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## A commercial monovalent canine parvovirus vaccine performs better than a commercial combination vaccine in puppies

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**ABSTRACT:** Thirty puppies were randomly divided into 3 groups for comparative evaluation of two commercial canine parvovirus (CPV) vaccines. Each group was further subdivided into < 6 months and < 3 months -old puppies and either vaccinated with a monovalent vaccine: Primodog, a combination vaccine: Duramune or maintained as a non-vaccinated control. Humoral immune response was determined by Hemagglutination Inhibition (HAI) on 21 and 35 -days after vaccination. The geometric mean titer (GMT) induced by Duramune, 21 and 35 -days post-vaccination was GMT 73.3 and 137.2, respectively. Comparatively, Primodog demonstrated higher GMT on 21 and 35 -days after vaccination: 97.0 and 168.9, respectively. The older puppies (< 6 months old) demonstrated higher seroconversion to both vaccines. Together these data suggest that monovalent CPV vaccines perform better compared to a combination vaccine in inducing humoral immunity to puppies. Moreover, older puppies demonstrate better seroconversion to CPV vaccines.

**Keywords:** Dogs, Primodog, Duramune, enteritis, and serum antibody.

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## INTRODUCTION

Dogs have been working closely with human beings as companion and assistive animals. Dogs' health and services are adversely affected by infectious diseases including Canine parvovirus (CPV). Canine parvovirus typically infects dogs < 6 months of age. Based on antigenic differences CPVs can be classified into CPV-1 and CPV-2. The CPV-2 can be further classified into CPV-2a and CPV-2b.

Canine parvovirus-1 affects neonatal puppies and can cause fetal deaths (Mochizuki et al., 2002). Canine parvovirus-2 (CPV-2) causes a disease that has both enteric and cardiac forms (Pratelli et al., 2001). The enteric form of the disease is marked by severe vomiting and hemorrhagic gastroenteritis in 2-6 months-old puppies. The cardiac form of the disease leads to respiratory or cardiovascular failure in young puppies. Mortality due to this highly contagious disease can reach up to 91% in untreated cases.

In Pakistan, based on clinical diagnosis, the prevalence of CPV enteritis ranges from 21 to 22.7 % (Jafri and Rabbani, 1999). The presence of both CPV-2a and CPV-2/2b has been confirmed in Pakistan (Towakal et al. 2010; Shabbir et al., 2017). Both monovalent and combination CPV vaccines are available for prophylaxis of CPV in Pakistan. The sustainability of a protective titer against CPV depends on a booster dose of vaccine. Research on optimal vaccination strategies including the type of vaccines and the best age of vaccination in puppies is limited in Pakistan. Expanding basic research on the vaccination of puppies in locally relevant conditions can help in streamlining vaccination schedules. Additionally, vaccine trials help in devising control strategies that could be translated into practice (Anderson and Smith, 2019).

The present study evaluated the comparative humoral immune response of puppies to two commercial canine parvovirus vaccines under experimental conditions. The study was designed with the follow-

ing specific objectives: a.) To compare humoral immunity in puppies vaccinated with commercial CPV vaccines under experimental conditions and b.) To monitor the trends of antibody levels against CPV in different age groups of puppies.

## MATERIALS AND METHODS

All experimental protocols were approved by the UVAS Bio-Ethical Committee.

The study comprised of puppies (n = 30) maintained at the Pet Centre, University of Veterinary and Animal Sciences (UVAS), Lahore, Pakistan. Antibody titer induced by a monovalent CPV vaccine: Primodog and a combination CPV vaccine: Duramune were monitored and compared.

### Vaccines

A monovalent CPV vaccine: Primodog (Merial / Boehringer Ingelheim, Ingelheim am Rhein, Germany) and a combination CPV vaccine: Duramune (Elanco US Inc., Fort Dodge, IA, USA) were used in the present study. Both Primodog and Duramune contain CPV vaccines, but they differ in a couple of ways. Firstly, Duramune contains inactivated canine parvovirus (CPV), but Primodog contains a modified live CPV vaccine. Secondly, Duramune contains CPV combined with other antigens such as canine adenovirus, canine parainfluenza virus, canine leptospira (*L. canicola*, *L. icterohaemorrhagica*, *L. grypotyphosa*, and *L. pomona*), and canine distemper virus, but Primodog contains CPV vaccine alone.

### Experimental Animals

Thirty seronegative healthy Alsatian puppies < 6 months of age were randomly divided into 3 groups. Each group comprised 10 puppies (Table 1). For age-wise comparison, each group was sub-grouped into < 3 months (approximately 6 weeks) and < 6 months (approximately 18 weeks) -old puppies. The approximate weight of puppies in the first sub-group

**Table 1.** Experimental design and vaccination schedule for comparison of a combination (Duramune) and monovalent (Primodog) CPV vaccines in puppies

Age	Group A Combination Vaccine: Duramune (n=10)	Group B Monovalent vaccine: Primodog (n=10)	Group C Non-vaccinated Control (n=10)
< 6 months	5	5	5
< 3 months	5	5	5
Day 0	Initial Vaccination	Initial Vaccination	Not vaccinated
Day 21	Booster vaccination	Booster vaccination	Not vaccinated

CPV: Canine parvovirus

(< 3 months) was 3 kg per animal and the approximate weight of puppies in the second sub-group (< 6 months) was 8 kg per animal. Deworming was done one week prior to the start of vaccination. The puppies were housed in the Pet Centre Kennels of UVAS and fed milk and bread for the entire study period. Clean drinking water was supplied to the experimental puppies at all times.

