

Evaluation of Postvaccinal Antibody Responses to Three Brands of Combined Vaccines Containing Canine Parvovirus Type 2 Following Single Administration in Nigerian Local Dogs (*Canis lupus familiaris*)

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THE current level of morbidity and mortality among dogs due to canine parvovirus infection despite vaccination raises concerns about the effectiveness of commercially available polyvalent modified live vaccine containing Distemper, Adenovirus, Leptospira, Parvovirus and Parainfluenza antigens (DHLPPi) in Nigeria. This study was conducted to evaluate canine parvovirus type 2 (CPV-2) antibody responses to three different brands of DHLPPi vaccines. Fifteen Nigerian local dogs, age 8- 14 weeks; were randomly selected. The dogs were divided into 3 groups (A, B and C) each containing 5 dogs. The dogs were vaccinated with respective polyvalent modified live vaccines containing Distemper, Adenovirus, Leptospira, Parvovirus and Parainfluenza antigens (DHLPPi) tagged A, B and C. Blood samples were collected before vaccination weekly for four weeks and on day 90 post vaccination. The sera from the collected blood samples were subjected to Indirect Enzyme Linked Immunosorbent Assay (ELISA). The mean titre values were analysed using ANOVA test and student t-test. Group A had a statistical significant ($P<0.05$) higher antibody titre when compared with that of group B on day 21. A statistical significant ($P<0.05$) increase in antibody response was found in group B when compared to group A on days 14 and 28 and group C on day 14. Group C had significantly higher antibody titre when compared to group A on days 7 and 28 and group B on day 21.

The group C antibody titres were found to be the consistently higher than those of groups A except on days 14 and 21 and and group B on every other days except on days 14 and 90.

In conclusion, the three vaccines were found to be immunogenic, but vaccines B and C gave adequate and consistently high immune response while vaccine A gave the least response. The occurrence CPV enteritis in dogs despite vaccination may therefore, be attributed to vaccine failure as a result of poor immunogenicity of some vaccines used in routine vaccination of dogs and other factors that may affect the quality of vaccine or ability of the host to produce adequate antibody to administered vaccines.

Keywords: Canine parvovirus 2, Dog, ELISA, Antibody, Immunogenicity.

Introduction

Canine parvovirus is a small, non-enveloped and single stranded DNA virus belonging to the family Parvoviridae [1]. Canine parvovirus (CPV2) belongs to genus Parvovirus and it is related to feline pan-leukopenia virus (FPV), mink enteritis virus (MEV) and raccoon parvovirus (RPV) [2].

CPV2 causes acute, highly contagious, sometimes fatal enteritis in dogs [3] and acute haemorrhagic enteritis and myocarditis in puppies over the age of 3-4 months [4]. The virus replicates in the intestinal crypts and the lymphoid organs and it can spread to all tissues [5], including the brain [6, 7]. After penetration through the oronasal

route, the virus replicates in gastro-enteric associated lymphoid tissues and is disseminated by infected leukocytes to the germinal epithelium of the crypts of the small intestine, causing diarrhoea and acute lymphopenia [5].

The disease is more severe in younger animals, it may be asymptomatic in adult dogs. Infected adult dogs may be apparently healthy but may act as carriers of the virus to other susceptible animals [8]. It was reported that breeds like Rottweiler, America pit bull terriers, Doberman pincher and German shepherd dogs (GSDs) are at higher risk of CPV infection than breeds like Toy poodles, cocker spaniel and mixed breeds [9].

The most common form of the disease is enteritis [9] which is clinically characterized by vomiting, diarrhoea, dehydration, dark or bloody faeces, fever and leukopenia counts [10]. The most dramatic manifestation of CPV-2 myocarditis is sudden death in young pups usually about 4 weeks of age [11]. The mortality may be very high (up to 70%) in pups but much less in adult dogs. The haemorrhagic enteritis, majorly of the small intestine and enlargement of the mesenteric lymph nodes and payer patches are the main gross signs found in dogs at necropsy [12].

