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Full Length Research Paper

Evaluation of post-vaccination immunity to canine distemper and parvoviruses in Benin City, Nigeria

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A study was conducted to determine the immune status of dogs vaccinated against Canine Parvovirus (CPV) and Canine Distemper Virus (CDV) by a clinic-based immunoblot enzyme-linked immunosorbent assay (ELISA) using a commercially available 120 sample immunocomb® test kit for canine parvo and distemper IgG in Benin City, Nigeria. Out of 120 dogs sampled, 63 (52.5%) were females while 57 (47.5%) were males. 84 (70%) dogs were exotic breeds while 36 (30%) were mongrels. Majority of the dogs (35, 29.17%) were less than one year old and the age range of all dogs sampled was between 9 weeks and 10 years. Analysis of data showed that there was no significant difference ($P < 0.05$) between sex, breed, age and level of immunity. A non-parametric chi-square contingency analysis of antigens (CPV and CDV) and level of immunity revealed that there was a high significant association ($p < 0.001$) between CPV and CDV antigens and level of immunity of the vaccinated dogs sampled.

Key words: Immunoblot ELISA, post-vaccination immunity, canine distemper, parvoviruses.

INTRODUCTION

Canine distemper is a highly contagious, multi-systemic, and potentially fatal viral disease that was once recognized as the leading cause of death in dogs. It affects primarily dogs and several other animals' species including of recent members of the Felidae family - lions and tigers, both in nature and in captivity (Morell, 1994; Harder et al., 1996; Roelke-Parker et al., 1996). It is an enzootic disease with a wide host range and has been reported in most parts of the world including Nigeria (Abdullahi, 1979). The disease is caused by a canine distemper virus (CDV), which is a member of the genus morbillivirus in the Paramyxoviridae family. The virus is antigenically related to human measles virus (HMV), rinderpest virus, pestedepetits ruminant virus (PPRV) and dolphin distemper virus (Appel, 2000). The control of the disease has been throu-gh annual vaccinations in dogs especially in those coun-tries where prophylaxis has only been limited to the reduction of incidence (Appel and Summers, 1999).

In recent years, the incidence of distemper in dogs appears to have increased despite the significant strides made in reducing the frequency of this disease through the use of modified-live CDV vaccines and the development of several diagnostic tests for specific viral diagnosis. Waner et al. (1998) reported that the persistence of canine distemper is as a result of insufficient vaccinetions in cases where multiple vaccinations are a common practice and or vaccination failures probably due to faulty vaccine and interference by maternally derived antibody (MDA).

Canine parvovirus (CPV) like CDV is also a contagious disease of dogs and other Canidae such as wolves, coyotes, Smith American dogs and Asiatic Raccoon dogs. It is an important cause of mortality and morbidity in young dogs (Waner et al., 2004). The virus is a widespread, resistant and highly contagious agent capable of causing enteritis and myocarditis and is excreted in faeces both in naturally infected and vaccinated dogs (Brunner and Swango 1985). The disease is both controlled by monovalent and multivalent vaccines. However, sporadic cases do occur particularly in the young population when initial vaccinations fail because of interference by maternally derived antibodies (MDA) (Waner et al., 2004).

Interference by maternally derived antibodies is regarded as a major cause of Canine Parvovirus and Canine distemper virus vaccination failures in young dogs (Baker et al., 1959; Pollock and CarMichael, 1982; Buonavoglia et al., 1992). Veterinarians and researchers have come to

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the conclusion that the surest way to know that a puppy has adequately responded to vaccination or to confirm the immune status in a mature dog is to check the antibody levels in the dog's blood or serum. An easily accessible in-clinic procedure called immunoblot ELISA assay (rapid dot-ELISA assay or immunocomb ELISA test) has been developed for the semi-quantitative assay of CDV and CPV IgG antibody titres in the sera of vaccinated mature and young dogs using the enzyme-linked immunosorbent assay (ELISA) technology (Naveh et al., 1995; Waner et al., 1996; Waner et al., 1998; Truyen, 2001).

In Benin City there have been several records of mortalities and morbidities in dogs despite immunization. Clinical records revealed that these dogs manifested high fever, vomiting, diarrhea and weakness as common signs. In this study, the Biogal's Immunocomb$^{(R)}$ antibody test kit for canine parvovirus and distemper IgG was used to evaluate the immune status of dogs vaccinated against these diseases.

