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Preparation and Evaluation of Anti Rota IgG Conjugated With Fluorescein

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Hyper immune serum against *bovine rota virus* was prepared in guinea pigs through its inoculation with complete and incomplete Freund's adjuvant and without adjuvant in successive doses into susceptible guinea pigs. Serum neutralization (SNT). ELISA and indirect fluorescent assay (IFA) tests were used to evaluate the hyper immune sera before conjugation with fluorescent , the antibody titre reach 32 by SNT and 5984 by ELISA and identified by IFA to be Bovine rota. Separation of anti B.rota immunoglobulins IgG were done using activated sepharose 4B followed by conjugation with fluorescein isothiocyanate at PH 9.6. The conjugated IgG was test against reference B.rota antigen using direct and indirect fluorescein assay [FA, IFA]. Both assay gave good result as the working dilution of the fluorescein isothiocyanate conjugated B.rota IgG was 1: 30.

INTRODUCTION

Neonatal calf diarrhea has a complex etiologies among which are Rota viruses (Radostists, 1991).

Rota viruses are unique viruses that produce an acute diarrhea 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 viruses infection constitute a serious economic problem threatening animal production in Egypt (Arwa *et al.*, 2003). Diarrhoeal disease are considered one of the most important causes of death especially during the early few weeks of life in which a considerable number of newly born animals could be lost (Daoud *et al.*, 2003).

MATERIALS AND METHODS

Materials

1-Virus

Egyptian tissue culture adapted strain on screened MDBK. Was kindly suppied from Rinder pest like diseases department, Abbassia, Cairo.

2-Bovine Rota antigen and antisera

Standard purified reference Bovine rota virus antigen and antisera were kindly supplied from Rinder pest like diseases department, Abbassia, Cairo

3-Guinea pigs

Six susceptible guinea pigs tested against Bovine Rota were used for preparation of Rota hyper immune sera.

4-Fluorescein isothiocyanate: (Sigma ,USA).

5-Dialysis bag: (Sigma ,USA).

6-Sepharose 4B cyanobromid : (Sigma ,USA).

7-Antiguinea pig Fitic : (Sigma ,USA).

8-Cell culture:

Monolayers screened MDBK cell cultures were grown and maintained as

described by Chaseey. (1977) and Dea *et al.*, (1980) .

9- Vaccine:

Entero-3. vaccine , kindly supplied from the Rinderpest like disease Department at the Veterinary Serum and Vaccine Research Institute (VSVRI),Egypt .

Methods

1-Preparation of Rota hyper immune sera

Each G.pig was inoculated 0.5ml (contain 6 log10 TCID50) intramuscularly (I/M) from mixture of (1ml Rota antigen and 1ml of complete Freud's adjuvant). A booster dose (0.5ml) was given 2 week later from (equal amounts of antigen and incomplete Freund's adjuvant). Two weeks later, a final dose of antigen alone was given via the same route .After a period of two weeks, serum samples were collected and subjected to evaluation.

2-Evaluation of Guinea pig Rota hyper immune sera:-

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-Identity test

The B.rota antigen was identified by IFA using reference B.rota antisera as described by Elian *et al.*, (1996).

C-Measurement the amount of microgram (ug) of protein of purified reference B.rota antigen

Quantitation of ug of protein of the B.rota antigen was done by Bradford (1976).

D-Serum neutralization test (SNT)

This was carried on micro titer plate 96 well using MDBK cell culture method according to Dauver- gene *et al.*, (1983); Castrucci *et al.*, (1984) and wassel (1996).

E-Enzyme linked immuno sorbent Assay (ELISA)

BRV Antigen preparation against and the test technique were described by Eman *et al.*, (1995) and Mohamed (1995) and Arwa , (2002).

F-Immuno fluorescent assay (IFA)

The technique was described by (Snodgrass *et al.*, 1976) where, 96 well tissue culture plates containing MDBK cells were infected with 100 TCID50of reference Rota virus. The appearance of cytopathic effect on the MDBK cells occurred 48 hours post-inoculation of the virus, then, the plates were fixed with absolute ethanol. Two fold dilution from the tested G.pig sera were added to the plate which was incubated at 37oC for 45 minutes , washing with phosphate buffer saline (PBS) of PH 7.2 for 3 times . Anti-G.pig fittic was used for the identification of the tested G.pig sera.

