Vet.Med.J.,Giza. Vol.46, No. 4B. (1998): 737-742.

PREPARATION OF SPECIFIC FELINE PANLEUKOPENIA VACCINE

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SUMMARY

A cell culture living attenuated feline panleukopenia vaccine was prepared on VERO cell culture. The results obtained demonstrated the safety and effectiveness of such vaccine. Safety was determined by the inability of the vaccine to cause disease, and efficacy was determined by the ability of the product to induce antibody production in susceptible cats and by the immunity challenge. Among the use of two different routes of vaccination subcutaneously or intramuscularly, it was found that there was no significant difference in antibody titre induced in cats vaccinated using the two routes.

INTRODUCTION

Feline panleukopenia, feline infectious enteritis or cat distemper is a highly contagious disease affecting family (Felidae). In young cats it causes mortality rate of (60% - 70%) but when infection occurs in pregnancy the virus passes the placenta barrier and causes cerebellar hypoplasia resulting in spontaneous ataxia in the kitten (Greene, 1990). The disease is caused by feline panleukopenia virus (FPLV) which is classified as a member of the family parvoviridae (Porterfield, 1989). The first vaccine for FPL was reported by Leasure, et al (1934). Early in the 1960's at least one vaccine manufacturer started work on an attenuated live virus vaccine (Goff, 1971). In Egypt all FPLV vaccines are imported so Attyat, et. al (1998) studied the growth characteristic of FPLV in tissue culture as one step to produce a vaccine of a living attenuated vaccine instead of the imported vaccines to vaccinate cats and zoo animals as lion, leopard and tiger.

In the present work trials were conducted for production of living attenuated FPL vaccine.

MATERIAL AND METHODS

1- Viruses and cell culture :

FPLV vaccinal and virulent strains (Cu3) were

kindly supplied from James Baker Institute for Animal Health. Cornell University NY. USA.

The vaccinal and virulent strains were propagated in VERO (African green monkey cell line) and NLFK (Feline kidney cell line) respectively according to Attyat, et. al. (1998).

The infectivity titre was calculated according to Reed and Muench (1938).

2- Vaccine preparation :

A feline panleukopenia vaccine was prepared on VERO cells as living attenuated vaccine in a lyopholized form. The used stabilizer was composed of sucrose (50 mg/ml) and lactalbumin hydrolysate (25 mg/ ml) such vaccine was subjected to all quality control measures, including the freedom of foreign contaminants, safety and potency test according to the British Pharmacopia (1990).

3- Animals and Vaccination :

15 Balady cats of about (12 - 16) weeks old, were used in the present study. These cats were chose to be free from external and internal parasites, seronegative to FPLV and clinically normal. All of them were housed under hygienic measures and kept under observation for 15 days preinoculation.

Two cats were inoculated with a double dose of the prepared vaccine as a safety test according to the British Pharmacopia (1990).The other 13 cats were divided into 3 groups, where the 1st group (5 cats) was inoculated each with 1 ml of the vaccine containing 104.2 TCID50 of the vaccinal virus given intramuscalary (I/M). The 2nd group (5 cats) each was inoculated with the same dose given subcutenously (S/C).The 3rd group (3 cats) was kept without inoculation as a test control.

All animals were clinically examined daily for 21 days post vaccination. Blood and serum samples were obtained from them on the 7th, 14th and 21th days post vaccination.

All vaccinated and non vaccinated cats were challenged orally with 103 TCID50 of the virulant FPLV, 21 days post vaccination according to Scott and Glauberg (1975).

4- Virus recovery :

Fecal swabs were obtained from all animals after vaccination and challenge in trails of virus recovery, when FPLV was recovered, it was confirmed by virus neutralization test using known hyperimmune serum.

5- Total leukoytic count:

It was carried out according to Schalm's (1986).

6- Serum neutralization test : (SN)

Microtitre serum neutralization test was done according to Rossiter and Jessett (1982). The serum neutralization antibody titre was calculuted as the reciprocal of serum dilution which neutralize 100 - 200 TCID50 of the virus according to Sing, et al (1967).

RESULTS AND DISCUSSION

Live virus vaccines are usually perferred as immunizing agents against many viral diseases because they induce longer lasting protection (Beve-

ridge, 1967). This study aimed to produce a modified living attenuated FPL vaccine. The prepared vaccine proved to contain the required titre of FPL vaccine which should be not less than 1000 TCID50 per ml (Tissue Culture Infective Dose) according to British Pharmacopia (1990). It was also safe in mice and cats where it had no adverse effect on inoculated animals.

As shown in table (1), it was demonstrated that vaccination of cats with FPL vaccine is effective to immunize cats against the disease when inoculated S/C or I/M. There was no significant differance between the serum neutralizing antibody titre obtained by the two methods of vaccination. These results come in agreement with Davis, et. al. (1970), Bittle, et. al. (1970) and Schultz and Scott (1973).

The serum neutralizing antibody titre began to appear in the 1st week with an average of 1 : 15.8 by I/M route and 1 : 14.2 by S/C route and reached its maximum on the 21th day with an average of 1 : 230 and 1 : 205 by I/M and S/C route respectively. These serum neutralizing antibody titre protected cats from experminal infections by the challenge virus. Scott and Glaubery (1975) found that maximum titre of FPL antibodies obtained on the 14th day post vaccination. The titre obtained in this experminte was found to be similar to that obtained by Scott, et. al (1970) Scott and Glauberg (1975) and Arciuch and Gorski (1985/1986a).

