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Research Article

Evaluation of Post-vaccinal Antibody Response to Canine Distemper Virus Vaccine Following a Single Dose of Multivalent (DHLPPi) Vaccines to Nigerian Local Breeds of Dogs (*Canis lupus familiaris*)

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ABSTRACT

The current level of morbidity and mortality among dogs due to canine distemper virus infection raise concerns about the effectiveness of commercially available DHLPPi vaccines. The prevalence of the disease despite vaccination warranted the evaluation of the potency of vaccines that are used for routine vaccination in Nigeria. This study was conducted to investigate antibody responses to the three common brands of DHLPPi vaccines for dogs and to recommend the best immunogenic brand for routine vaccination in dogs in Nigeria. Twenty local breed of dogs, age 8 -14 weeks were purchased from dog breeders in Ibadan, Oyo-State, Nigeria. The dogs were screened for heamoparasites and endoparasites. Those that were positive were treated appropriately and they were acclimatized for three weeks in the University of Ibadan Veterinary Teaching Hospital kernels. They were divided into four groups tagged A, B, C and D. They were fed with rice and meat and formulated rations and served fresh clean water *ad-libitum.* Groups A, B and C were vaccinated while Group D was not vaccinated and served as the control. Blood samples were collected before vaccination (day 0) and weekly for four weeks and 90 days post-vaccination. The sera of collected blood samples were subjected to ELISA test. Mean values of ELISA antibody titers were calculated and the mean values obtained were compared for significant differences using ANOVA test and student t-test. The antibody titres of the three groups A, B and C were observed to increase within a week of vaccination, and the three vaccinated groups showed variable antibody responses on different days of samplings.characterised with rising and waning of antibodies. Group D was observed to be low titres of antibody throughout the study period. From these findings, all the vaccines were potent, however, comparatively vaccine C was the best, vaccine B was better than A. Vaccine C is therefore strongly recommended for use in dogs for routine vaccination and a booster dose should be administered 4-5 weeks after first dose for optimum humoral immunity against canine distemper virus infection. Seromonitoring is essential in planning vaccination regimen for dogs. Other factors that can affect the effectiveness of vaccine during storage, transportation and administration should be considered for a desirable result

Keywords: *Canine distemper virus, DHLPPi vaccine, ELISA, Antibody, Vaccination*

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INTRODUCTION

Canine distemper, (sometimes termed *hardpad disease* in canine), is a highly contagious viral disease that affects a wide variety of animal families, including domestic and wild species of dogs, coyotes, foxes, pandas, wolves, ferrets, skunks, raccoons, and large cats, as well as pinnipeds, some primates, and a variety of other species (Ikeda *et al.,* 2001). The canine distemper virus (CDV) is a member of the genus *Morbillivirus*, of the family *Paramyxoviridae*, and order *Mononegavirales* (Ikeda *et al.,* 2001). CDV is related antigenically to dolphin distemper virus (DDV), human measles virus (HMV), peste de petits ruminant virus (PPRV) and rinderpest virus (RPV) (Eghafona *et al*., 2007)

In dogs, canine distemper affects several body systems (i.e. it is a multi-systemic disease) (Kapil *et al.,* 2008) especially the gastrointestinal and respiratory tracts and central nervous system. The common symptoms include high diphasic fever, conjunctivitis, oculo-nasal discharge, labored breathing and coughing, vomiting and diarrhea, loss of appetite, lethargy, and hardening of nose and footpads (hyperkeratosis) (Kapil *et al.,* 2008).

Morbilliviruses cause an acute disease characterized by generalized immunosuppression, rash, respiratory and gastrointestinal signs, and occasional but devastating neurological complications (Schneider & Schneider, 2008). The advent of preventive vaccination programmes has gone a long way to reduce the incidence of the disease in recent years (McCaw *et al*., 1998).

Histopathologically, canine distemper is reported to be characterized by cellular infiltration of the central nervous system, diffuse encephalitis of the forebrain, perineuronal and perivascular degeneration in the brain, presence of inclusion bodies in different cell types and interstitial bronchopneumonia (Tipold *et al.*, 1999, Ezeibe, 2005). There is also presence of hyperaemia and depopulation of splenic corpuscles, hyperaemia and degeneration of renal tubular epithelia and hyperkeratosis of skin (Ezeibe, 2005).