Effective vaccines like modified live (ML) and inactivated parvovirus vaccines have been used to fully protect susceptible sero-negative pups [13]. The only concern is the ability of the vaccine to protect against challenges of any of the three serotypes, namely CPV-2a, CPV-2b, and CPV-2c [14]. Greenwood *et al.* [15] however reported that live attenuated CPV2 vaccine is able to protect dog against CPV 2a and CPV 2b field challenges.

Commercially available CPV-2 vaccines are marketed and administered in combination with distemper, hepatitis, leptospirosis, and para-influenza vaccines (DHLPPi) [16].

It is recommended that puppies be given a series of vaccinations to stimulate active immunity as maternally derived immunity declines [17].

However, the usefulness of annual revaccination of vaccinated dogs is still widely debated because of high occurrence of CPV enteritis with attendant high mortality and morbidity [18, 19 and 20]. Early studies have also demonstrated interference of vaccination of puppies by maternally derived antibody (MDA) [21]. It was also reported that colostral transfer account for approximately 90% of MDA [22, 23]. Puppies and kittens from unvaccinated dams have no inhibitory MDA and may be easily susceptible to infection [24]. The interference with the immune response to vaccination by the MDA may result in vaccine failure and CPV infection. MDA interference in such puppies may last up to 40 - 69 days [25]. In the absence of MDA blockade, the onset of protection against CPV infections is as early as 3 days post vaccination [17]. Pups with low protective antibody can be vaccinated by six weeks while those with higher protective antibody should be delayed for more weeks [17]. Optimum age at which a pup should

be vaccinated should be determined by serologic tests of MDA taking into consideration the half-life of MDA which is about 9-10 days [17]. The fact that canine parvovirus enteritis still occurs in dogs despite vaccination informed this study.

This study was carried out to investigate the immunogenicity of CPV-2 vaccines in three brands of polyvalent DHLPPi vaccines commonly used for routine vaccination of dogs in Nigeria. The study also aimed at determining the trend of antibody titres to the vaccines in other to determine the optimum time for a booster dose in indigenous dogs.

Material and Methods

Experimental animals

Fifteen apparently healthy Nigerian local puppies, age 8 - 14 weeks, were procured from a dog breeder in Ibadan, Oyo-State, Nigeria. The dogs were acclimatized for a period of 5 weeks during which they were dewormed and certified to be free of infection. They were thereafter divided into three groups; A, B and C in separate pens with each group containing five dogs. They were fed on home cooked meals and provided water *ad-libitum*.

Blood sample was collected from each dog before the vaccination of the dogs and thereafter, dogs in groups A, B and C were administered vaccines labelled A, B and C, respectively. A single dose of their respective brands of polyvalent modified live vaccines containing Distemper, Adenovirus, Leptospira, Parvovirus and Para-influenza antigens (DHLPPi) were administered subcutaneously. After the vaccination, blood samples were collected weekly for 4 weeks and thereafter on day 90 post vaccination. 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 [26].

Sample Collection and serum preparation

Blood was collected via jugular venipuncture using 21-gauge needles and 5 ml syringes.

Blood was collected into plain and EDTA tubes for serology and haematology respectively.

Blood samples collected in plain bottles were centrifuged at 5000 rpm for 15 minutes and serum samples were harvested and stored at -20°C till they were analysed.

Serological Analysis

A commercial antibody detection ELISA (indirect) test kit manufactured by INGENASA, C/Hnos, Garcia Noblejas, 39 28037 – MADRID, SPAIN was used for the detection of antibodies to CPV in serum following the manufacturer's protocol.

Test sera were diluted using 1/100 dilution factor in the diluent and wash solution was diluted using 1 part concentrated wash solution and 9 parts distilled water. About 100 µl each of control negative and positive samples were dispensed into duplicate wells. Test samples (100 µl) were also dispensed per well and incubated for 10 minutes at room temperature. Excess reagent was tapped-off the plate and washing was done by dispensing 300 µl of wash solution per well as instructed by the manufacturer. The plate was emptied and the excess was tapped out on paper towel. The procedure was repeated four times after which 100 µl of conjugate (Peroxidase- labelled MabanticanineIgG) was immediately dispensed into each well and allowed to incubate for 10 minutes at room temperature. The plate was emptied and washed as earlier described. Thereafter, 100 µl of substrate, 3, 3', 5, 5', Tetramethyl [1, 1',-biphenyl]-4, 4'-diamine (TMB), was immediately dispensed into each well and allowed to incubate for another 5 minutes at room temperature. Stop solution (100 µl) was immediately dispensed into each well. The absorbance of each well was read using an ELISA plate reader at 450 nm within 5 minutes after the addition of stop solution.

