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ISOLATION AND PREPARATION OF BOVINE ROTA VIRUS (BRV) VACCINE IN MINNESOTA, USA

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SUMMARY

Nine cytopatic bovine rota virus stratins were isolted in MA104 cells from fecal specimens of calves.

Plaque assay was used for selection, titration and purification of small and large plaques of selected rota virus strains.

Electron microscopy was used to detect the presence of particles with rota virus morphology in MA104 cells. Virus neutralization test was used for further identification of strains of high immunogenic response.

Pooling of these isolates and their use in making a formalized-inactivated bovine rota vaccine was tried.

INTRODUCTION

Rota viruses infect a variety of animal species and cause diarrhea in young animals. Rota virus from one species has been shown to infect members of certain other species, thus propagation of bovine rota virus in cats, dogs, pigs, lambs, calves, rabbits, mice and human beings has been reported (Castrucci et al., 1984).

Bovine Rota Virus (BRV) is one of the most common pathogenic agents causing diarrhea in young calves (Castrucci et al., 1983) and the disease occurs allover the world including the USA (Kenneth and Christine, 1989), Italy (Castrucci et al., 1988), Argentina (Rodolfo et al., 1989) and Egypt (Shalaby et al., 1981 and Wafaa, 1994). The infected animals develop anorexia and a severe watery diarrhea, the feces is yellowish and contains mucus. The affected animals usually die within 72 hours of the onset of the disease (Castrucci et al., 1985). The detection of rota virus particles in feces of infected animals is usually done by electron microscopy (Bryden et al., 1976 and Morisse, 1982).

Most rota virus strains were found to be difficult to grow well in cell culture, the strains that do grow in cell culture produce rounding of cells in 48-72 hours post infection. The destructionof the entire monolayer generally occurs from 3-5 days after infection (Castrucci et al., 1985).

The present study was initiated to adopt several strians from field isoaltes of bovine rota virus in purified form with high titre and good immunogenic response for preparation of inactivated vaccine

MATERIAL AND METHODS

Virus and Antisera:

Simian rota virus SA-11 and Bovine Rota antisera were kindly supplied from National Veterianry Services Laboratories, Ames Iowa, USA.

Field Isolates:
Field isolates of bovine rota virus were obtained from diarrhoeic calves (faecal specimens) for veterinary diagnostic laboratories, Minnesota (VDL). The local specimens were positive by electron microscops for rota virus.

Animals:
Six susceptible calves, 6 months old, and four guinea pigs were used in this study.

Tissue Culture:
Fetal Rhesus monkey kidney cells (HA104) were grown and maintained as described by Castrucci et al., (1988).

Virus Propagation:
For culturing rota viruses 5 ug/ml of trypsin were added to the maintenance media to enhance the rota virus ability to infect the cells (Babiuk et al., 1977).

Plaques assay:
Plaque assay using serial dilutions of isolates and MA104 cells was done according to (Crandell and Gomes, 1970).

Virus Neutrialization test (VNT):
It was carried out in MA104 cells in 96-well microtitre plates as described by Castrucci et al., (1985).

Serum Neutralization Test (SNT):
It was carried out according to Sato et al., (1981).

Electron Microscopy:
The virus particles from MA104 were deposited on copper grids coated with a collodion film and carbon. They were negatively stained with 2% solution of phosphotungstic acid, pH 6.5. The particles were viewed with a Zeiss 10 electron microscope at 60 KV accelerating voltage (Brussow et al., 1987).

Inactivated Bovine Rota Vaccine (BRV-V):
The adapted homogeneous small plaques isoaltes of high titre and good rota particles with electron microscope were pooled together after 18 passages in MA104 cell line and gave tite of 2×10^9 PFU / ml. The virus was inactivated by overnight (14 hours) incubation at 4°C with 0.5% formaldehyde, then emulsified in an equal volume of Freund's incomplete adjuvant and drown in volume of 2.0 ml in disposable plastic syringes. The vaccine preparations were stored at 4°C till use (Castrucci et al., 1988).

Vaccine Evaluation and Control:
The inactivated Bovine Rota Vaccine was tested for the following:

a- Purity in accordance with the United State Code of Federal Regulations testing, 9 CFR 113.2b

b- Safety;

1. In Guinea Pigs:
Two guinea pigs were inoculated with 1/4 dose (0.4 ml) subcutaneously and observed for 7 days and two left as a control.