### Vaccination schedule

The vaccination schedule is presented in Table 1. Both the vaccines were administered (@ dose rate of 1mL per vaccine) via subcutaneous route. The puppies in the Control group were injected with phosphate buffered saline at the same dose rate and route as were the vaccines.

### Blood sample collection and processing:

In order to determine their seronegative status, serum samples from the puppies of all groups were collected on the day of vaccination (Day 0). Serum samples were also collected from puppies in all the groups on 21 and 35 -days post-vaccination. Briefly, a 3mL blood sample was obtained from the cephalic vein of each puppy using a 27 gauge butterfly needle (Crow et al., 2009). The serum samples were harvested by placing syringes in a slant position at ambient temperature for 30 minutes. The serum samples were transferred to sterile centrifuge tubes and centrifuged at 1000 x g for 10 minutes. After centrifugation, supernatants were transferred to 1.8mL cryogenic vials and stored at -80 °C, till further testing.

### Hemagglutination Inhibition

A standardized field CPV available at the Institute of Microbiology of the University of Veterinary and Animal Sciences, Lahore, Pakistan was used to titrate the canine serum samples. The virus strain has previously been characterized as CPV-2/2b (Towakal et al. 2010). A Hemagglutination Inhibition (HAI) test following Palmer *et al.* (1975) was used for the titration (Palmer et al., 1975).

### Statistical methods

The geometric mean titer of anti-parvoviral antibodies on 21 and 35 -days after vaccination for each group was calculated and the lowest tested dilution factor was incorporated in a log<sub>2</sub> transformation (Nauta 2011). The one-way analysis of variance (ANOVA) was applied and the statistical significance was set as P values < 0.05 (Gueorguieva and Krystal, 2004).

## RESULTS

### Comparison of monovalent and combination vaccines for induction of anti-CPV antibodies

The day 0 sampling of puppies in all groups demonstrated their seronegative status for anti-CPV antibodies. On 21 days post-vaccination, the GMT of puppies (n=10) inoculated with Primodog: 97.0, was markedly higher compared to the ones (n=10) inoculated with Duramune: 73.3. Similarly, on 35 days post-vaccination, the GMT of puppies inoculated with Primodog: 168.9, was markedly higher compared to the ones inoculated with Duramune: 137.2. The puppies of the control group (n=10) did not show any antibody response on either day. Statistical analysis revealed significant differences in antibody titers of both vaccines (P < 0.05). The comparison of antibody titers induced by both vaccines demonstrated that the puppies receiving the monovalent CPV vaccine: Primodog demonstrated a higher titer of anti-parvoviral antibodies compared to the puppies that received the combination vaccine: Duramune.

### Age-wise comparison of anti-CPV antibodies induced by CPV vaccines

Age-wise comparative results of antibody titers are presented in Table 2. On 21 days post-vaccination in < 6 months old puppies vaccinated with Duramune, 60% of puppies showed an HAI antibody titer of 1/128 and the remaining showed a titer of 1/64. However, in the same age group the puppies vaccinated with Primodog, 100% of puppies showed a titer of 1/128. On 21 days post-vaccination, in < 3 months old puppies vaccinated with Duramune, 80% of puppies showed a titer of 1/64 and the remaining showed a titer of 1/32. However, in the same age group, in the puppies vaccinated with Primodog, 20% of puppies showed an HAI titer of 1/128 and the remaining showed a titer of 1/64. The age-wise comparison of antibody titers 35 days after vaccination followed similar trends and those data are presented in Table 2. The statistical analysis revealed significant differences (p-value<0.05) between the groups of dogs vaccinated with both vaccines.

In summary, a monovalent CPV vaccine induced higher anti-parvovirus antibodies compared to a combination vaccine. Older pups (< 6 months) exhibited better seroconversion to CPV vaccines compared to the younger ones (< 3 months).

**Table 2.** Hemagglutination inhibition antibody titer resulting from an age-wise comparison of a combination (Duramune) and monovalent (Primodog) CPV vaccines in puppies.