MATERIALS AND METHODS

Dogs

One hundred and twenty (120) dogs of both sexes; females (63, 52.50%) and males (57, 47.5%), of different ages; less than one year (35, 29.17%), 1-2 years (31, 25.83%), 3-4 years (32, 26.67%), 5 - 6 years (10, 8.33%), 7 - 8 years (10, 8.33%) and 9 – 10 years (2, 1.67%) and intervals since last vaccination were enrolled in this study. Seventy (53.33%) of the dogs were owned by clients where they were being maintained under domestic setting and assessed under conditions encountered in every day life. Nineteen (15.83%) were clinic-owned and thirty-one (25.83%) were those raised by breeders where they were maintained under relatively controlled conditions. Selection for studies was done based on thorough evaluation of individual dog medical records of routine clinic visits and vaccinations for two and half (2½) years (between November, 2003 and May, 2006) in Benin City, Nigeria.

Serum collection and storage

Blood was collected from the cephalic vein of each dog on presentation. Injection sites were aseptically prepared using swabs soaked in methylated spirit. Each blood sample was transferred to a sterile, non-heparinized plastic test tube and kept on a slanting position at 45°C under room temperature of 20 – 25°C and left overnight to allow for proper clotting. Sera, which were collected from the 120 dogs sampled, were centrifuged (Sigma Ultra centrifuge, USA) at 2500 rpm for 5 min for purification and stored at –20°C. The processing of the serum samples was carried out at the Department of Medical Microbiology (Virology unit) of the University of Benin Teaching Hospital (UBTH, Benin City, Nigeria.

Vaccine

A multivalent vaccine (Duramine® Forte Dudge Animal Health, Forte Dodge, IOWA, USA) containing Modified-live Distemper, Parvo, Leptospira, Canine Adenovirus and Parainfluenza antigen was used prior to sampling. Vaccination schedule employed in the area of study (Benin City) indicated that dogs received their primary vaccination at 6, 10 and 14 weeks old, then annual revaccination using this vaccine. Out of the 120 dogs sampled, 29 (24.17%) did not receive any of the primary vaccination, 47 (39.17%) received single primary vaccination, 11 (9.17%) received double primary vaccination, 32 (26.6%) received triple primary vaccination while one (0.83%) was not documented. For the annual revaccination, 39 (32.52%) did not receive any annual revaccination, 25 (20.83%) received single, 33 (27.50%), received double and 16 (13.33%) received triple annual revaccinations while 7 (5.83%) cases were not documented.

ELISA

A 120 sample immunocomb® test kit for canine distemper and parvo IgG (immunocomb biogal laboratories Kibbutz Galed, Israel) was used for the evaluation of CPV and CDV IgG antibodies in sera of dogs collected using the Immunoblot Enzyme-linked Immunosorbent Assay (ELISA) method. It is a semi quantitative procedure based on color comparison between a standard and a test sample result usually expressed in "S" units on a scale of 0 – 6.

Statistical analysis

The test of association between types of antigens (CPV and CDV) and level of immunity was measured by a non-parametric chi-square contingency analysis at 95% level of confidence and values of $P < 0.05$ were considered significant.

RESULTS

The data of the 120 dogs examined for 2½ years (from November, 2003 to May, 2006) and results of the immunocomb test performed on the samples collected, summary of results of the test performed and the result of the test association between types of antigens (CPV and CDV) and level of immunity were represented in the data provided. Both sex and breed showed no statistical significant difference ($P > 0.05$) with level of immunity against both CPV and CDV. However, in this study there was a significant difference ($P < 0.05$) between age and level of immunity to CDV. Both sexes showed high levels of immunity (high IgG titer ranging from 1:80 – 1:640) to both CPV and CDV antigens (Figures 1 and 2). The age of dogs ranged from 9 weeks to 10 years with the majority of dogs less than one year 35 (29.17%). All ages of dogs sampled showed significant levels of IgG titres, ranging from 1:80 – 1:640 to both CPV and CDV antigens (at $P < 0.05$) as shown in (Figures 3 and 4). Alsatian breeds of dogs were sampled more followed by the mongrel breed. All breeds showed remarkable levels of IgG titres (ranging from 1:80 – 1:640) to both CPV and CDV antigens as shown in Figures 5 and 6.

