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REPLICATION OF SOME PORCINE PARVOVIRUS STRAINS IN VARIOUS TISSUE CULTURES

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The goal of our study was to determine which of the porcine parvovirus strains NADL-1 NADL-2, NADL-8 and FS 59e/63 could be propagated best, i. e. which of them gave the highest haemagglutination activity after propagation in primary tissue culture of pig kidney cells or in the cell lines PK-15, SB and ST. The highest yields were obtained when strains NADL-2 and NADL-1 were propagated in the continuous cell line SB and in primary pig kidney cellus. These values varied from 11–12 (log_2) HA units /(0.020 ml. We also studied the influence of medium enriched with fetal calf serum or calf serum upon virus propagation, including the optimum time for collecting the viruses from the tissue cultures. The results showed that the growth medium which contained 10% fetal calf serum as the protein substrate was more favorable for the propagation of all strains examined in the primary pig kidney cell cultures. The results obtained indicated that the optimum time for harvesting viruses from tissue culture is after 3–4 days incubation, regardless of the strain tested or tissue used.

Key words: Parvovirus, Cell culture, Propagation of virus, Calf serum.

INTRODUCTION

Porcine parvovirus (PPV) is one of the most important causative agents of infertility and reproductive failure in swine. (being as such characterised by SMEDI-symptomatocompex). Since the virus exhibits a strong destructive effect upon tissues with a high mitotic index, the direct result of the disease is a lethal effect on blastocytes, embryo and the early fetus (Mengeling et al., 1981; Cartwright et. al., 1971; Joo et al., 1976.) PPV is a member of the family Parvoviridae and is approximately 20 nm in size. It consists of an icosahedral shaped capsid containing 32 capsomeres which round up a single DNA strand of 5,2 kpb in length (Joo et al., 1986.) The virus replicates within the cellular nucleus and requires the functional state of the cell designated as the late "S" or early "G_2" phase of the cellular cycle. The enzymes coded during these phases are necessary for viral replication. Thus PPV has a trophic requirement

for actively dividing cells (embryonal, fetal tissue, the intestinal epithelium, lymphatic tissue, bone marrow; (Morimoto et al., 1972; Tinsey et al., 1973.) The ratio of "full" and "empty" viral particles affects viral replication. "Empty" particles interfere by blocking cellular receptors (Molitor et. al., 1972; Choi et. al., 1987).

The most efficient protection against parvoviral infection can be obtained by vaccination, based upon prevention of transplacentary infection and fetal infection. According to most investigators, vaccinal efficiency is related to the haemagglutination (HA) activity of the viral suspension. For this reason, determination of HA activity of viral suspensions is very important in vaccine preparation, because the haeminhibition titer of vaccinal antibody directly correlates with it (Paul et. al., 1987). Lei et. al., (1980.) found that the main portion of the antigenic mass and the immunogenic activity of a vaccine was bound to the "empty" viral particles. In vaccine preparation one should not ignore the difference in immunogenic properties of various viral strains. Different strains have been used for the production of vaccine. The strains were propagated on primary pig kidney cultures and continuous cell lines (Lei et. al., 1980; Rivera et. al., 1986a; Joo et. al., 1982; Paul et. al., 1980; 1987; Mengeling et. al., 1979; Izumida et. al., 1986; Fujisaky et. al., 1982), and 45 day-old pig fetus (Wrathall et. al., 1984).

Bearing in mind all the aforementioned, the aim of our study was to determine which of the PPV strains examined propagated best, i. e. which of them developed the highest HA activity in different tissue culture systems. We also studied the influence of medium enriched with fetal calf serum (FCS) or calf serum (SC) upon PPV virus propagation, as well as the optimum time for harvesting the viruses.

MATERIALS AND METHODS

Viruses – Four PPV strains: NADL-1, NADL-2, NADL-8 and FS 59e/63 were included in the study.

