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LABORATORY IMMUNE RESPONSE AGAINST ROTA VIRUS FREEZE DRIED INACTIVATED VACCINE IN BUFFALO CALVES

(With 2 Tables and 2 Figures)

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الأستجابة المناعية للقاح فيروس الروتا المجفف المخمد فى عجول الجاموس

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فى هذه الدراسة تم تقييم لقاح فيروس الروتا المخمد فى خمسة عجول جاموس قابلة للعدوى. تم تحصين كل حيوان بالحقن بالعضل بجرعتين من اللقاح كل منهم ٢ مل و بينهم ثلاثة أسابيع. لم يظهر أعراض مرضية بعد التحصين ولم يتم عزل الفيروس من المسحات الأنفية و الشرجية. وقد تكونت الأجسام المناعية المعادلة المتكونة المتخصصة لفيروس الروتا مبكراً عند اليوم العاشر بعد التحصين و استمرت حتى نهاية الشهر السابع بعد التحصين و تم قياس الأجسام المناعية عيارياً باستخدام اختبارى التعادل المصلى و الاليزا.

SUMMARY

In this study, inactivated Bovine Rota virus (BRV) vaccine was evaluated in five susceptible vaccine. Each animal was vaccinated intramuscularly (I/M) with two doses of 2 ml of the vaccine (3 weeks apart). No clinical signs of illness as post-vaccination (PV). No shedding of the virus through secreta and excreta. Specific BRV neutralizing antibodies developed early at the 10th day PV. The antibodies were monitored till the 7th month PV. The developed antibodies were quantitavely measured by both Serum Neutralization Test (SNT), and Enzyme Linked ImmunoSorbent Assay (ELISA).

Key words: *Buffalo-calves - Vaccination- Rota-virus - Immune Response.*

INTRODUCTION

Rota viruses have a wide host range, as indicated by their recovery from the newborn of many animal species (Ashley et al., 1978; Bryden et al., 1976; England and Poston, 1980; Hoshino et al., 1981; Jones et al., 1979; Mebus et al., 1969; Much and Zajac, 1972 and Tzipori and Walker, 1978).

Most of these Rota viruses were detected in newborn animals with diarrhoea. Rota viruses have also been associated with respiratory illness in some species (Eugster et al., 1978). Morbidity is very high among the infected calves and mortality is also high in certain locations in the world so a correct protection program should be directed to active immunization of the pregnant dams during the last stage of pregnancy to increase levels of the immunoglobulin against Rota virus in their colostrum and milk (Snodgrass and Wells, 1978).

In Egypt, specific BRV antibodies were detected for the first time, to the extent of our knowledge, in buffalo and cattle sera by Hafez et al. (1980). Shalaby et al. (1981), isolated the virus from newborn calves suffering from diarrhoea. The incidence of BRV infection in newborn calves were detected by Shalaby et al. (1987). Ikram et al. (1995), vaccinated pregnant buffalo dams with trivalent inactivated vaccine (Scour-grad 3), against Rota, Corona and E. coli K-99 in two private farms. The antibody levels were estimated quantitatively using ELISA technique.

The aim of the present study is to investigate efficacy of a monovalent inactivated BRV vaccine in inducing an immune response in buffalo calves. The antibody level in the sera of vaccinated and control calves was estimated using both SNT and ELISA technique.

MATERIAL and METHODS

Materials:

1. **Animals:** Five susceptible, apparently healthy, three months old buffalo calves. Clinical examination and observations, including rectal temperature, for both vaccinated and control buffalo calves for 21 days were recovered.
2. **Inactivated BRV vaccine:** A lyophilized BRV inactivated vaccine, that was prepared at Department of Rinderpest like diseases in Serum and Vaccine Research Institute, Abbasia (Iman et al., 1997), was used to vaccinate three buffalo calves. The vaccine was reconstituted with 2 ml of

balanced salt solution and inoculated I/M as a vaccinal dose. The second dose was given after three weeks.

3. **Virus:** BRV (Nebraska calf diarrhoea strain), was used as antigen for both SNT and ELISA technique.
4. **Samples:** Samples of sera, buffy coat, nasal and rectal swabs were collected pre- and post-vaccination (PV) to study safety, potency and immune response of the respective vaccine.
5. **Tissue culture:** Madin Darby Bovine Kidney (MDBK) cell line (Nagesha et al., 1985) was used for SNT and trail of virus isolation, while infectivity of the virus was enhanced by using trypsin according to method described by Theil et al. (1977).
6. **Media and Reagents:**

A. **Eagle's media:** It was prepared according to Polantick and Bachrach (1964) and was used for growing and maintaining of tissue culture.