Horst (1975) reported that the most effective method of controlling canine distemper is mass vaccination of dogs and other carnivores. Most commercial canine distemper vaccines are made from the Onderstepoort strain of the virus isolated in South Africa (Yoshida *et al*., 1999). However, strains different from the Onderstepoort strain have also been isolated (Iwatsuki *et al*., 2000). Although vaccination against CDV with attenuated virus can protect the majority of animals, this protection does not necessarily extend to the field strains (Wang *et al*., 2011).

In Nigeria, commercially available CDV vaccines are marketed and administered in combination with hepatitis, leptospirosis, parvovirus and parainfluenza vaccines (DHLP+Pi combined vaccine) (Greene and Appel, 2006). It is recommended that puppies be given a series of vaccinations to stimulate active immunity as maternally derived immunity declines. This should then be followed by annual revaccination to maintain immunity (Greene, 1990).

However, the usefulness of annual revaccination of dogs is still widely debated. Smith (1995) has suggested that a more cost-effective and beneficial approach is to first measure serum antibody titres to determine the necessity of revaccination.

MATERIALS AND METHODS

Study Population: Twenty local dogs, age between 8 and 14 weeks were purchased from dog breeders in Ibadan, Oyo-State, Nigeria. They were fed for five weeks in the dog kennel of the Veterinary Teaching Hospital, University of Ibadan and were acclimatized for the period. During the period of acclimatization, the dogs were screened for heamoparasites and endoparasites, the infected ones were treated appropriately and they were separated into four groups of 5 dogs per group, tagged A, B, C and D in separate kennel units. The dogs were fed with cooked rice, beans, meat and formulated diet containing indomie waste, fish meal, maize and soya meal. Clean water was given ad-libitum and 12 hours of daylight and darkness were maintained through the period of the experiment.

Blood samples were collected before the dogs were vaccinated (day 0). Vaccines labelled A, B and C were administered to the respective groups A, B and C while group D (control) was not vaccinated. After vaccination, blood samples were collected weekly for four weeks and lastly on day 90. All the animals received humane care according to the criteria outlined in the Public Health Service Policy on Humane Care and the Use of Laboratory Animals (P.H.S., 1996).

Sample Collection and serum preparation

Dogs in each groups were bled via jugular venipuncture using 21-guage needles and 10 ml syringes. Four milliliters (4ml) of blood were collected into plain bottles for serum. The bottles were left slanted on the bench at room temperature for the blood to clot.

Blood samples collected into plain bottles were spun in centrifuge at 5000 rpm for 15 minutes and serum samples were harvested into new plain sample bottles, then stored at -20°C till all samples were collected. The serum samples per group were subjected to CDV indirect antibody

ELISA: An Indirect ELISA test was carried out on the sera using the ELISA antibody test kit for Canine distemper manufactured by INGENASA, C/Hnos, Garcia Noblejas, 39 28037 – MADRID, SPAIN. The recommended protocol by the manufacturer was followed.

Calculations and interpretation of results

The serum CDV antibody titre was calculated as described in the ELISA kit manufacturer's protocol (INGENASA, C/Hnos, Garcia Noblejas, 39 28037 – MADRID, SPAIN)

The cut off optical density (O.D) was calculated by multiplying the O.D of positive control by 0.2 and calculating the mean of the two wells.

Every samples with O.D lower than the cut off are regarded as negative while those with O.D higher than the cut off are regarded as positive.

The positive samples were categorized into three as follows:

- Low titres (corresponding to IFI values of 1/20-1/40). These samples show O.D between 0.2 × O.D of positive control and 0.4 × O.D of positive control.
- Medium titres (corresponding to IFI values of 1/80-1/160). These samples show O.D between 0.4 × O.D of positive control and 0.8 × O.D of positive control.
- High titres (corresponding to IFI values of ≥ 1/320). These samples show O.D higher than 0.8 × O.D of positive control.