2. In Calves:
Two calves were inoculated with 10X dose of field dose.

c- Potency: Two calves were inoculated with 2 ml of the vaccine intramuscularly (I/M) and second dose was given 2-3 weeks later following first dose. Blood samples were collected 2 weeks and 3 weeks following the second dose.

d- Two calves were kept as control.

RESULTS

Virus Isolation:
Nine out of 25 isolates of rota virus were grown in

tissue culture and produced cytopathic effect on MA104 cell culture in the presence of MEM enriched with 1% fetal bovine serum and containing 5 ug/ml of trypsin, as shown in table (1).

The CPE was characterized by the presence of round foci of rounded cells which tend to aggregate linearly, forming a sort of net-work on the surface of monolayer. The cells showed partial detachement from the plastic surface. This effect was at about 48 hours and the destruction of the entire monolayer generally eccurred 5 days after incculation.

Plaque formation and strain selection:

Plaques appeared 6 days after inoculation with the isolted strains, the plaques were small and large, rounded with irregular border and varied from 1-3 mm in diameter. As shown in Table (2) the titre of small palques was higher than that of large ones.

The highest titre reached $10^{8.4}$ PFU/ml in /M6 (Minnesota6) SPP (Small Plaque Purified) strain and the lowest titre reached 10^{4} PFU /ml as in M22 (Minnesota22) LPP (Large Plaque Purified).

Table (1) : Cytopathic appearance of BR field isolates post infection in MA104 cells.

Strain Number	Days of the observation following infection of MA104 cells with field strains of Rota virus				
	1	2	3	4	5
1	-	-	-	-	-
2	-	+	+	+	+
3		ND			
4	-	+	+	+	+
5	-	+	+	+	+
6	-	+	+	+	+
7	-	+	+	+	+
8	-	-	-	-	-
9	-	-	-	-	-
10	-	+	+	+	+
11	-	-	-	-	-
12	-	-	-	-	-
13	-	-	-	-	-
14	-	-	-	-	-
15	-	-	-	-	-
16	-	+	+	+	+
17	-	-	-	-	-
18	-	-	-	-	-
19	-	-	-	-	-
20	-	-	-	-	-
21	-	+	+	+	+
22	-	+	+	+	+
23	-	ND	-	-	-
24	-	ND	-	-	-
25	-	-	-	-	-

\+ = Cytopathic effect which begin to the prephery with rounding after 24 hours then death and detach of the cell within 22 hours post infection.

\- = No CPE.

ND = Not Done.

Table (2) : Titre and size of plaque purified of BRV isolates and its morphology with E.M.

Tissue culture Rota strains	Titre expressed as Log_{10} PFU / ml	Size of plaque *	Electron microscope appearance
M2 origin	7.5	-	many good rota particles
M2 SPP	7.7	1 - 2 mm in diameter	many good rota particles
M2 LPP	5.3	› 2 mm	many good rota particles
M4 origin	7.0	-	many good rota particles
M4 SPP	7.3	1 - 2 mm in diameter	many good rota particles
M4 LPP	5.0	› 2 mm	many good rota particles
M5 origin	6.0	-	many rota most lysed
M5 SPP	7.0	1 - 2 mm in diameter	many rota most lysed
M5 LPP	6.0	› 2 mm	many rota most lysed
M6 origin	7.5	-	many good rota particles
M6 SPP	8.4	1 - 2 mm in diameter	many good rota particles
M6 LPP	ND	› 2 mm	many good rota particles
M7 origin	6.0	-	many lysed rota particle
M7 SPP	6.0	1 - 2 mm in diameter	many lysed rota particle
M7 LPP	4.0	› 2 mm	many lysed rota particle
M10 origin	6.5	-	many good rota particles
M10 SPP	7.3	1 - 2 mm in diameter	many good rota particles
M10 LPP	5.5	› 2 mm	many good rota particles
M16 origin	5.0	-	many lysed rota particle
M16 SPP	6.0	1 - 2 mm in diameter	many lysed rota particle
M16 LPP	4.0	› 2 mm	many lysed rota particle
M21 origin	6.0	-	Good rota particles
M21 SPP	6.0	1 - 2 mm in diameter	Good rota particles
M21 LPP	4.0	› 2 mm	Weak rota particles
M22 origin	6.0	-	Good rota particles
M22 SPP	6.0	1 - 2 mm in diameter	Good rota particles
M22 LPP	4.0	› 2 mm	Good rota particles

PFU = Plaque Forming Unit.
PP = Plaque Purified.
M = Minnesota.
S = Small.
L = Large.
* = Plaques were rounded with irregular border.
› = More than.