Calculations and interpretation of results

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

Percentage increase in antibody was calculated using the formula $A_1 - A_0 / A_0 \times 100\%$. Where $A_1 - A_0$ is the difference in antibody titres and A_0 is the initial antibody titre.

Statistical analysis

The result of the ELISA antibody titres were calculated using proportion and descriptive statistics. The mean values obtained were compared for significant differences using ANOVA and student t-test.

Results

The mean CPV antibody titres for all the groups from day 0 to day 28 and day 90 post-vaccination is presented in table 1 below. At day 0, the mean CPV antibody titre of group A was 3168.50 ± 75.39 , while those of B and C were 2645.75 ± 190.23 and 3238.75 ± 161.94 respectively.

On day 7, a statistically significant ($p < 0.05$) decrease in mean CPV antibody titre was observed in group A (3159.2 ± 167.05) when compared to that of group C (3608.5 ± 268.11). The mean titre of group B (3450.50 ± 270.10) was higher than that of group A, but less than that of group C (3608.5 ± 268.11) although these differences were not statistically significant. On day 14 also, the mean CPV antibody titres in groups A, B and C were 3507.0 ± 285.48 , 3822.75 ± 336.22 and 3264.0 ± 143.42 respectively (Table 1). The mean CPV antibody titre value of group B was statistically significantly higher ($P < 0.05$) than that of groups A and C.

On day 21, the mean titres in groups A, B and C were 3732.75 ± 183.02 , 3114.75 ± 154.34 and 3689.5 ± 212.38 respectively, with value of group A being statistically significantly higher ($P < 0.01$) when compared to that of group B. Also, mean titre value of group B had a significantly lower ($P < 0.01$) value than that of group C.

At day 28 also, the mean CPV antibody titres in groups A, B and C were 3008.75 ± 282.74 , 3512.0 ± 182.32 and 3587.50 ± 204.19 respectively, with value of group A being statistically significantly lower ($P < 0.05$) than that of group B, and group C ($P < 0.01$).

At day 90, the mean titres in groups A, B and C were 3342.3 ± 208.84 , 3517.667 ± 143.81 and 3393.33 ± 137.31 respectively. The mean CPV antibody titre value of group B was higher than those of both groups A and C. The mean CPV of group C is next to that of group B while that of group A was the least. These differences were however not statistically significant (Table 1)

The trend and relative percentage changes in vaccinal antibody response to the brands of vaccines administered are presented in figure 1 and table 2 below. Dogs in group A showed an initial rise in mean CPV antibody titre from day 7 (-0.29%) till day 21 (10.7%) followed by a drop on day 28 (-5.04%) before another steady rise till day

90 (5.49%). In group B, there was a significant rise in mean CPV antibody titre from day 0 through day 7 (30.42%) till day 14 (44.49%). It however declined on day 21 (17.73%) but rose steadily from day 28 (32.74%) to day 90 (32.96%). CPV

antibody titre in group C rose from the basal level by 11.43% on day 7. It however dropped on day 14 (0.78%) but rose to the peak on day 21 (13.92%) after which it began to drop through day 28 (10.79%) to day 90 (4.77%) (Table 2).

TABLE1. ELISA CPV antibody titres (Mean \pm SD)in Nigerian Local dogs vaccinated with different brands DHLPPi vaccine.