I-Micro agglutination Test

Antigen preparation and the test techniques were according to Collins *et al.*, (1988), and geometric mean titres were calculated according to Max (1977).

RESULTS AND DISCUSSION

Our results showed that the guinea pigs produced specific antibodies against inoculated bovine Rota antigen ,was sterile, and

Table (1) : Evaluation of Bovine Rota Fraction in (Entero -3 .Vaccine)Identity test using prepared anti Guinea pig IgG conjugated with fluorescein.

Type of vaccine	Direct Fluorescent assay using prepared material .	Control indirect immunofluorescent assay using anti guinea pig
	Identity	Identity
1.Entero -3 vaccine: a. Batch No. 1 b. Batch No. 2 c. Batch No. 3	 Bovine Rota Bovine Rota Bovine Rota	 Bovine Rota Bovine Rota Bovine Rota
2. Reference control Rota antigen .	Rota virus	Rota virus
3.Negative control antigen	Negative	Negative

Table (2) : Evaluation of prepared guinea pigs Rota antibodies before conjugation with fluorescin

No. of guinea pigs	Susceptibility of G.pigs against bovine Rota pre-vaccination	Purity test	Identity test	Amount of serum protein per-0.1 ml	Indirect fluorescent assay (IFA)using specific anti-guinea pig fitic 6 weeks post inoculation with Rota V antigen.		Agglutination test		Serum neutralization Test		Average ELISA titer before conjugation	
2	0	Ster.	Rota virus	4.2µ g/0.1	+	+	+	+	16	32	4560	6180
2	0	Ster.	Rota virus	3.1µ g/0.1	+	+	+	+	16	32	4448	5984
2	0	Ster.	Rota virus	4.0µ g/0.1	+	+	+	+	16	32	4744	5776
Mean				3.76µ g/0.1	+	+	+	+	16	32	4460	5904

+:Presence of antibodies against Bovine Rota.
Control positive : using specific reference Rota antibodies and Bovine Rota antigen.
Log SNT :Log serum neutralizing index .
Ster. :Free from contaminants (virus .bacteria and mycoplasama)

Table (3) : Titration of conjugated anti Bovine Rota IgG with fluorescein reference using Bovine Rota antigen

Antigen	Anti Bovine Rota IgG conjugated with fluorescein					
dilution	1/2	1/4	1/8	1/16	1/32	1/64
Reference Bovine Rota antigen	+	+	+	+	+	
Negative control	–	–	–	–	–	–
Control negative cells	–	–	–	–	–	–