Table (3) : Virus neutralization test of (BRV) isolates against known BR antibodies.

Tissue culture rota viruses	Bovine rota virus neutralization test								
	Titre								
	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512
M2 SPP	-	-	-	-	-	-	+	+	+
M2 LPP	-	-	-	-	-	-	+	+	+
M4 SPP	-	-	-	-	-	-	+	+	+
M4 LPP	-	-	-	-	-	-	+	+	+
M6 SPP	-	-	-	-	-	-	+	+	+
M10 SPP	-	-	-	-	-	-	+	+	+
M10 SPP	-	-	-	-	-	-	+	+	+
M21 LPP	-	-	-	-	-	-	+	+	+
M21 SPP	-	-	-	-	-	-	+	+	+
M22 LPP	-	-	-	-	-	-	+	+	+
M22 LPP	-	-	-	-	-	-	+	+	+
Control	-	-	-	-	-	-	+	+	+

M = Minnesota.
PP = Plaque Purified.
S = Small.
L = Large.
\+ = CPE.
\- = No cytopathic effect (CPE).

Table (4) : Mean of bovine rota (BR) neutralizing titre in calves following inoculation with inactivated bovine rota vaccine.

Animal	Bovine rota serum antibodies titre *						
	Before vaccination	Weeks Post Vaccination					
		1	2	3	4	5	6
Calves vaccinated with BR vaccine	0	0	2.0	2.0	4.0	4.0	8.0
Non - vaccinated control calves	0	0	0	0	0	0	0

* = Titre expressed as reciprocal of serum dilution.