Statistical analysis

Mean values of ELISA antibody titers were calculated and the mean values obtained were compared for significant differences by using ANOVA test and student t-test.

RESULTS

As shown in Fig. 1, the mean CDV antibody titers were interpreted using the model provided by the ELISA kit manufacturers as follows:

Titre value of positive control = 0.094 while Titre value of negative control = 0.0014. (Low titres = Titres between 0.019 – 0.037; Medium titres = Titres between 0.037 – 0.075 while High titres = Titres greater than 0.075).

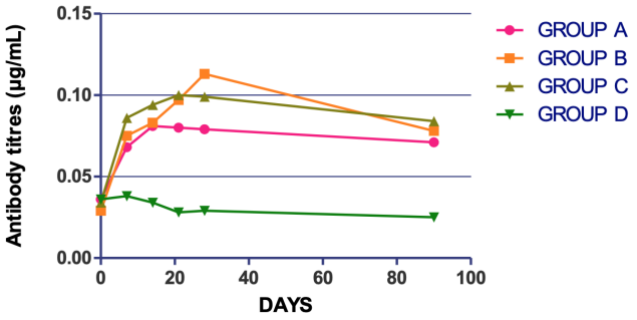

Figure 1.
Line graph showing Canine distemper vaccinal antibody titers in Nigerian local dogs on three types of vaccines.

Dogs in groups A, B, C and D had low titres of antibody before the vaccination as shown in the respective serum antibody titres of 0.036±0.010, 0.029±0.008, 0.034±0.018 and 0.036±0.010 on day 0. There was no significant difference in the mean titre values among the groups of dogs used in this study (Figure 2).

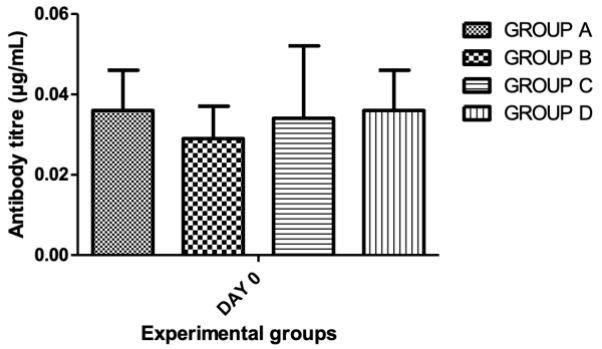

Figure 2.
Graph showing the mean ± standard deviation (SD) serum antibody titers to CDV in dogs sampled on day 0 prior to vaccination. Significance at P<0.05.

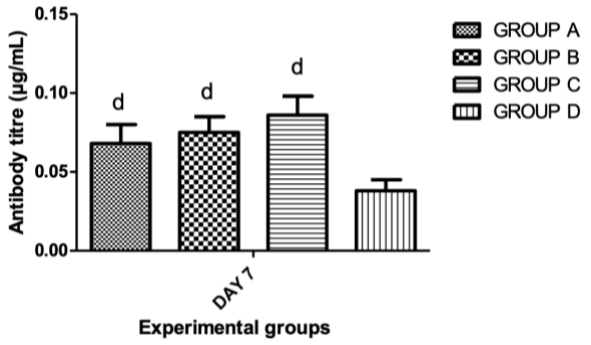

Figure 3:
Graph showing the mean ± standard deviation (SD) serum antibody titers to CDV in dogs on day 7 following a single dose of three types of DHLPPi vaccines. Significance at P<0.05 and 'd' indicates significance when titres of other groups are compared to group D. Group A, Band C received vaccines tagged A,B and C respectively while Group D was the control.

On day 7, the mean antibody titres were 0.068±0.012, 0.075±0.010, 0.086±0.012 and 0.036±0.001 respectively for groups A, B, C, and D. In comparison to the mean titre of group D, a significant (P<0.05) increase was observed in groups A, B, C. The antibody titre of group A was observed to be within medium titre range, those of groups B and C were within high titre range while that of group D was within low titre range (Figure 3)

On day 14, the mean antibody titres for groups A, B, C and D were 0.081±0.015, 0.083±0.013, 0.094±0.017 and 0.038±0.007 respectively. The antibody titres of group A, B and C was observed to be within high titre category while that of group D was of low titre category. A significant (P<0.05) increase was observed in the titres of groups A, B, and C when compared to group D (Figure 4).