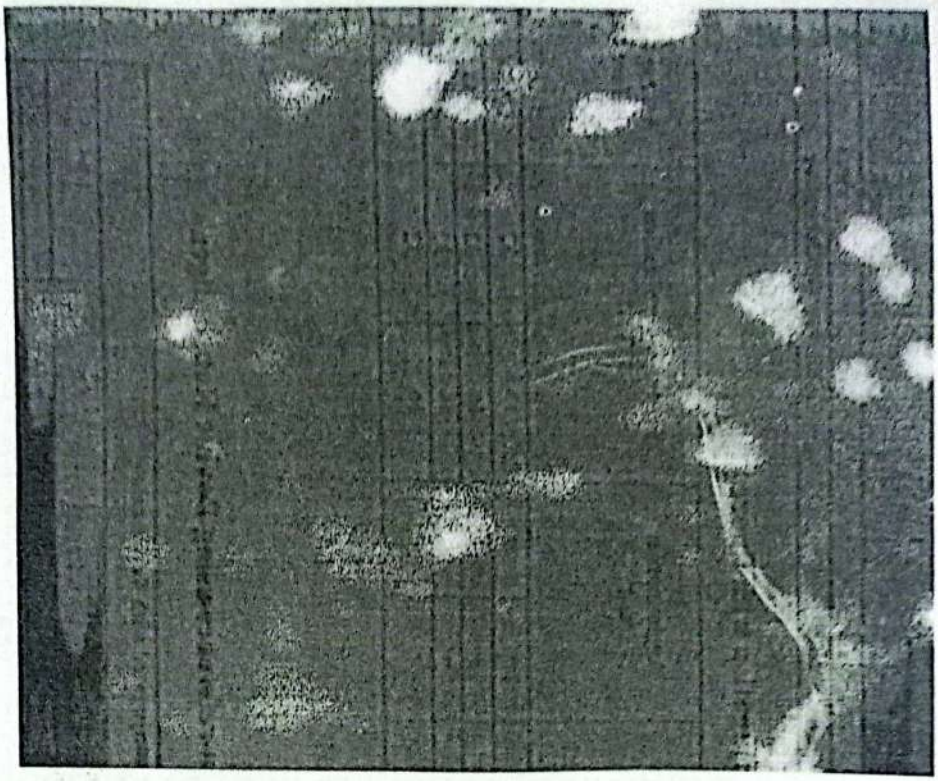

Fig. (1): IFA x 140 Bovine Rota antigen detected by conjugated guinea pig IgG immunoglobulin for B.rota. (Direct FA)

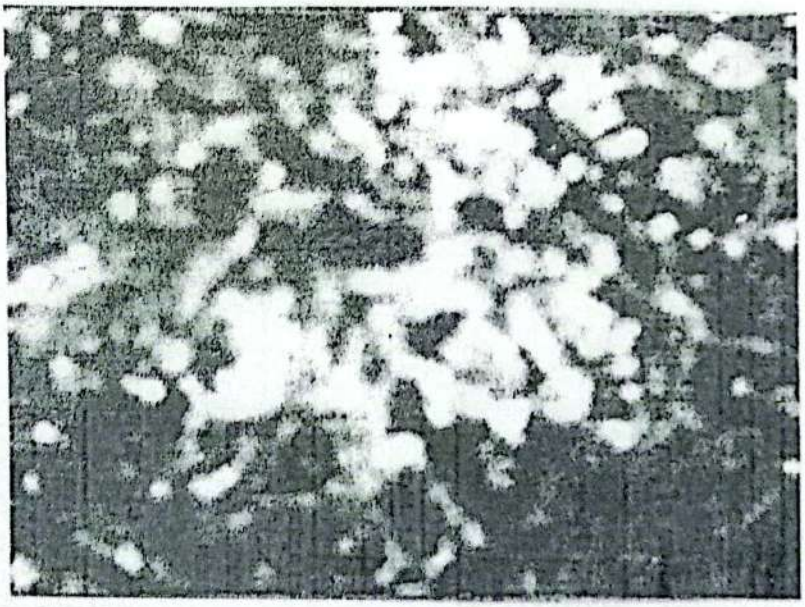

Fig. (2): FA x 140 Bovine Rota detected by guinea pig IgG conjugated with fluorecein after addition of B.rota antibodies .(IFA)

identified using IFA technique agreed with that finding in WHO (1966,1973) shown in table (1). The SNT and ELISA titre results which obtained for evaluation of Bovine Rota Fraction were ranged from (16-32) for SNT and (4448-5984) for ELISA, agreed with (Arwa *et al.*, 2003 and Daoud *et al.*, 2003), (table 2). IFA and micro agglutination tests shown in table (2) and photo (1, 2) these come in harmony with (Eman *et al.*, 1995 and Daoud *et al.*, 2003).

G.pigs IgG immunoglobulins were extracted by using sepharose-4 cyan bromide, the protein content of the IgG extracted ranged from (4.2-3.7µg /0.1ml) before onjugation with, and this amount of protein was satisfactory to use in conjugation with fluorescnein agreed with (Anderson *et al.*, 1975 and Nowotony 1979).

The titre of conjugated Bovine Rota IgG antibodies reached 1: 32 which of good titre to use for conjugation (Nowotony, 1979).

From the last result we can say that we could prepare anti bovine Rota IgG conjugated with fluorescin of low price, good titre and good quality and quantity in pure form to be use for diagnosis of Bovine Rota infection and for identity evaluation of produced vaccine.

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