Tissue cultures – The following cell cultures were used for viral propagation: primary tissue culture of pig kidney cells and the continuous cell lines PK-15 (porcine kidney), SB (porcine kidney) and ST (porcine testis). Primary tissue cultures and continuous cell lines were prepared and maintained by the usual procedure. MEM Hank's solution with 10% inactivated fetal calf serum (FCS-Sarva) or calf serum (CS) was used as the growth medium.

Production of stock virus – When Roux flasks were used to produce viral antigen the cells were inoculated with 5 ml PPV suspension, immediately after trypsinisation. The flasks with infected cell cultures were examined daily. After incubation at 37°C for 3, 4, 5 and 6 days, the supernatant from each culture was tested for haemagglutination activity. The virus was harvested by freezing and thawing three times. The fluids were centrifuged at 3.000 rev./min. for 15 min. and the supernatant was stored at -20°C.

Haemagglutination test (HA test) – Serial double dilution of the test samples was made in PBS solution using standard U-bottomed plates. An equal volume fo 0,5% guinea-pig erythrocytes was added and the result was read after leaving the plates overnight at 4°C.

RESULTS

1.1. The propagation of PPV in different tissue cultures.

The results obtained for propagation of four PPV strains: NADL-1, NADL-2, NADL-8 and FS 59e/63 in tissue cultures of primary pig kidney cells and the following continual cell lines: PK-15, SB and ST are shown in table 1.

Table 1. Viral haemagglutination titer in 0,020 ml expressed as $\log_2$.

PPV strains	TISSUE CULTURE			
	Primary pig kidney cells	SB	PK-15	ST
NADL-1	11	11	8	7
NADL-2	11	12	7	9
NADL-8	6	7	4	5
FS59-e/63	9	9	8	7

It can be seen that the highest yields were obtained when NADL-1 and NADL-2 strains were propagated in the SB continuous cell line and in primary pig kidney cells. These varied from 11-12 HA/0,020 ml. The occurrence of a cytopathic effect was detected after the formation of a continual layer in all cell cultures inoculated with all four strains examined, but the intensity of the effect was different. The most pronounced cytopathic effect occurred 3 days following inoculation when the NADL-8 strain was grown. These results show that the HA activity of the strains studied did not correlate with the occurrence of the cytopathic effect.

1. 2. The optimum time for harvesting the viruses.

The results regarding the optimum time for harvesting the PPV strains from different tissue culture systems are given in table 2 and 3.

They indicate that the optimum time for harvesting viruses is 3–4 days following inoculation, regardless of the strain tested or tissue culture system used. Thus the HA titer for strain NADL-1 varied within the range of 10–11, for NADL-2 within the range 11–12, for NADL-8 within the range of 5-6 and for strain FS 59e/63 within the range of 8–9 HA/0,020 ml at this time (Figure 1).