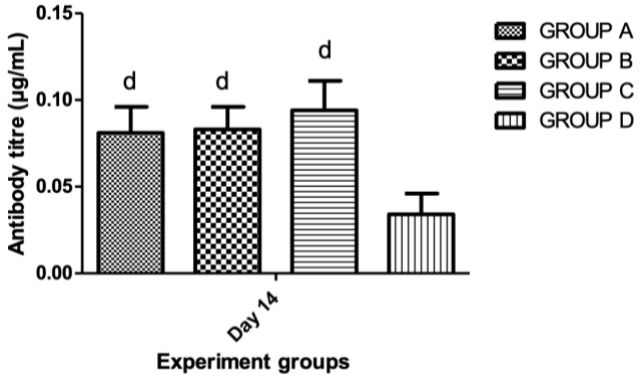

Figure 4:
Graph showing the mean ± standard deviation (SD) serum antibody titers to CDV in dogs on day 14 following a single dose of three types of DHLPPi vaccines. Significance at P<0.05 and 'd' indicates significance when titre of other groups are compared to group D. Group A, Band C received vaccines tagged A,B and C respectively while Group D was the control.

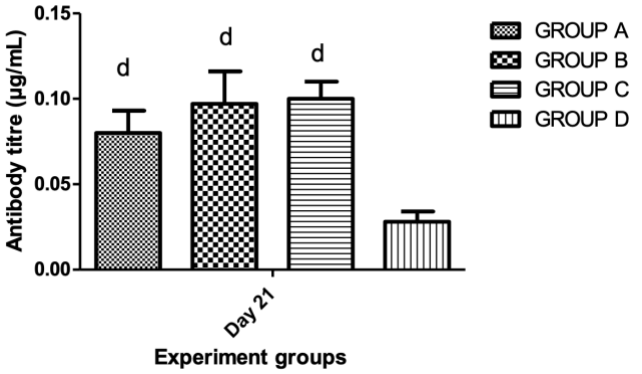

Figure 5:
Graph showing the mean ± standard deviation (SD) serum antibody titers to CDV in dogs on day 21 following a single dose of three types of DHLPPi vaccines. Significance at P<0.05 and 'd' indicates significance when titres of other groups are compared to group D. Group A, B and C received vaccines tagged A, B and C respectively while Group D was the control.

On day 21, the mean antibody titres for groups A, B, C and D were 0.080±0.013, 0.097±0.019, 0.100±0.010 and 0.034±0.012 respectively. The antibody titre of group A, B and C were observed to be of high titre category while that of group D was of low titre category. A significant (P<0.05)

increase was observed in the titres of groups A, B, and C when compared to group D (Figure 5).

On day 28, the mean antibody titres for groups A, B, C and D were 0.079±0.011, 0.113±0.011, 0.099±0.018 and 0.029±0.005 respectively. The antibody titres of group A, B and C were observed to be of high titre values while that of group D was of low titre value. A significant ($P<0.05$) increase was observed in the titers of groups A, B, and C when compared to group D. The antibody titre of group B was significantly ($P<0.05$) higher than that of group A (Figure 6).

On day 90, the mean antibody titres for groups A, B, C and D were 0.071±0.005, 0.078±0.006, 0.084±0.007 and 0.025±0.003 respectively. The antibody titre of group A was observed to be of medium titre, those of groups B and C were of high titres while that of group D could be categorized as low titre. A significant ($P<0.05$) increase was observed in the titers of groups A, B, and C when compared to group D. The antibody titre of group C was significantly ($P<0.05$) higher than that of group A (Figure 7).