B. **Hank's balanced salt solution:** It was prepared according to the method of Weller et al. (1952) and used for viral dilution and tissue culture preparation.

Methods:

1. **Serum Neutralization Test:** The test was performed according to Robson et al. (1960).
2. **ELISA technique:** ELISA microplates were coated with BRV antigen with 1 : 50 dilution in carbonate-bicarbonate buffer (pH 9.6) and ELISA procedure was completed as described by Folken et al. (1980). ELISA titre was calculated according to Syndor et al. (1984).
3. **Trials of virus isolation:** Trials of viral isolation from swabs and buffy coat samples, collected from vaccinated and control buffalo calves, were conducted on MDBK cell line according to method described by Zeidan (1986).

EXPERIMENTS and RESULTS

Clinical Observation:

No clinical signs of illness appeared during the period of the experimentl; temperature records indicated normal limits.

Trials of viral reisolation:

Samples of swabs, buffy coat were collected from vaccinated and control buffalo calves at days 3, 7, 10, 14 and 21 PV. Each sample was

inoculated into 4 MDBK cell culture tubes. Microscopical examination of tissue culture was carried out daily for 14 days to detect any cytopathic effect (CPE). Results of this pointed out that no reisolation of the virus ensued from different samples of swabs and buffy coats that collected for 21 days PV from vaccinated and control buffalo calves.

Seroconversion:

Serum samples were collected from both vaccinated and control buffalo calves at days; zero, 3, 7, 10, 14, 21, 28, 35, and 60 then monthly till the 7th month PV. Specific developed BRV antibodies were quantitavely measured by SNT and ELISA technique.

Results of seroconversion in vaccinated and control buffalo calves are represented in Tables (1 and 2) and illustrated in fig. (1 and 2). It showed that specific developed BRV neutralizing antibodies were first detected as early as 10 days PV with a mean serum neutralizing titre of 0.5 $\log_{10}$. The titres were gradually increased reaching the peak at the 35th day PV (two weeks after the second dose), and remained high till the end of the second month PV with a mean ELISA titre of 9120, then gradually decreased till the end of the experiment.

DISCUSSION

Rota virus antibodies were detected in cattle and buffalo sera (Hafez et al., 1980 and Shalaby et al., 1981). Specific colostral antibodies may protect the offspring against viral diarrhoea for the first several days of life. Maternal antibodies are most protective if present in the intestinal tract rather than its circulation (Wood et al., 1978). The calf often becomes naturally infected with Rota virus and Corona virus when over 4 days of age, as the maternal antibody titre decline. Whether the infection remains subclinical or results in mild or severe diarrhoea, that depends on a number of factors, including immune status of the calf, virulence of the virus, quantity of the virus infection, quality of the management and infection with other pathogenic agents, so the protection program should be directed to active immunization of pregnant dams during the last stage of pregnancy (Snodgrass et al., 1978).

Previous studies proved that the inactivated BRV vaccine is safe and efficious when used in cattle (Iman et al., 1997). In this study, the results showed that the vaccine is safe in buffalo where all vaccinated animals showed neither signs of disease nor elevation in temperature. It was noticed that no shedding of the virus in secreta and excreta of the vaccinated or the

control calves took place. This statement adds another proof for the safety of the used vaccine.

Results of seroconversion studies are presented in table (1 and 2), and illustrated in fig. (1 and 2). It was proved by SNT that specific BRV antibodies begins to appear at the 10th day PV with a mean titre of 0.5 $\log_{10}$ neutralizing antibodies titre (NAT). These results go in harmony with that obtained by ELISA technique where it was increased to a mean of 966 ELISA antibody titre. Specific developed BRV antibodies were gradually increased reaching the peak at the 35th day PV with a mean ANAT of $\log_{10}$ 2.1 and remain high till the end of the second month PV and these results go in harmony with that obtained by ELISA technique where it reached to the peak on the end of the second month PV with a mean ELISA titre of 9120.

REFERENCES

Ashley, C.R.; Caul, E.G.; Clark, S.K.R.; Covner, B.D. and Dunn, S. (1978): Rota virus infection of apes. Lancet, 2 : 477.

Bryden, A.S.; Thouless, M.E. and Flewett, T.H. (1976): A rabbit Rota Virus. Vet. Rec., 99 : 323.

England, J.J. and Poston, R.P. (1980): Electron microscopic identification and subsequent isolation of Rota virus from a dog with fatal neonatal diarrhoea. Am. J. Vet. Res., 14 : 782 - 783.