1.3. Protein substrate of the medium and its influence upon the activity of PPV strains tested.

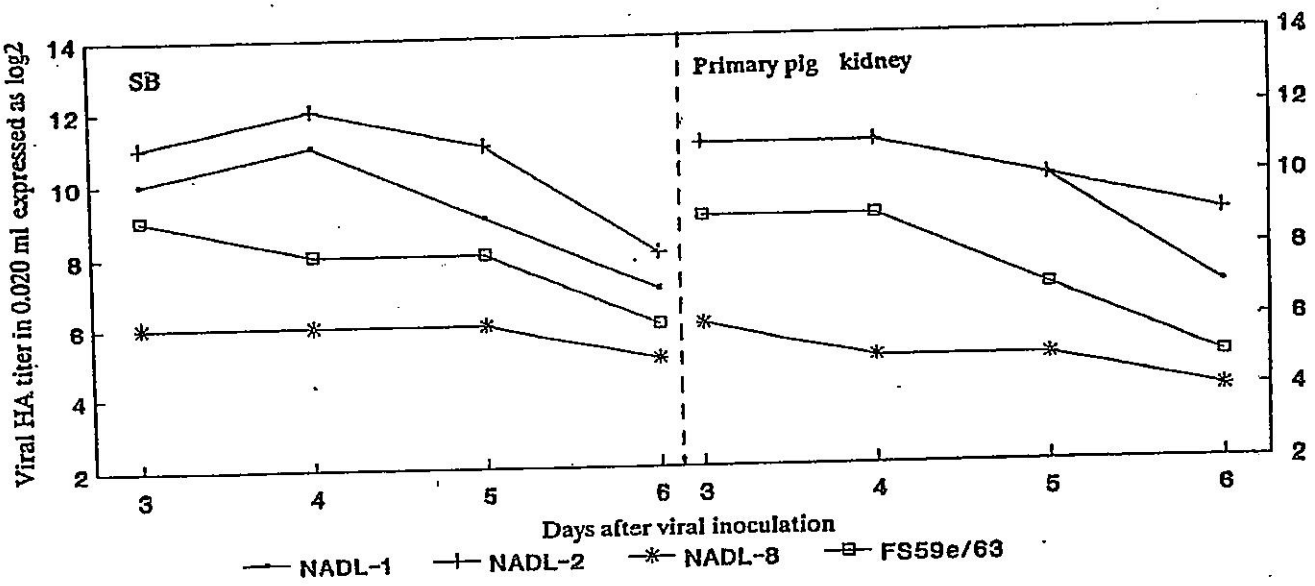

Figure 1. Changes in viral HA titer with time after the inoculation of two different tissue cultures. with four different strains.

The test results obtained regarding the influence of FCS or CS on HA activity of PPV replication in primary pig kidney tissue culture are shown in table 4. for three strains.

Table 2. Viral haemagglutination titer in 0,020 ml expressed as log_2. in relation to time of harvesting for strains NADL-1 and NADL-2

Days after the inoculation	NADL-1		NADL-2	
	Primary pig kidney cells	SB	Primary pig kidney cells	SB
3	11	10	11	10
4	11	11	11	12
5	10	9	10	11
6	7	7	9	8

Table 3. Viral haemagglutination titer in 0,020 ml expressed as log_2. in relation to time of harvesting for strains NADL-8 and FS 59c/63

Days after the inoculation	NADL-8		FS59e/63	
	Primary pig kidney cells	SB	Primary pig kidney cells	SB
3	6	6	9	9
4	5	6	9	8
5	5	6	7	8
6	4	5	5	6

Table 4. Viral haemagglutination titer in 0,020 ml expressed as log_2. for three strains propagated in two different media.

PPV strains	FCS	CS
NADL-1	7	4
NADL-2	7	4
FS59e/63	6	3

These results show that the growth medium which contained 10%FCS as the protein substrate was more favourable for the propagation of all three PPV strains. HA activity values for strains NADL-1 and NADL-2 were 7 and for strain FS 59e/63 it was 6, when the growth medium contained 10% FCS. The HA activity values of the suspensions of virus were much lower and varied between 3 and 4 HA/0,020 ml for all examined strains when the growth medium contained 10% CS.

DISCUSSION

It is known that some strains of certain virus groups may possess a different affinity for replication in different tissue culture, systems, regardless of their biological characteristics. In that case, the rate of their replication is different. The study of the biological properties of PPV strains: NADL-1, NADL-8 and FS 59e/63, show that these strains have the ability of penetrating the transplacental barrier as well the ability to induce fetal death till the age of 56 days (Cartwright et. al., 1971; Mengeling et. al., 1984). The NADL-2 strain is not able to penetrate the placenta but can cause fetal death if directly inoculated into the amniotic fluid (Mengeling et. al., 1984). All the strains studied up to now exhibit antigenic homology (Joo et. al., 1976).