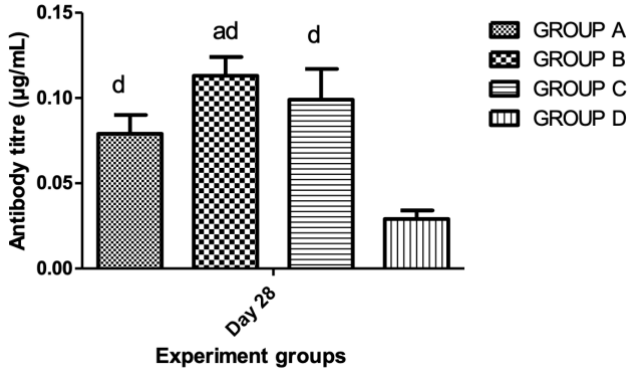

Figure 6:
Graph showing the mean ± standard deviation (SD) serum antibody titers to CDV in dogs on day 21 following a single dose of three types of DHLPPi vaccines. Significance at $P<0.05$ and 'd' indicates significance when titres of other groups are compared to group D. Group A, B and C received vaccines tagged A, B and C respectively while Group D was the control.

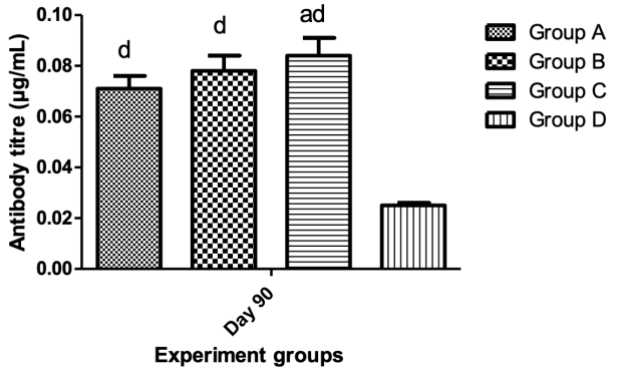

Figure 7:
Graph showing the mean ± standard deviation (SD) serum antibody titers to CDV in dogs on day 21 following a single dose of three types of DHLPPi vaccines. Significance at $P<0.05$ and 'd' indicates significance when titres of other groups are compared to group D. Group A, B and C received vaccines tagged A, B and C respectively while Group D was the control.

DISCUSSION

This study was carried out to quantify and compare serum CDV antibodies responses to three different types of DHLPPi vaccine used in the vaccination of dogs in Nigerian local dogs (Canis lupus familiaris) using enzyme-linked immunosorbent assay (ELISA). This is to evaluate the efficacy of different brands of the imported DHLPPi vaccines used for routine vaccination of dogs and to determine the level of antibody protection within the period of our study.

ELISA is an antibody detection and quantification technique that has been used for seropositivity test in a study carried out by Waner *et al*. (1998). Gill *et al*. (2004) also described ELISA as a highly sensitive test for CDV-specific antibodies independent of biological function.

The purpose of vaccination is to protect animals from infectious diseases by enhancing a specific immune response (Taguchi *et al*., 2011). This immune response takes advantage of memory B cells produced as a result of a primary exposure to an antigen in order to produce a secondary immune response following a second exposure to the same antigen.

The antibody responses to vaccination of the experimental dogs with a single dose of three different types of DHLPPi vaccine suggest significant increase in the antibody titres across all groups when compared with the control group. This observation agrees with a similar study by Durrani *et al*. (2012) on Mono- and polyvalent rabies vaccines in Dogs.

The antibody titres post-vaccination in the three groups of dogs showed that the dogs produced CDV specific antibodies to the respective vaccine administered. There were significant differences in the antibody titre of the dogs in the three groups A, B, and C compared to the control group from day 7 through the entire period of the study. This agrees with the study of Wilson *et al*. (2017) who found antibody production in animals after vaccination and Gill *et al.* (2004) who reported antibody response from day 4 to a multivalent vaccine containing CDV.

The trend of antibody titres in Groups A, B, and C showed differences in the duration of attaining peak antibody level based on the administered vaccines and declining. A booster dose will therefore be necessary to enhance the antibody production through secondary immune response. The timing of a booster dose of vaccine should however be based on serologic titre check (Wellborn *et al*., 2011). The mean antibody titre in group A dogs reached its peak on day 14 through 21 and began to decline thereafter, Group B on the other hand had a steady rise in antibody titre till day 28 and subsequently declined. Group C also reached its peak on day 21 and maintained it to day 28 but subsequently declined. Group D antibody titre was low through throughout the period of this study. From this trend of antibody titre, it can be inferred that a booster dose could safely be given to group A dogs at 4 weeks while groups B and C could be revaccinated from 5-6 weeks after the primary vaccination. This findings is different from the vaccination regimen recommended by Gill *et al*. (2004). They recommended revaccination on day 21 for a long lasting immunity based on their finding that the antibody titre reached its peak on day 12 but declined thereafter.