Groups (Mean and Standard Deviation of Antibody Titres)			
Sampling Period (Post-Vaccination)	Group a	Group b	Group c
DAY 0	3168.50 \pm 75.39	2645.75 \pm 190.23	3238.75 \pm 161.94
DAY 7	3159.2 \pm 167.05	3450.50 \pm 270.10	3608.5 \pm 268.11 ^a
DAY 14	3507.0 \pm 285.48	3822.75 \pm 336.22 ^{a,c}	3264.0 \pm 143.42 ^b
DAY 21	3732.75 \pm 183.02	3114.75 \pm 154.34 ^{a,**}	3689.5 \pm 212.38 ^{b,**}
DAY 28	3008.75 \pm 282.74	3512.0 \pm 182.32 ^a	3587.50 \pm 204.19 ^{a,**}
DAY 90	3342.3 \pm 208.84	3517.667 \pm 143.81	3393.33 \pm 137.31

Values with different superscripts within columns are significantly different ($P < 0.05$). Asterisks within rows show significant difference.

TABLE 2. Percentage serum antibody production to CPV antigen in Local dogs immunised with different brands of DHLPPi vaccines.

Sampling Period (Days)	0	7	14	21	28	90
Group A (%)	0	-0.23	10.70	17.81	-5.04	5.49
Group B (%)	0	30.42	44.49	17.73	32.74	32.96
Group C (%)	0	11.43	0.78	13.92	10.77	4.77

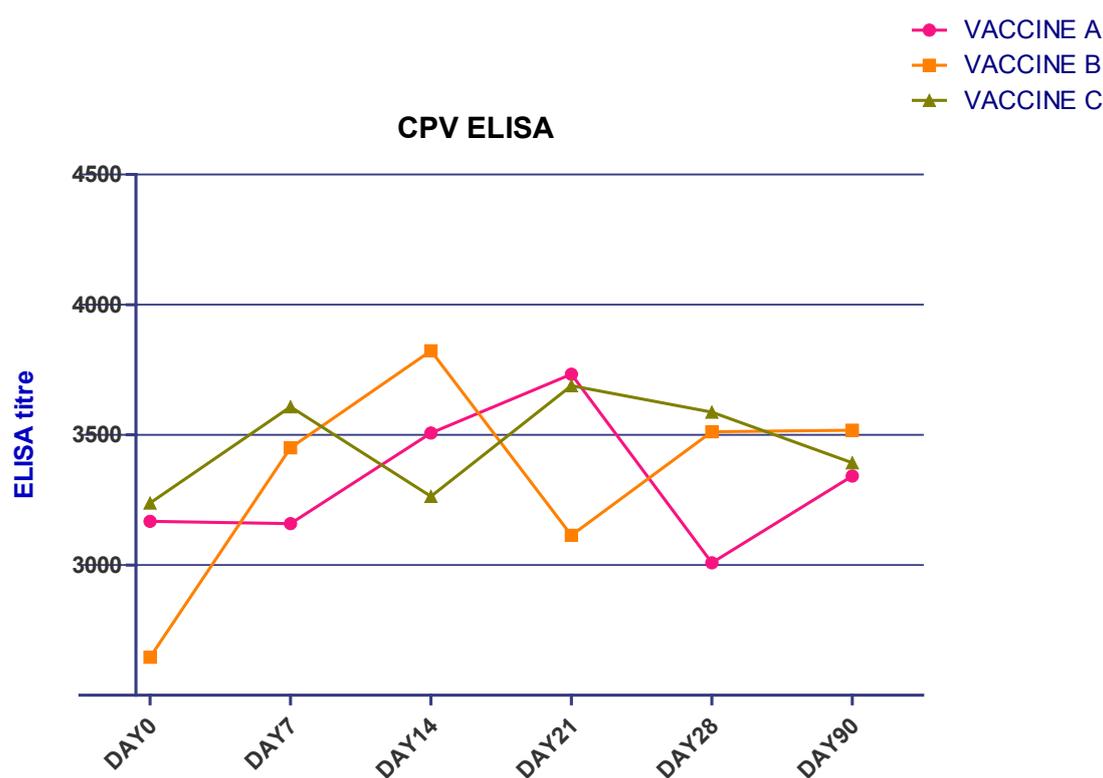


Fig. 1. Line graph showing Canine parvovirus vaccinal antibody titres in Nigerian local dogs vaccinated with a single dose of three brands of DHLPPi Vaccines.