The results of our study show that the examined PPV strains exhibited different HA activity following propagation on the different tissue cultures. Thus, the HA titer for strains NADL-1, NADL-2 and FS 59/63 varied within the range of 7 to 12 HA/0,020 ml. The values for strain NADL-8 were significantly lower, i. e. from 4 to 6. However, viral replication was influencd to a great extent by the tissue culture selected. The highest values for HA activity were obtained when strains NADL-1 and NADL-2 were propagated in tissue cultures of the primary pig kidney cells and the cell line SB, with simultaneous inoculation with cell suspension and they varied between 11–12 HA/0,020 ml.

Most PPV have a trophic requirement for actively dividing cells. Cartwright et. al. (1971) found that growth of the virus was most obvious in young actively growing porcine primary cultures, inoculated 3–4 days after preparation.

The occurrence of a cytopathogenic effect did not correlate with the haemagglutination activity of the strain studied. The most pronounced cytopathogenic effect was observed 3 days following inoculation of strain NADL-8 and it showed the same intensity regardless of the tissue culture employed. Other strains produced more modest cytopathogenic effects.

The optimum time for harvesting the strains varied from 3 to 4 days following inoculation. After that period the HA titer decreased by 2–4 HA/0,020 ml over the next 2 days. Rivera et al. (1986) obtained the highest values for HA titer 6 days following inoculation. Our results do not correlate with these.

The propagation of PPV in tissue culture depends on the protein substrate in the medium. Some bovine serum samples used appeared to contain inhibitors of viral growth. It has been shown that a great majority of bovine serum samples including those of fetal origin possess inhibitors of HA activity as shown by the HA test (Joo et. al., 1976). Our results confirm that the growth medium enriched with 10% FCS was more favourable for the replication of the examined strains then medium containing calf serum.

The data of Paul et. al. (1980; 1982) and Mengeling et. al. (1979) showed that the HA titer of a suspension of virus NADL-2, which was used for the preparation of inactivated vaccine was 256–512 /ml. The HA titer of strain CVL-1243 propagated in 45 day-old pig fetus was 8192/0,20 ml. (Wrathall et. al., 1984). Strain 893/86 propagated in the PK-15 cell line gave an HA titer of 512/0,050 ml (Rivera et. al., 1986a; 1986b) and it was reported that the vaccine consisting of a suspension of virus contained HA activity of 2048/0,050 ml. and stimulated a marked serum antibody response in vaccinated gilts.

Bearing in mind the aforementioned, it could be concluded that tissue cultures of primary pig kidney cells and the SB cell line were more favourable for the propagation of the sudied strains than the other systems, as well as growth medium enriched with FCS. Regarding the higher HA titer of strains NADL-1 and NADL-2, they might be the strains of choice in the preparation of inactivated vaccine.

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REZULTATI UMNOŽAVANJA NEKIH SOJEVA PARVOVIRUSA SVINJA NA RAZLIČITIM KULTURAMA TKIVA

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SADRŽAJ

Cilj rada je bio da se utvrdi koji od sledećih sojeva parvovirusa svinja: NADL-1, NADL-2, NADL-8 i FS 59e/63 se najbolje umnožava na kulturi tkiva primarnog prasećeg bubrega i kontinuiranim ćelijskim linijama PK-15, SB i ST. Najviće vrednosti hemaglutinacione aktivnosti virusne suspenzije dobijene su umnožavanjem sojeva NADL-1 i NADL-2 na kontinuiranoj ćelijskoj liniji SB kao i kulturi tkiva primarnog prasećeg bubrega, i one su iznosile 11–12 ($10g_2$) HA/0,020 ml.

Ispitivanjem proteinskog supstrata u medijumu za rast ćelijske kulture i umnožavanja virusa, utvrđeno je da je fetalni teleći serum u finalnoj koncentraciji 10%, bio pogodniji od telećeg seruma, za umnožavanje virusa jer su hemaglutinacione vrednosti virusne suspenzije bile više. Optimalno vreme za sakupljanje virusa bilo je 3–4 dana od inokulacije, bez obzira na ispitivani soj, odnosno kulturu tkiva.