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Elicitation of Immune Response in Pups against Parvovirus Infection

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Abstract

In the present study, 24 unvaccinated healthy pups of either sex preferably in the age group of 8- 18 week were be enrolled and divided in to 4 groups, each contained 6 pups. The 1st group was administered with Megavac 6, 2nd group with Vanguard 5L, 3rd group with Nobivac DHPPiL anti-canine parvovirus vaccines and the 4th group was considered as control. Blood was collected from all the pups on 0th day and vaccinated (treatment groups) on the same day. On the 28th day blood was collected from all the pups followed by administration of the booster dose to all the treatment groups. Again on 35th day only blood was collected from each group without vaccination. From the present study, it was observed that the efficacy of Megavac 6 was significantly ($P<0.01$) higher as compared to Vanguard 5L and Nobivac DHPPiL. All the three vaccines were safe and did not show any adverse reaction during the entire period of study. The overall health status of the vaccinated animals was sound and better than control pups.

Keywords: Canine parvovirus, Pups, Vaccines

Contents

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1. Introduction

Canine parvovirus 2 (CPV2) is a relatively new disease that appeared in the late 1970s. It was first recognized in 1978 and spread worldwide within one to two years [1]. There are 2 types of canine parvovirus called minute virus, CPV 1 and CPV 2 and three antigenic variants of CPV2 called CPV-2a 2b and 2c. Types 2a and 2b are distinct from

the original CPV. Canine parvovirus type 2 (CPV2) is a contagious virus mainly affecting growing pups. The disease is highly infectious and spreads from infected to healthy ones by direct or indirect contact through their feces. The infection is severe in puppies that are not protected by maternal antibodies or vaccination. It has two distinct presentations, cardiac and intestinal form. The disease is both controlled by monovalent and multivalent vaccines. However, sporadic cases do occur particularly in young population when initial vaccination fails because of interference by maternally derived antibodies [2]. Actually there will be a period of about a week when there is not enough maternal antibody to protect puppy but too much to allow a vaccine to work. This period is called the "window of vulnerability". Therefore, the present study was designed to determine comparative efficacy and overall safety of administration of Megavac 6 vaccine with competitor vaccines, Vanguard 5L and Nobivac DHPPiL in vaccinated animals.

2. Materials and Methods

In this study, 24 unvaccinated healthy puppies (non-descript breed) in the age group of 8-18 weeks of either sex were selected .They were divided into four equal groups each containing 6 apparently healthy pups and maintained by implementing all the necessary management measures to avoid the risk of parvovirus infection at the College of Veterinary Science & Animal Husbandry (BAU), Ranchi. All the pups were monitored closely for 2 weeks before their inclusion in the study group. The pups were dewormed 7 days prior to vaccination. The pups belonging to the three treatment groups 1, 2 and 3 were vaccinated with the Megavac 6, Vanguard 5L and Nobivac DHPPiL vaccines respectively. Blood sample were collected aseptically from the pups in sterile glass tubes without anticoagulant on 0, 28^{th} and 35^{th} day. The blood samples were allowed to clot at room temperature. The clear supernatant was poured off in to vial along the side of the tube. Supernatant which were collected in to vial from 24 pups, centrifuged at 1,500 rpm for 5 min for purification and were decomplemented by keeping the serum samples in hot water bath at 56˚C for 30 min. The serum sample thus obtained, were transferred to a sterile clean plastic vials, screw capped, marked properly and stored at -20˚C. All the vaccines were reconstituted according to the manufacturer's instruction.

Serological tests

Hemagglutination inhibition (HI) and serum neutralization test (SNT) tests were performed according to Buxton and Frazer [3] with certain modifications.The HI titers were expressed as the reciprocal of the highest dilution of the serum, inhibiting agglutination of the RBC. Here in this case it was given by 8HA Units of viral antigen. The SNT titer was calculated as the reciprocal of the highest serum dilution which completely neutralized the virus. The data obtained on Antibody titer of HI and SNT were converted in to log 10 value and these converted values were subjected to statistical analysis.

Statistical analysis

Statistical analysis for different observation of different parameters done as per method by Snedecor and Cochran [4].