Discussion

The purpose of vaccination is to protect individuals from infectious diseases by enhancing a specific immune response [27]. This immune response takes advantage of memory B cells produced by the B cells on primary exposure to an antigen in and this help the animal to produce a faster and better secondary immune response following a second exposure to the same antigen. ELISA test used in this study was to quantify the specific antibody to CPV vaccines from the multivalent vaccine administered. ELISA test was reported to be a veritable tool that can be used to confirm protective level of antibody at a relatively low cost [28]. Elisa test was recommended to be primarily used to validate vaccine efficacy induced by primary vaccination and to establish vaccination protocol suitable for each dog [28, 29].

Antibody titre observed on day 0 was possibly due to waning maternal antibody (MDA) passed from their dams through colostrum. The mothers however had no history of vaccination but may have acquired the antibody from natural sub-clinical infection.

Following vaccination, antibody was produced within a week as evident by the significant increase in the antibody titres across the three groups. This observation is similar to a report that stated that multivalent vaccine induce different immune responses to different immunogenic agents [30]. Some other researchers also reported that live attenuated CPV-2 vaccine is able to protect dogs against CPV-2a and CPV-2b field challenge [15]. Spibey *et al.* [31] also reported a cross reactivity and protection of CPV-2 vaccine on CPV-2c virus despite the antigenic variance

Antibody production following vaccination was observed after day 7 in group A in contrast to groups B and C which had earlier onset of antibody response shortly after the administration. The observed delay in antibody response till after day 7 in group A compared to the other two groups showed a prolonged lag phase of immune response. The reason for this prolonged lag phase in group A can be attributed to lower immunogenic of the vaccine A compared to vaccines B and C. Group A antibody titre was observed to increase steadily from day 7 through days 14 and 21. It

however wane on day 28, but rose again on day 90. Group A however, had the least antibody titre on day 90

The mean antibody titre values of vaccines B increased steadily from day seven thus showing a shorter lag period between exposure and antibody production and high immunogenicity. The antibody titre however declined on day 21 but the value rose again on day 28 and was sustained till day 90. Group B had the highest titre on day 90. Group B consistently had the highest percentage increase in antibody production when compared with those of groups A and C. This findings showed that vaccine B had a short lag period and a prolonged protection from parvovirus disease steadily for a prolonged period up to 90 days post vaccination.

Group C also increased steadily on day seven (7) post vaccination to day 28, although the value declined slightly on day 14 to almost the basal value but soon increased again on day 21 to day 28 to maintain a mean titre value lower than that of group B but higher than that of group A on day 90.

Comparatively, Group B vaccine offer the best protection against the parvovirus disease because of the early onset of antibody production, consistently highest relative increase in antibody production and maintenance of the highest of the antibody titre on day 90 when compared to others.

The antibody titres of the three groups were observed to be higher than those of their respective basal values in the groups used in this study.

This trend of antibody production titres justify the need for booster doses to be administered at intervals of 3-4 weeks, however this is dependent on the result of titre checks [32].

Persistent levels of morbidity and mortality due to CPV 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 [33]. 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 falling below protective levels but which retains high enough levels to block an active immune response by the vaccinated puppies [34]

This persistence may also be caused by infection due to field strains of the virus (i.e. strains different from the ones used in producing the vaccine). More work needs to be done on this to know if the vaccine virus strain is protective for all the local field strains

Puppies with poor MDA may be vulnerable (and capable of responding to vaccination) at an earlier age, while those with high titre MDA will have protective antibodies against natural infection and they will invariably poorly respond to vaccination till 12 weeks of age [35]

This study has shown that all the three vaccines elicited production of protective levels of antibody but vaccine B stimulates greater antibody production for a longer period. Although the antibody level of group B dogs decreased till day 28, it is of a greater possibility that a longer lasting protection against canine parvovirus is conferred by this type of DHLPPi vaccine. A researcher, in his report, attributed variation in antibody titres to differences in maternal antibody titre, vaccine types, animal immune competence, environmental factors or individual biological differences [36]

Conclusion and Recommendations

From the findings of this present study we concluded that vaccine B brand ranked highest of the three vaccines, its antibody titres were better than those of both Vaccines A and C in immunogenicity as it elicited higher titres of antibody production in vaccinated dogs for a period of 90 days. Furthermore, a period of 21 days interval seems optimum for administration of booster dose of vaccine as deduced from the results of the antibody pattern to parvovirus vaccines in this experiment.