Eugster, A.K.; Strother, J. and Hartfiel, D.A. (1978): Rota virus infection of neonatal ruminants in zoo nursery. J. Wildl. Dis., 14 : 351 - 354.

Folken, R.N.; Stops, P.J. and Harris, C.C. (1980): Enzymatic immunoassay for detection of Rota virus antigen and antibody, pp. 692 - 699. In Rose, N.R. and Freedman, M., Manual of Clinical Immunology, 2nd ed. Amer. Soc. for Micro. Washington USA.

Hafez, S.M.; Lange, S.; Niebubr and Liess, B. (1980): Serological survey for prevalence of antibody to Rota virus and Bovine leukemia virus among buffalo and cattle in Egypt. Bul. Int. Epiz., 92 : 1193 - 1203.

Hoshino, Y.; Baldwin, C.A. and Scott, F.W. (1981): Isolation and characterization of feline Rota virus. J. Gen. Virol., 54 : 313 - 323.

Ikram, A. Karim; Mervat, M. Mahmoud; Abdel-Gawad, A.A.; El-Ebeedy, A.A. and Saleh, S.M. (1995): Estimation of Rota virus antibody level in vaccinated and non vaccinated buffalo dams and their neonates. Beni Suef Vet. Med. Res., V (1) : 54 - 62.

Iman, K.A. Kassem; Thanaa, I. Baz and Wassel, M.S. (1997): Production of freeze dried inactivated vaccine for Bovine Rota virus. Vet. Med. J., Giza, Under Press.

Jones, R.C.; Hughes, C.S. and Henry, R.R. (1979): Rota virus infection in commercial laying hens. Vet. Res., 104 : 22.

Mebus, C.A.; Underal, N.R.; Rhodes, M.B. and Twiehaus, M.J. (1969): Calf diarrhoea scours reproduced with a virus from a field outbreak. Bull. Neb. Agric. Exp. Station. 233 : 1 - 16.

Much, D. and Zajac, I. (1972): Purification and characterization of epizootic diarrhoea of infant mice virus. Infect. Immun., 6 : 1019 - 1024.

Nagesha, H.S.; Ragha Van, R. and Lai, S.M. (1985): Madin Darby Bovine Kidney cell line (MDBK) for isolation of BRV from clinical cases of neonatal calf diarrhoea. Ind. J. Virol., 1 (2) : 224 - 235.

Polantick, J. and Bachrach, H.L. (1964): Production and purification of milligram amount of FMD virus from baby hamster kidney cell culture. Applied Microbiology, 12 : 368 - 373.

Robson, D.S.; Gillespie, J.H. and Baker, J.A. (1960): The neutralization test as an indicator of immunity to virus diarrhoea. Cornell Vet., 50 : 503 - 509.

Shalaby, A.A.; Ikram, A. Karim; Sohair, M. Sokkar; Shehata, H.A. and Reda, I.M. (1987): Isolation of BRV from calves in Kaliobia. J. Egyp. Vet. Med. Ass., 47 (12): 209 - 218.

Shalaby, M.A.; Saber, M.S. and El-Karamany, R.M. (1981): Rota virus infection associated with diarrhoea in calves in Egypt. Vet. Res. Communications, 5 : 165 - 170.

Snodgrass, D.R. and Wells, P.W. (1978): Passive immunity in Rota viral infections. J. Am. Vet. Med. Assoc., 173 : 565 - 568.

Synder, P.B.; Marquart, W.W.; Mallinson, E.T.; Savage, P.K. and Alley, D.C. (1984): Rapid serological profiling ELISA. Avian Dis., 28 : 12 - 24.

Theil, K.W.; Bohleh, A. and Agness, A.G. (1977): Cell culture propagation of porcine Rota virus (Reo virus like agent). Am. J. Vet. Res., 38 : 1765 - 1768.

Tzipoiri, S. and Walker, M. (1978): Isolation of Rota virus from deer. Vet. Rec., 99 : 398.

Weller, T.H.; Enders, T.C.; Robbins, F.C. and Stoddord, M.D. (1952): Studies on the cultivation of poliomyelitis viruses in tissue culture. J. Immunol., 69 : 645 - 671.

Wood, G.N.; Bew, M.E. and Dennis, M.J. (1978): Studies on cross protection induced in calves by Rota in calves, children and foals. Vet. Rec., 103 : 32 - 34.

Zeidan, S.M. (1986): Clinicopathological studies on bovine virus diarrhoea in sheep. Thesis for M.V.Sc. degree, Fac. Vet. Med., Cairo Univ.

Table (1) : Serum neutralizing antibody titre of buffalo calves post vaccination with inactivated BRV vaccine*.