3. Results and Discussion

24 unvaccinated healthy puppies of either sex preferably in the age group of 8-18 weeks were be enrolled and divided in to 4 groups, each contained 6 pups. The 1^{st} treatment group was administered with Megavac 6, 2^{nd} group with Vanguard 5L, 3^{rd} group with Nobivac DHPPiL and the 4^{th} group was kept as control. Blood was collected from all the pups on 0 day and vaccinated on the same day. On 28^{th} day blood was collected from all the pups after which vaccine was given (except control) on the same day. Again on the 35^{th} day post-vaccination, blood was collected from each treatment group. Serum samples collected on 0, 28^{th} and 35^{th} day from all the groups were put to HI and SNT using CPV-2 (Ka/BE Strain) vaccine virus as test antigen. The data recorded were converted to $\log_{10}$ values and statistical analysis was done to assess the efficacy as well as safety of vaccine for parvovirus immunization

Effect of vaccination on HI titer

Average hemagglutination inhibition (HI) antibody titer of sera after parvovirus vaccination of pups in different treatment groups have been presented in Table 1. On 0 day the critical difference test showed there was no significant difference between the different groups. On 28^{th} day the highest titer was observed in the pups treated with Megavac 6 which showed a significant difference ($P<0.01$) with the control group. Rest of the group did not show significant difference among themselves. On 35^{th} day the lowest antibody titer was observed in Group 4.

Effect of vaccination on serum neutralization antibody titer

On the 28^{th} day, group 1 showed a highest antibody. Group 1 showed significant difference ($P<0.01$) between the treatment groups. On the 35^{th} day, the highest antibody titer was observed in group 1 and the lowest titer in Group 4 (Table 2). Common problem in vaccinating canines, such as dogs and puppies, is the maternal antibody interference during immunization. Maternal antibody interference is the most common cause of vaccine failure in weaning pups. Maternal antibody neutralizes vaccine and suppresses pup´s active immune response. This common immunization

problem occurs with all disease ,but is of particular concern in the case of CPV´s enteritis because of the explosive nature of disease transmission and because pups are at greatest risk of CPV-induced mortality [5].

Tizard [6] reported a significant increase in antibody titer on 3 wks of post first vaccination. After 3 weeks of 2nd vaccination, it was observed that a significant decrease in HI antibody titer happened, but was above protective titer of 1:80. In the control group there was no significant variation between the different days. He concluded from his study that vaccination with Vanguard which contains a CPV2 strain help to protect dog against a virulent infection with CPV 2c-type.

Bergman et al. [7] reported that the puppies vaccinated with commercially available modified live vaccine against CPV, provided adequate protection with final vaccination at 10 weeks age.

Reddy et al. [8] vaccinated the pups with Megavac 6 and collected serum sample on 720 day post vaccination to assess the serum antibody response and observed the HI titer 600.

Wanner et al. [5] vaccinated pups with modified attenuated CPV vaccine at 6 and 9 wks of age observed 39% of the pups responded to vaccination at the HI titer is 1:10, 30% of pups at 1:20, 26% at 1:40 and 5% at 1:80.

Table 1. HI antibody titer (log_{10} values) of pup sera

Day	Group 1	Group 2	Group 3	Group 4 (Control)
0 day	1.95±0.21	1.65±0.25	1.60±0.25	2.20±0.11
28th day	2.91±0.23^{b}	2.66±0.29b^{c}	2.40±0.27^{bc}	1.70±0.15^{a}
35th day	3.86±0.15^{b}	2.71±0.17^{c}	2.61±0.19^{c}	1.35±0.05^{a}

Values bearing same superscript in a row did not differ significantly. All the values were expressed in Mean ± S.E, (P<0.01)

Table 2. SNT antibody titer (log_{10} values) of pup sera between groups

Day	Group 1	Group 2	Group 3	Group 4 (Control)
0-Day	2.00±0.15	1.60±0.19	1.70±0.13	1.75±0.10
28-Day	2.81±0.25^{c}	2.30±0.13^{b}	1.95±0.16^{ab}	1.60±0.11^{a}
35-Day	3.36±0.12^{c}	2.40±0.24^{b}	2.30±0.15^{b}	1.45±0.07^{a}

Values containing same superscript in row did not differ significantly. All the values were expressed in Mean ± S.E, (P<0.01)

4. Conclusion

Megavac 6 was better than Vanguard 5L and Nobivac DHPPiL and was more active during maternal antibody interference. Megavac 6, Vanguard 5L and Nobivac DHPPiL were safe and did not show any adverse reaction during and after vaccination. The health status of the pups vaccinated with Megavac 6 Vanguard 5L and Nobivac DHHPiL were sound and better in comparison to unvaccinated animals.

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