Out of the total number of dogs sampled, 47 (39.17%) dogs received single primary vaccination at either 6, 10 or 14 weeks while 39 (32.5%) dogs did not receive any or all of the total annual revaccinations up to the time of sampling. Figures 7 and 8 showed the level of immunity and total annual revaccinations. These results indicated that some of the dogs showed high level of immunity (at value of 1:80 – 1:640) despite not being revaccinated while some did not show some level of immunity (at value of 0 – 1:20) despite being revaccinated.

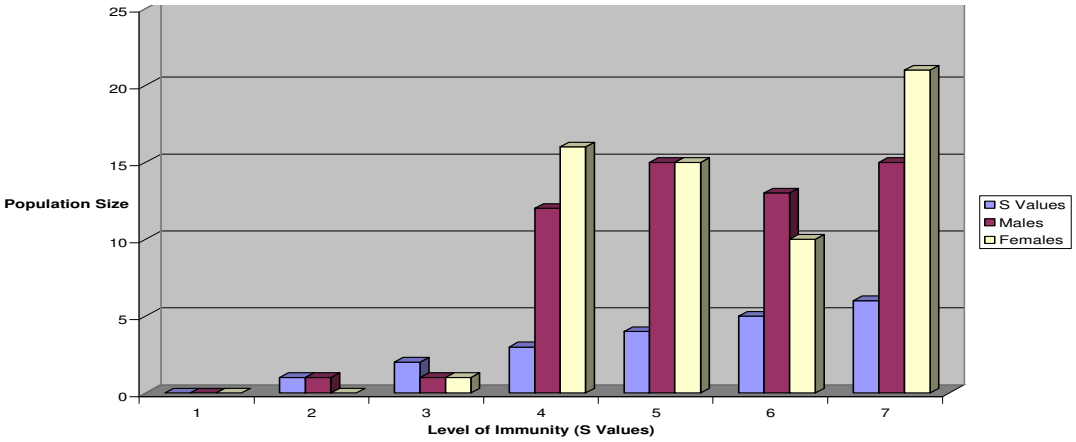

Figure 1. Relationship between sex and level of immunity against CPV.

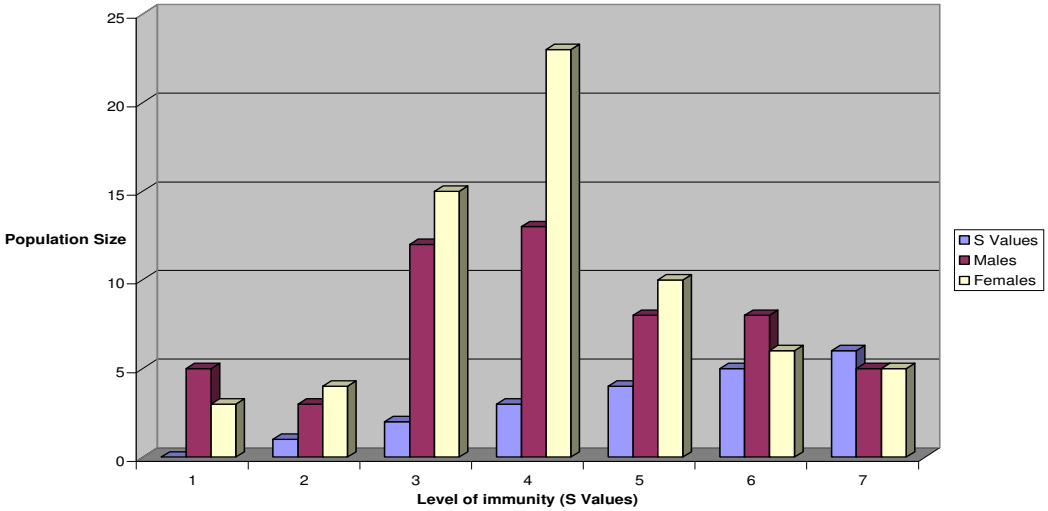

Figure 2. Relationship between sex and level of immunity to CDV.