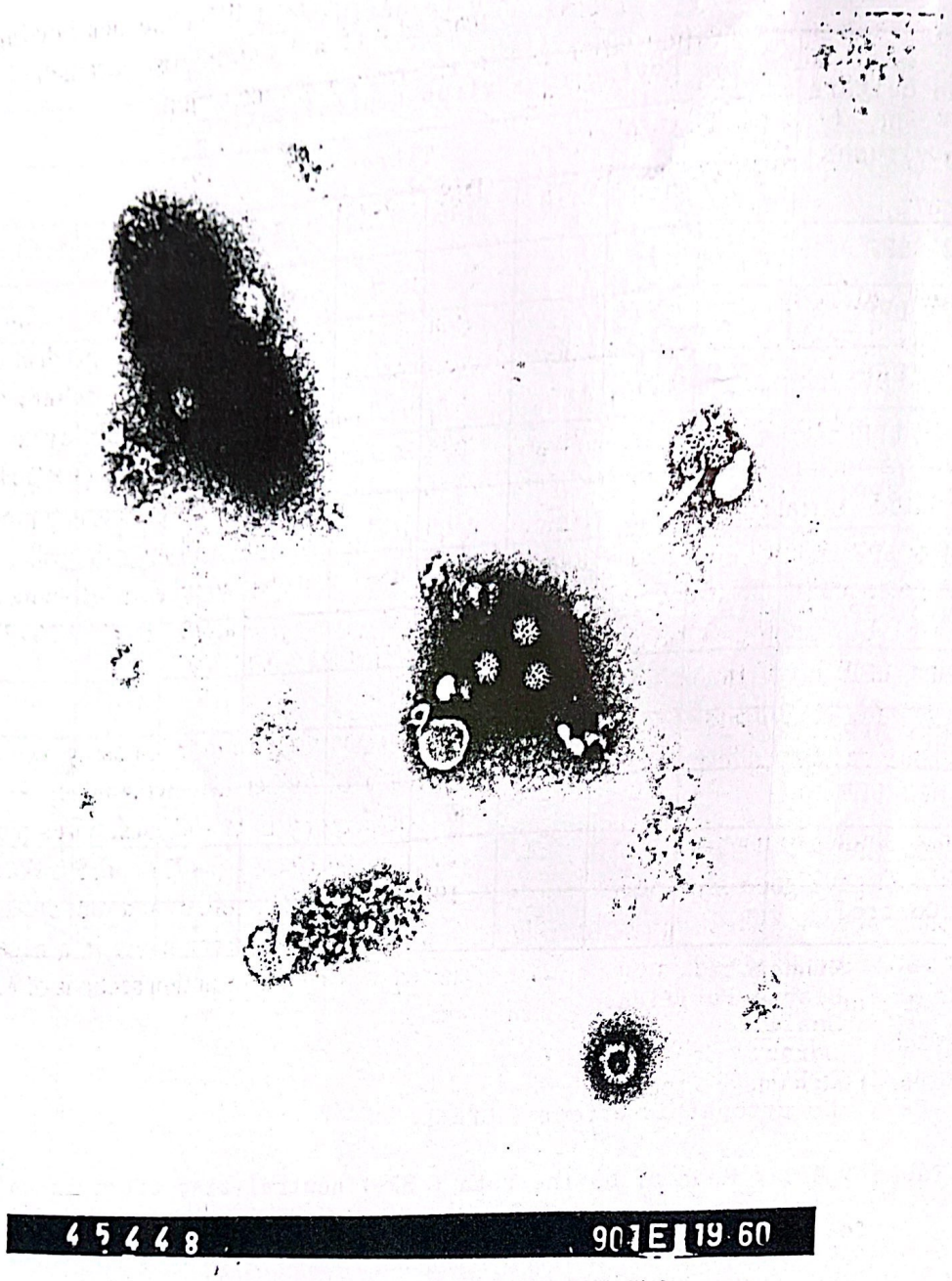

Figure (1) : Electron micrographs of extra cellular bovine rota purified strains spin 16,000 RPM with no sonication , as seen intact virions and inner shell particle (X 100,000).

Electorn Microscope Examination (E. M.):

E M was used to make sure that intact virions of rota viruses of small plaque purified strian as shown in Fig. (1) and Table (2). We kept the strains that have a good rota particles and neglect the strains that have lysed rota particles (like M5, M7 and M9 strains).

Virus Neutralization Test:

When we use the 100 tissue culture infectious dose 50 ($TCID_{50}$) of each purified strains against equal volume of 2 fold dilution from 1:4 through 1:512 of bovine antisera we found that all the purified isolated strians have the same immunogenic power against the used antisera, indicating the presence of specificity against the used antisera of bovine rota virus and these purified isoaltes still carry their immunogenic determinants set after the process of tissue culture purification. The titres ranged form 1:4 to 1:128 .

The preliminary studies of inactivated bovine rota vaccine (BRV-V) gave good satisfactory results of sterility and safety in calves (no rise of body temperature and it remained within thee normal 38.6 for 10 days and no diarrhoea) and guinea pigs. The results of potency test in calvs were shown in Table (4) the immune response of calves to the inactivated (BRV) the neutralizing titre reached 4 after 1 month post vaccination and 8 after 6 week post vaccination.

DISCUSSION

The present studies described that some field isolates produce a CPE strictly dependent on the presence of trypsin in the cell culture as the trypsin enhance the rota virus ability to infect cells, as some of the outer proteins (VP4) cleaved by the enzyme trypsin, during uncoating processes of rota virus replication to inter the cells. These finidings are in harmony with Mohamed and Saunders (1977); Babiuk et al., (1977) and Castrucci et al., (1988).

By using the plaque formation assay, we select the strain of higher titre with intact virion as demonstrated by E M and these virus particles described by Chasey (1977) and Suzuki et al., (1981) as single-shelled and double-shelled particles. They were seen either in a negatively stained preparations or in thin sections of MA104 - cells.

The resutl of virus neutralization proved the presence of antigenic relationship between these isolated they did not lose their immunogenic characters during the process of plaque purification selection. These findings agreed with those obtained by Castrucci et al., (1985).

So, through a suitbale culture system such as MA. 10^4, rota virus strain with high titre ($10^{8.4}$ PFU/ML) and good antibody response it waspossible to prepare a vaccine (monovalent or polyvalent) against bovine rota virus infection. These findings are in harmony with Castrucci et al., (1983) and Brussow et al., (1987).

The results of SN of tested vaccine of purified bovine rota tissue culture pooled isolates gave neutralizing titres of 8 which were considered of good immunity (since the achieved neutralizing titre 4) in the 6th week post vaccination, these results agreed with those reported by castrucci et al., (1985).

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