Based on the findings of this study, we recommend vaccines B and C for routine vaccination of Nigerian local dogs and revaccination with a booster dose of the vaccine to be done 3 weeks after the first dose. The use of booster dose is to elicit optimum, longer lasting and more protective secondary humoral immunity against Canine Parvovirus.

Further study into the antibody response to repeated administration of these vaccines is recommended.

Sero-monitoring to design vaccination program based on available vaccines is also

advised. It is further recommended that the DHLPPi vaccine virus strain should be correlated to the field or local strain of CPV within the geographic region to ensure adequate protection of dog against the specific Canine Parvovirus in the environment.

Conflict of Interest

There is no conflicting interest among the authors and there was no external funding that could influence the outcome of the result.

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تقييم استجابات الاجسام المضادة بعد التحصين بثلاثة انواع من فاكسينات فيروسات البارو PARVOVIRUS للكلاب بعد إعطاء جرعة واحدة في الكلاب المحلية النيجيرية (Canis (lupus familiaris

أوه. أ. ادجيموبي ، دي. اوه أولاوبا ، أوه. تي. أومويوالي و أتش . أوه نوتجدي
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إن المستوى الحالي لمعدلات المرض والنفوق بين الكلاب بسبب الإصابة بعدوي فيروس parvovirus على الرغم من ارتفاع التحصين يثير المخاوف بشأن فعالية اللقاح الحي المعدل متعدد العترات المتوفر تجارياً والذي يحتوي على مستضدات "Distemper" و "Adenovirus" و "Lptosira" و "Parvovirus" و "Parinfluenza" في نيجيريا. أجريت هذه الدراسة لتقييم استجابة الأجسام المضادة من نوع parvovirus 2 ((CPV-2)) لثلاثة انواع مختلفة من لقاحات DHLPPi.

اجريت هذه التجربة على عدد خمسة عشر كلبًا نيجيريًا محليًا ، تتراوح أعمارهم بين 8-14 أسبوعًا ؛ تم اختيارها عشوائيًا. تم تقسيم الكلاب إلى 3 مجموعات (A ، B ، C) تحتوي كل منها على 5 كلاب. تم تحصين الكلاب باللقاحات الحية المتعددة المعدلة التي تحتوي على كل من Distemper و Adenovirus و Leptospira و Parvovirus و DHLPPi (Parinfluenza antigens) في المجموعات A و B و C. تم جمع عينات الدم قبل التطعيم وبعد ذلك أسبوعيا لمدة أربعة أسابيع وتجميع آخر بعد 90 يوم. تم استخدام اختبار الاليزا لتقدير القياسات المختلفة في مصل الدم للكلاب تحت التجارب . تم تحليل قيم المتوسطات باستخدام اختبار ANOVA واختبار الطالب t.

تم العثور على زيادة ذات دلالة إحصائية ($P < 0.05$) في استجابة الأجسام المضادة للقاح B في الأيام 7 و 14 وكانت أعلى باستمرار من الاستجابات للقاحات A و C.

في الختام ، أعطى اللقاح B استجابة كافية من الأجسام المضادة للحماية والثبات ، وكان اللقاح A عادلاً بينما أعطى اللقاح C استجابة مناعية ضعيفة. ومن ثم ، يمكن أن يُعزى حدوث التهاب الأمعاء الخلقي المكتسب (CPV) في الكلاب على الرغم من التطعيم إلى فشل اللقاح نتيجة ضعف المناعة لبعض اللقاحات المستخدمة في التلقيح الروتيني للكلاب.

الكلمات الرئيسية: كلاب parvovirus 2 ، الكلب ، ELISA ، الأجسام المضادة ، المناعية.