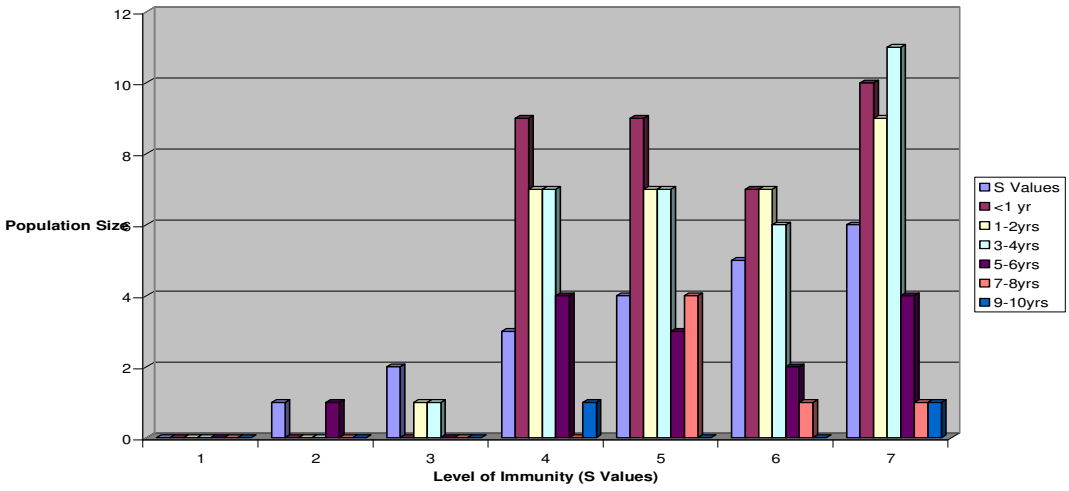

Figure 3. Relationship between Age and level of immunity to CPV.

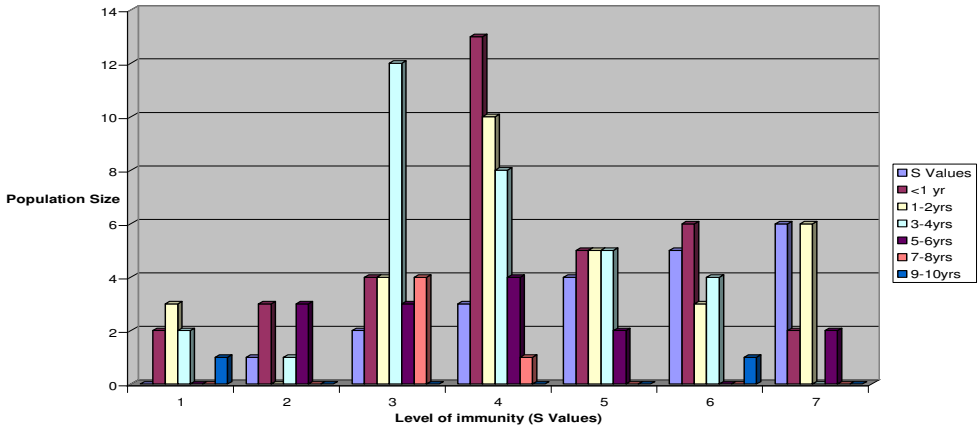

Figure 4. Relationship between Age and level of immunity to CDV.

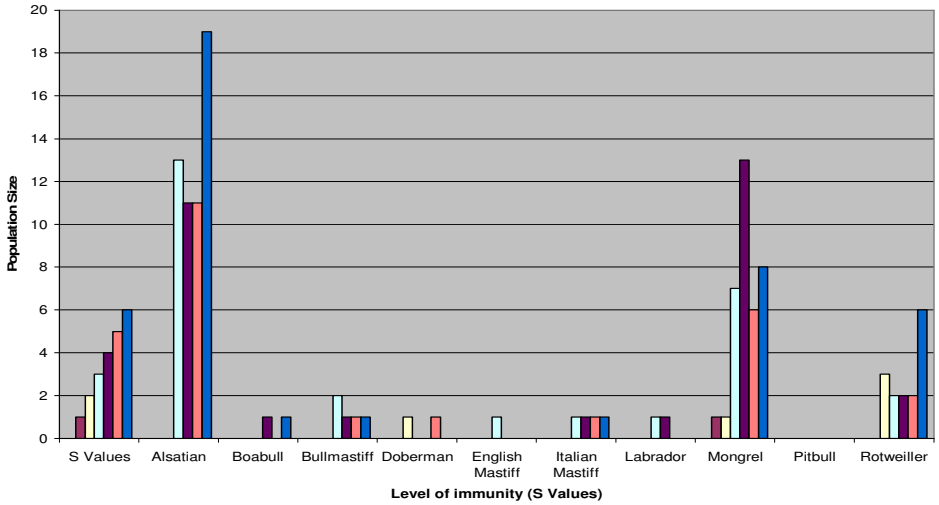

Figure 5. Relationship between breed and level of immunity to CPV.

