dans le lait de vaches traitees en fin de gestation soit par le vaccine anti rota complet, soil par L'adjuvant seul. Comp. Immun. Micro. Infect. Dis., 4: 293-300.

24. Wassel, M.S. 1996. Isolation and preparation of bovine rota virus (BRV) vaccine in Minnesota, USA. Vet. Med. J., Giza, 44 (3): 583-591.

دراسات مناعية لتجهيز وتقييم لقاح جامع ميت لعدوي فيروسات الروتا والكورونا والتهاب القولون المعدي في مصر

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المعمل المركزي للرقابة – مركز البحوث الزراعية وزارة الزراعة – الجيزة – مصر.

تم في تلك الدراسة تجهيز وتقييم لقاح جامع ميت لعدوي فيروسات الروتا والكورونا والميكروب القولوني عترة ك ٩٩ باجراء اختبار النقاوة في الاوساط الغذائية المختلفة والسلامة في الفئران الرضيعة واختبار القوة العيارية في العجول الخالية من الأجسام المضادة النوعية. وقد أعطي اللقاح مستوي مناعي مرضي (٤ – ٨ لو٢) باختبار التعادل للسيرم لكل من فيروس الروتا والكورونا و ١١٣٠ – ٦٠٨٦ باختبار الاليزا لفيروس الروتا و ١٠١٦ – ٥٤٣٣ لفيروس الكورونا و ١١٣٥ – ٨٤٧٢ للميكروب القولوني عترة ك٩٩ بعد ٢ و ٨ أسابيع من التحصين علي التوالي. وكما أعطي اختبار الحماية في الفئران البالغة ١٠٠٪ حماية بعد ٨ أسابيع. وعند استخدام اختبار التجمع الدقيق للسيرم وصل المستوي المناعي الي ٣٢٠ و ١٨١٠ بعد ٢ و ٨ أسابيع من التحصين وكانت جميع النتائج مطابقة للنتائج القياسية من حيث النقاوة والسلامة والقوة العيارية في البروتوكولات المرجعية.