EVALUATION OF INTRANASAL VACCINATION OF PUPS AGAINST CANINE PARVOVIRUS- 2*

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Canine parvovirus disease, caused by CPV-2, is a highly contagious disease of canines with acute haemorrhagic gastroenteritis and myocarditis and often become fatal. Since its report in 1977, it has been well established as an enteric pathogen of dogs throughout the world with high morbidity and frequent mortality. Usually immunisation is done by repeated parenteral administration of attenuated or inactivated vaccines.

Vaccination by nasal route has proven to be a very convenient and reliable method which resulted in rapid absorption and faster onset of action compared to oral and intramuscular administration (Werneling *et al*., 2002). Intranasal immunisation might lead to the development of local as well as systemic immunity (Zuercher *et al*., 2002). Hence the present study was carried out to evaluate the efficacy of intranasal canine parvovirus vaccination in pups.

Healthy unvaccinated pups of six to eight weeks of age were screened for maternal antibodies to CPV-2 using haemagglutination inhibition (HI) test and six pups with a maternal antibody titre <1:80 were selected for the study (Martella *et al*., 2005). They were vaccinated intranasally with a drop of attenuated canine parvovirus type- 2 (CPV-2) antigens, each dose containing $10^3 TCID_{50}$ antigenic mass on day zero and day two. Blood samples were collected on day seven, 14, 28, 60 and 90 days post vaccination. The faecal samples were collected from all the pups on fifth and sixth day post vaccination and subjected to haemagglutination test to detect shedding of viral antigen. All the vaccinated pups were monitored for a period of one year. Canine parvovirus antibodies in the serum were assessed by HI test (Carmichael *et al*., 1980) and serum neutralization test (SNT) (Pollock, 1981). The results were analysed statistically and interpreted (Snedecor and Cochran, 1994).

All the pups were seroconverted and had protective titre against CPV within a week or two after vaccination. Seroconversion was considered to have occurred when pups were found to develop a fourfold or greater increase in HI titre to a level $\geq$80. This was in agreement with the findings of Martella *et al*. (2005) who found seroconversion in 100 percent of pups with maternal antibody titres of $\leq$80 using intranasal administration of CPV-2b vaccine. The range of geometric mean HI titre from day zero to day 90 post vaccination was 30.58 to 2560 (Table 1, Fig.1 & 2). The peak titre attained was 2560 on day 90 post vaccination; but higher titres were reported by Martella *et al.* (2005), which might be due to the high vaccine titre, more volume and revaccination after two weeks. The differences between consecutive sampling intervals were statistically significant ($P < 0.05$). A similar pattern of seroconversion titres from day zero to day 90 post vaccination was observed by using SNT (Table 2, Fig. 3 & 4). Buonavoglia *et al*. (1994) reported similar results with intranasal vaccination of CPV-2. The SN titres obtained in this study were six times more than that of HI titres. This finding was similar to that of Wei *et al*. (2000) who found a linear positive correlation with the HI and SN titres. A slight fall in seroconversion titre was noticed on 60th day post vaccination in all pups which

may be due to any stress during that period or due to some immunogenic unresponsiveness as suggested by Czerkinsky *et al.* (1999).

Table 1. Geometric mean and range of antibody titres of pups in HI test.

Sl. No.	Day of bleeding	Geometric mean	Range
1	0	30.58	1:0-1:80
2	7	253.98	1:20-1:2560
3	14	640	1:160-1:2560
4	28	1015.95	1:320-1:5120
5	60	905.09	1:160-1:5120
6	90	2560	1:160-1:5120

None of the vaccinated pups developed anaphylaxis or other adverse reactions attributable to the vaccine. Faecal samples collected from all pups on fifth and sixth day post vaccination yielded negative result in HA test indicating no viral shedding in the faeces following vaccination. All the pups were survived in good health during the post vaccination monitoring period of one year and none of the pups developed any disease during this period. Results of the present study indicated intranasal vaccination as an effective, safe and non invasive route for canine parvovirus vaccinations and a minimal vaccine mass was able to induce active immune responses in all pups.

Table 2. Geometric mean and range of antibody titres of pups in SN test.