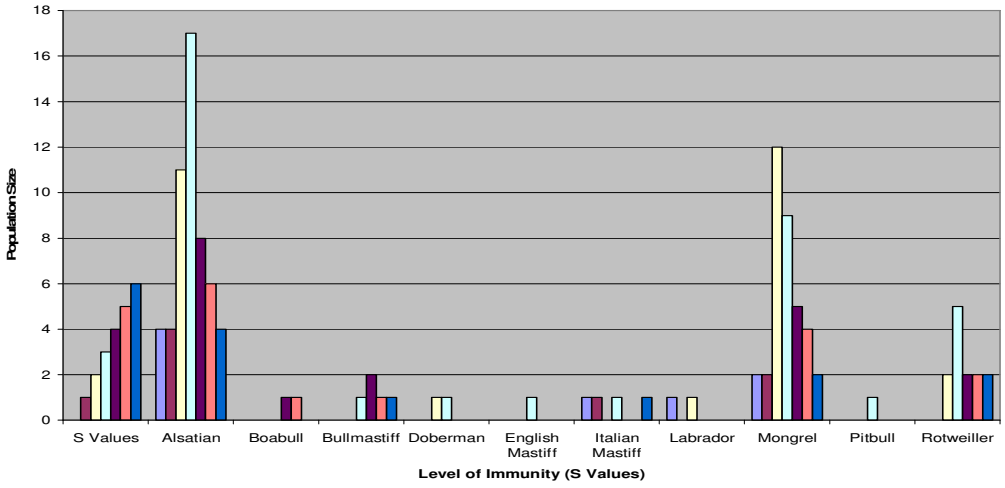

Figure 6. Relationship between breed and level of immunity to CDV.

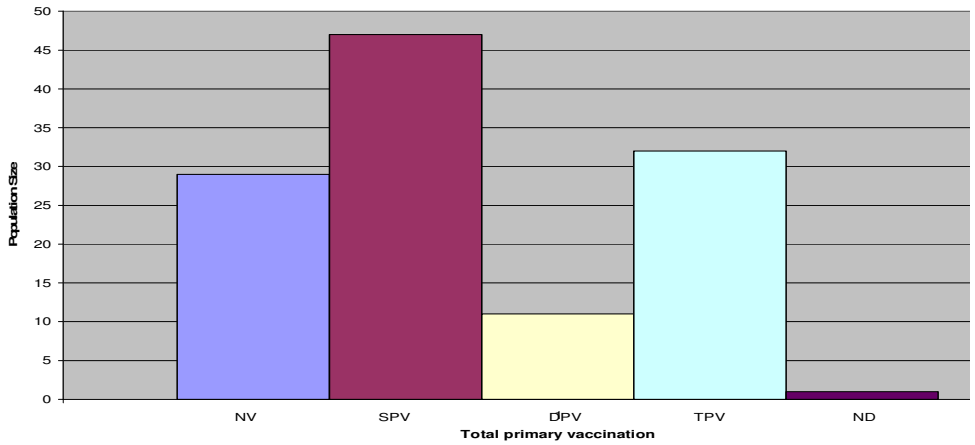

Figure 7. Relationship between total primary vaccination and number of dogs sampled. NV = Not vaccinated, SPV = single primary vaccination, DPV = double primary vaccination, TPV = triple primary vaccination and ND = not documented.