Time post vaccination	Log_{10} Serum Neutralizing Antibody Titre				
	Animal Nomber			Mean Titre	SD
	1	2	3		
Zero	-	-	-	-	-
3 days	-	-	-	-	-
7 days	-	-	-	-	-
10 days	0.6	0.6	0.3	0.5	± 0.17
14 days	0.9	0.9	0.6	0.8	± 0.17
21 days	1.2	1.2	0.9	1.1	± 0.17
28 days	1.5	1.5	1.2	1.4	± 0.14
35 days	2.1	2.1	1.8	2.0	± 0.17
2 months	2.1	2.1	1.8	2.0	± 0.17
3 months	1.8	1.8	1.5	1.7	± 0.14
4 months	1.5	1.5	1.2	1.4	± 0.17
5 months	1.2	1.2	0.9	1.1	± 0.17
6 months	0.9	0.9	0.6	0.8	± 0.17
7 months	0.6	0.6	0.3	0.5	± 0.14

BRV : Bovine Rota Virus. SD : Standard Deviation.

Control buffalo calves remained serologically negative allover the experiment.

Table (2) : ELISA antibody titre of buffalo calves post vaccination with inactivated BRV vaccine*.

Time post vaccination	Log_{10} Serum Neutralizing Antibody Titre				
	Animal Nomber			Mean Titre	SD
	1	2	3		
Zero	-	-	-	-	-
3 days	-	-	-	-	-
7 days	-	-	-	-	-
10 days	0.6	0.6	0.3	0.5	± 0.17
14 days	0.9	0.9	0.6	0.8	± 0.17
21 days	1.2	1.2	0.9	1.1	± 0.17
28 days	1.5	1.5	1.2	1.4	± 0.14
35 days	2.1	2.1	1.8	2.0	± 0.17
2 months	2.1	2.1	1.8	2.0	± 0.17
3 months	1.8	1.8	1.5	1.7	± 0.14
4 months	1.5	1.5	1.2	1.4	± 0.17
5 months	1.2	1.2	0.9	1.1	± 0.17
6 months	0.9	0.9	0.6	0.8	± 0.17
7 months	0.6	0.6	0.3	0.5	± 0.14

BRV : Bovine Rota Virus. SD : Standard Deviation.

Control buffalo calves remained serologically negative allover the experiment.

Fig. (1) : Mean serum neutralizing antibody titre of buffalo calves post vaccination with inactivated BRV vaccine

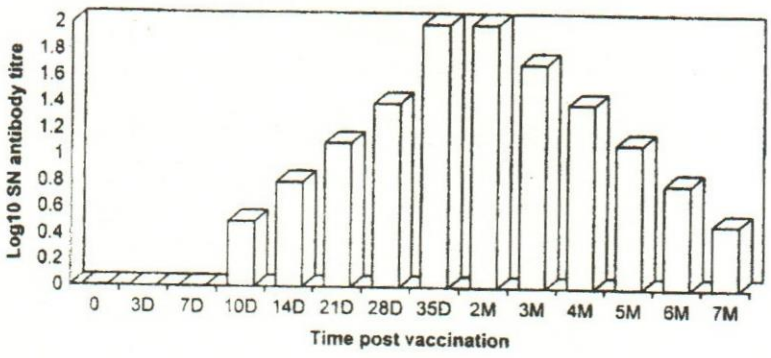
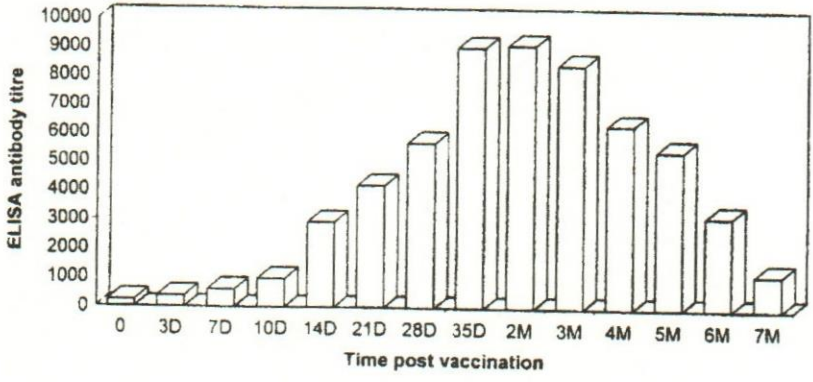


Fig. (2) : Mean ELISA antibody titre of buffalo calves post vaccination with inactivated BRV vaccine.



D : Day M : Month