Age	Group A Combination Vaccine: Duramune (n=10)		Group B Monovalent vaccine: Primodog (n=10)		Group C Non-vaccinated Control (n=10)	
	# of dogs	Titer	# of dogs	Titer	# of dogs	Titer
<b>21 days post-inoculation</b>						
< 6 months	3	1/128	5	1/128	5	0
	2	1/64	-	-		
< 3 months	4	1/64	1	1/128		
	1	1/32	4	1/64		
<b>35 days post-inoculation</b>						
< 6 months	2	1/256	3	1/256	5	0
	3	1/128	2	1/128		
< 3 months	4	1/128	1	1/256		
	1	1/64	4	1/128		

CPV: Canine parvovirus

## DISCUSSION

The present study was done to evaluate two commercial vaccines for inducing a humoral immune response in puppies to canine parvovirus (CPV) under experimental conditions. The puppies were randomly divided into vaccinated and non-vaccinated groups. The puppies were further subdivided according to their age. The puppies vaccinated with a monovalent vaccine demonstrated a higher immune response compared to the ones vaccinated with a combination vaccine. Additionally, older puppies demonstrated better immune response compared to the younger ones. Together these data suggest that in clinical practice monovalent CPV vaccines offer promising results in controlling the virus in puppies. Since the age of vaccination influences optimal immune response by puppies, the age to vaccinate should be determined by vaccination trials and backed by scientific data.

The monovalent CPV Primodog contains live virus alone at a high titer ( $10^{7.8}$  TCID<sub>50</sub> per 1 ml), hence it is expected to perform better in conferring humoral immunity to dogs (Willem, Lacheretz and Latour, 2001). The relatively lower seroconversion demonstrated by the combination CPV vaccine Duramune can be attributed to the presence of inactivated CPV. The inactivated virus may not effectively stimulate an immune response (Spickler and Roth, 2003). Some of the inactivating agents can lead to adverse effects on the development of humoral immunity (Razmaraii et al., 2012). Additionally, the use of combination vaccines can lead to adverse reactions in dogs (Miyaji et al. 2012). Together, these factors may have contributed to relatively poor seroconversion by puppies in-

oculated with a combination vaccine compared to the monovalent vaccine.

Better seroconversion to CPV vaccines exhibited by older pups (< 6 months) compared to the younger ones (< 3 months) seems to suggest that vaccination could be delayed till puppies reach at least 3 months of age. However, this delay in vaccination may not be practical when the field virus challenge is high. In field conditions, puppies are prone to CPV after maternally derived antibodies (MDA) have waned approximately after 10 days of their age (Nandi and Kumar 2010). However, there is a possibility of persistence of MDA in some puppies beyond 10 days, complicating scheduling of initial vaccination. Vaccination in the presence of MDA is a major challenge in conferring immunity to puppies. Therefore, the determination of MDA titer of 1 or 2 pups in a litter at the 5 or 6 weeks of age could be helpful, when designing a vaccination schedule (Nandi and Kumar 2010). In view of the above, the timing for the primary CPV vaccination should be carefully evaluated, keeping in view the presence of MDA and local virus challenge.

Field data on CPV vaccination suggests that a very high percentage of puppies respond to vaccination at 3 months of age (Nandi and Kumar 2010). In this regard, a prime-boost approach could be helpful. While the initial vaccination of puppies with a high titer monovalent CPV vaccine such as Primodog could be used to prime immune response (Nandi and Kumar, 2010), a combination vaccine such as Duramune can be used for boosting the response to initial vaccination with a monovalent live vaccine. Higher antibody

titer demonstrated by puppies vaccinated with both vaccines is suggestive of an anamnestic immune response and should be taken into account when designing vaccination schedules for canines (Anderson and Smith, 2019).

Evaluating post-vaccination responses in puppies is crucial in determining seroconversion by the inoculated vaccine. Vaccine efficacy studies help in determining immune response to a specified type, dose, and route of vaccine, as well as appropriate age to vaccinate and booster doses necessary to sustain the protective immune response. The serological response of puppies can be evaluated as a correlate of protection in domestic dogs, minimizing the need for challenge studies (Anderson and Smith, 2019; Nandi and Kumar 2010).

## CONCLUSIONS

Canine parvovirus is an emerging and re-emerging global pathogen. Parvoviral enteritis is a major cause of puppy mortality. To decrease the risk of this highly contagious virus, establishing the efficacy of a vaccine is warranted. The present study demonstrated that a monovalent CPV vaccine: Primodog, conferred better humoral immunity in puppies compared to a

combination vaccine: Duramune. Together, these data indicate higher seroconversion in puppies vaccinated with Primodog as compared to Duramune, irrespective of age. These data also suggest better seroconversion in older (< 6 months) dogs compared to the younger ones (< 3 months), irrespective of the vaccine type used. These findings highlight the need to choose a vaccine, based on its ability to seroconvert. Moreover, vaccinating at the proper age is likely to result in the induction of an effective immune response. The present study expands on the limited information available on CPV vaccination in Pakistan. This study also provides insights into the optimal use of vaccines to control CPV in domestic dogs. Future research on evaluating comparative cellular immune responses in vaccine-challenge studies could further elucidate immune responses conferred by both types of CPV vaccines. However, the present study provides enough support to make appropriate decisions for the selection of vaccine type and age for initial vaccination for the control of CPV in puppies.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest.

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