According to Davis, et. al. (1970) and Scott (1971) the serum neutralizing titre of 1 : 8 or

Table (1): FPL neutralizing antibody titre in cats after vaccination and subsequent challenge of immunity with the virulent FPL virus.

Cats group	Serum neutralizing antibody titre*												
	Days post vaccination				Days post challenge								
	0	7	14	21	7	14	21	28	35	42	49	56	63
Vaccinated I/M	< 2	15.8	80.8	230	29	325	325	325	325	325	325	325	325
Vaccinated S/C	< 2	14.2	76.2	205	237	294	294	294	294	294	294	294	294
Non vaccinated	< 2	< 2	< 2	< 2	80	106	128	192	192	192	192	192	192

* Antibody titre : Mean of the reciprocal of serum dilution inhibited the cytopathic effect of 100-200 TCID50 of the virus.

I/M : Intramuscularly.

S/C : Subcutenously

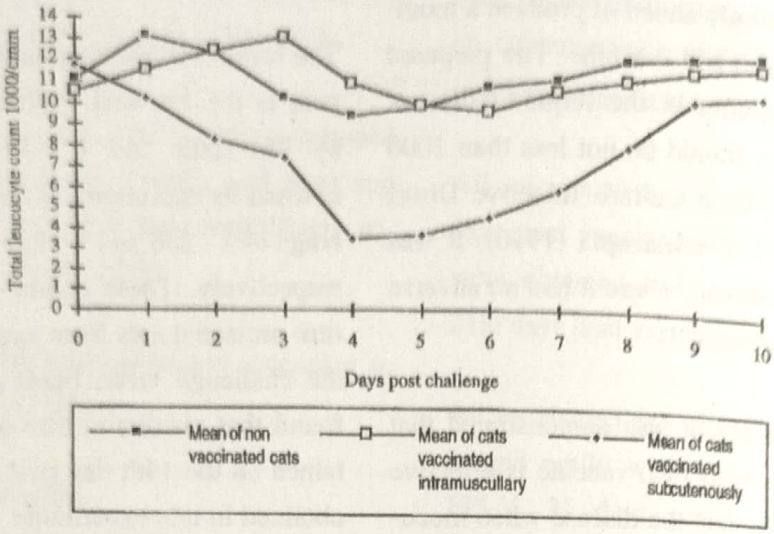

Fig. (1):Sequential changes of total leukocyte counts of vaccinated and non vaccinated cats after challenge with the virulent FPL virus.

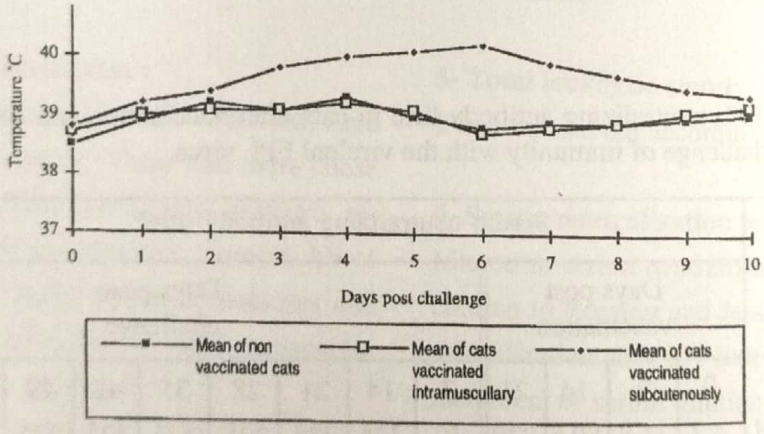

Fig. (2): Sequential changes of rectal temperature of vaccinated and non vaccinated cats challenged with virulent FPL virus.

more is protective against infection and the time required for vaccinated animals to develope resistance against contact infection was determined to be 3 days.

After challenge, the vaccinated cats remained clinically normal during the 14 days observation peroid of immunity challenge, while the 3 susceptible unvaccinated cats developed acute signs of panleukopenia including leukopenia (less than 4.000 cell/c.m.m) between the 4th and 8th days (Figure 1), depression, anorexia, loss of skin tonicity, vomiting, one of them had a bloody diarrhea followed by death within two days after challenge. The highest temperature responses (as shown in Figure 2) were recorded on the 3rd to the 6th day post challenge reached its maximum (40.20C)

These symptoms agree with those recorded by Scott and Glauberg (1975), Arciuch and Gorski (1985/1986b). Wosu (1988) and Greene (1990).

The vaccinated cats didn't shed the virus in their feces, while the infected cats shed the virus from the 2nd day till the 10th day post challenge. These findings come to be confirmed by Csiza, et. al. (1971) Schultz and Scott (1973) and Greene (1990) who mentioned that FPL virus could be shed from the infected cats up to 6 weeks.

In non vaccinated challenged cats, a high antibody titre was detected after 1st week post challenge this coincide with Sciza, et al. (1971), Johnson (1971), Schultz and Scott, (1973) and Scott et. al (1975), who mentioned that in infected cats detectable neutralizing antibodies were present 6 to 8 days after infection and maximum titre was reached by 10 to 12 days. There is apoint of interest, that Gaskell (1984) reported that recovery from infection with virulant virus, probably results in life long immunity.

In conclusion the prepared tissue culture live attenuated FPL vaccine is effective and safe to protect cats from the infection by FPL virus.

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