Sl. No.	Day of bleeding	Geometric mean	Range
1	0	68.12	1:10-1:100
2	7	4641.58	1:10-1:100000
3	14	46415.89	1:1000-1:1000000
4	28	100000	1:10000-1:1000000
5	60	46415.89	1:1000-1:1000000
6	90	681292.10	1:100000-1:1000000

Summary

A preliminary study was carried out to evaluate the efficacy of intranasal canine parvovirus vaccination in pups. Six healthy unvaccinated pups of six to eight weeks of age with a maternal antibody titre less than 1:80 were vaccinated intranasally with one drop of attenuated canine parvovirus type 2 (CPV-2) antigens, each dose containing $10^3 TCID_{50}$ antigenic mass on day zero and day

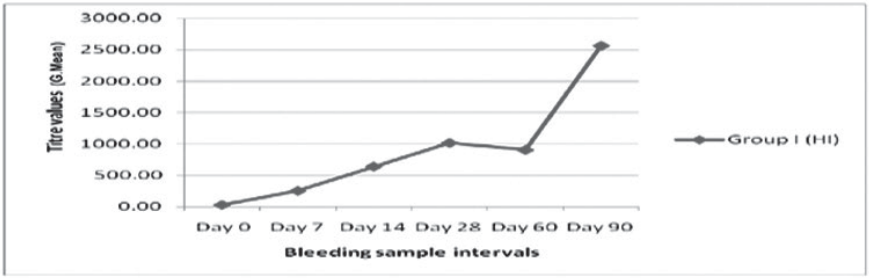

Fig.1. Antibody titres of group I animals in HI test

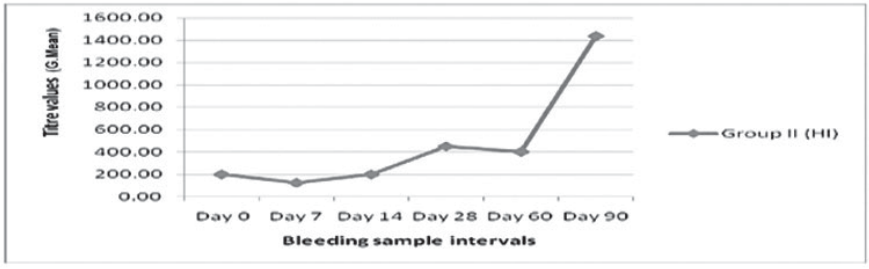

Fig.2. Antibody titres of group II animals in HI test

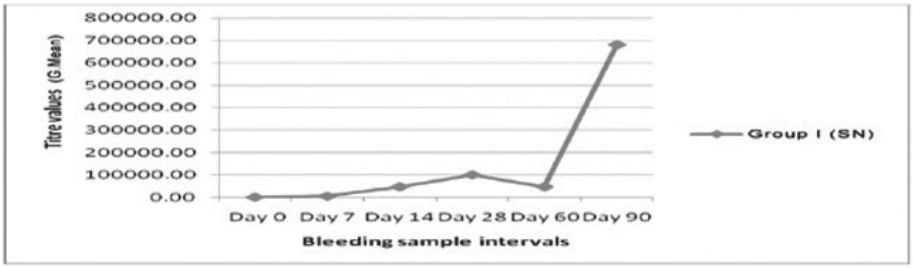

Fig.3. Antibody titres of group I animals in SN test

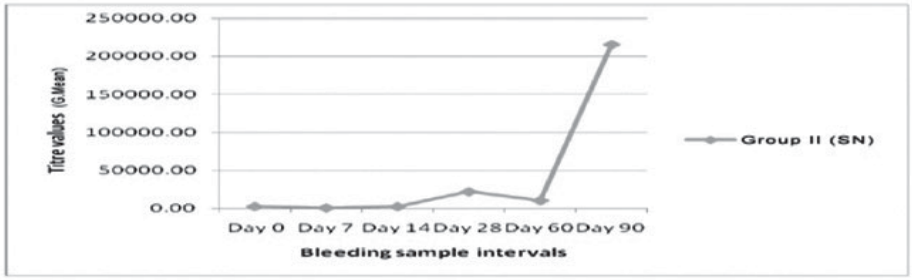

Fig.4. Antibody titres of group I animals in SN test

two and seroconversion was assessed by HI test and SNT on day seven, 14, 28, 60 and 90 days post vaccination. All the pups were seroconverted and had protective titre against CPV within a week or two after vaccination. Results of the present study indicated that intranasal vaccination can be used as an effective, safe and non invasive route for canine parvovirus vaccination and a minimal vaccine volume was able to induce active immune responses in all pups.

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