We also found from this study that the titres of the four groups of dogs were low on day 0 but by day 7, the antibody

titres in Groups A and B had increased to medium titre while that of Groups C was high titre. The three groups had high titres till day 90 except the group A that had the titre reduced to medium status. This finding showed that the dogs had adequate protective antibody to day 90. This findings agreed with the report of Gill *et al.* (2004) who said that the serological responses in vaccinated animals correlate reasonably with protective immunity for CDV. The further said that continuous detection of high titre antibody and resistance to infection on field challenge with specific antigen are indicators of protection from CDV infection.

Persistent levels of morbidity or mortality due to CDV infection despite vaccination could be as a result of vaccine failure which ensues mostly from interference with maternally derived antibody and break in cold chain of vaccine administered as reported by Waner *et al* (1998). Failure of vaccination as a result of the presence of maternally-derived antibody in puppies up to 16 weeks of age has been widely documented. It is the result of maternally-derived antibody titre falling below protective levels but high enough levels to block an active immune response by the vaccinated puppies (Dongoyaro, 2010)

The persistence of CDV may also be caused by infection due to challenge from local field strains of the virus (i.e. virus strains different from the ones used in producing the vaccine).

Puppies with poor MDA may be vulnerable (and capable of responding to vaccination) at an earlier age, while others may possess MDA at such high titers that they are incapable of responding to vaccination until 12 weeks of age (Friedrich & Truyen, 2000). Vaccination regimen commonly practiced in Nigeria involving revaccination of the animals twice on monthly interval, this agrees with the recommendation of Paul *et al.* (2006) that dogs can be revaccinated twice after primary exposure. This practice will assist in reducing vaccine failure caused by MDA especially in places where there are no facilities for seromonitoring.

Other factors like poor knowledge and attitude of dog owners towards vaccination and care of dogs, improper handling of vaccines and errors in administration may cause vaccine failure and may contribute to the prevalence of the disease despite availability of potent vaccines.

Public education through mass media and public enlightenment campaign, use of posters, seminars at religious centers and children education about animal diseases and prevention measures are recommended by Adejumobi *et al*. (2016) as means of increasing people's knowledge about diseases and steps to control vaccine preventable diseases of dogs.

In conclusion, this study showed that the three vaccines used in this study are immunogenic. However vaccine C ranked best, followed by vaccine B while vaccine A gave the least response. Vaccines C and B are therefore potent and can safely be used for routine vaccination in Nigeria. Furthermore, a period of 4-5 weeks interval seems optimum for administration of booster dose of vaccine as shown by the result from this study.

To effectively ensure effectiveness of vaccination and efficient control of CDV in Nigeria, we recommend public education on the importance of animal immunization and mass vaccination. In addition, those factors that can cause vaccine failure should be put into consideration and avoided. Veterinarians should therefore handle vaccine properly, vaccines should be kept from sunlight and stored properly at recommended temperature. Vaccines should be reconstituted with recommended solvent and with appropriate volume and reconstituted vaccine should be used within the specified time and at the right dosage. Appropriate vaccination regimen should be followed, also primary and secondary vaccination should be done at the stipulated time intervals. On the other hand, animal that are sick, immunosuppressed, stressed or on steroid medication should not be vaccinated to ensure optimum immune response. Dog owners should be educated on disease control, biosecurity and vaccination. Dogs should be properly fed because vitamin and protein deficiency result in suppression of immune system. Dogs should be housed in a well-ventilated and hygienic kennel to get a good immune response to vaccination.

We also recommend that further study be carried out on the antibody response to secondary exposure of these vaccines. Also seromonitoring for adult dogs and pre-vaccination titre check for puppies should be done routinely to design vaccination program based on available vaccines

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