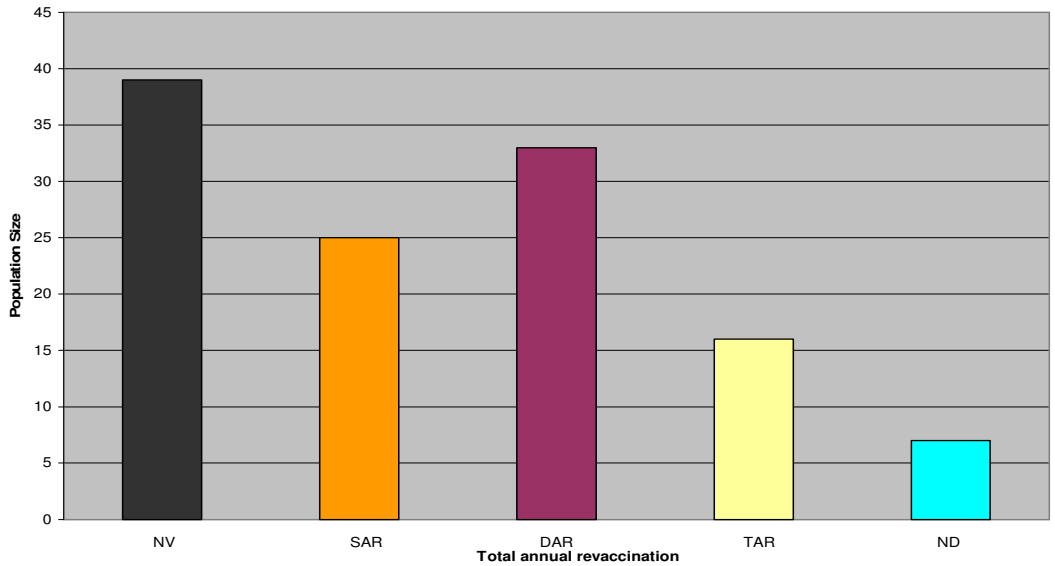

Figure 8. Relationship between total annual revaccination and number of dogs sampled. NV = Not vaccinated, SPV = single primary vaccination, DPV = double primary vaccination, TPV = triple primary vaccination and ND = not documented

All dogs sampled gave significant levels of antibody titre ($P < 0.05$) to both CPV and CDV antigens following immunocomb IgG antibody assay. However, there was a higher titer to CPV (with titre values of up to 1:640) than CDV following evaluation and analysis of results. A Chi Square t-test showed that there was a significant association ($P < 0.05$) between CPV and CDV antigens and level of immunity (with titre values ranging from 1:80 – 1:640) following immunoblot ELISA assay.

DISCUSSION

Results obtained from this study provided evidence that dogs vaccinated against CPV and CDV using commercially available CPV and CDV combination vaccine showed and maintained protective antibody titers. In this area of study, both CPV and CDV are endemic and are a cause of clinically important diseases in dogs associated with high mortality and morbidity rates. This correlated well with what was reported in literature (Abdullahi, 1979). Investigation of immune status following vaccination using standard procedures like the hemagglutination inhibition (HI), serum neutralization (SN) and immunofluorescent antibody (IFA) has not been practicable in Nigeria in view of the cost and other limitations associated with these tests (such as trained personnel and time constraint) as has been the case even in some advanced countries of the world (Waner et al., 1996; Waner et al.,

1998; Waner et al., 2004). Thus, the use of a rapid in-clinic immunoblot ELISA technique for the semi quantitative analyses of antibody titers to CPV and CDV provides solution to this limitation. This technique has been used to assess antibody response of pups after primary vaccination and the persistence of serum antibody titers to specific infectious diseases in adult dogs as revealed in literature (Naveh et al., 1995; Waner et al., 1996; Tizzard and Yawie, 1998 Waner et al., 2002; Waner et al., 2003; Waner and Keren-Kornblatt, 2006).

In this study, data was collected from 120 dogs of different sexes, ages and breeds so as to give a broader picture of dogs' antibody response to vaccination. Findings revealed that, some dogs were not vaccinated but showed some level of antibody against CPV and CDV as shown in Figures 7 and 8. Antibodies detected in this case must have been generated as a result of natural exposure to CPV and CDV antigens. This is common in mongrel breed of dogs (Figures 5 and 6) that stray or kept by owners that are either ignorant or not willing to present their pets for immunizations as revealed in literature (Waner et al., 1996) and personal experience. Conversely, some dogs showed no antibody despite vaccination (Figures 7 and 8). This is attributable to the fact that, there was vaccination failure. Reports have revealed that vaccine failure can result from the effect of maternally derived antibody or passively acquired antibodies at time of vaccination, delay in maturation of the immune system, poor vaccinal immunogenicity, genetic inability to respond to certain vaccine antigens, immuno suppression and ineffective lots of vaccine (Tizzard and Yawei, 1998; Schultz, 2000; Wise et al., 2006). It has also been reported that some dogs never appeared to mount an adequate antibody response to vaccination, but still remained healthy (Twark and Dodds, 2000). This is as a result of the persistence of immune memory cells and cell-mediated and mucosal immunity. It is thus advocated that revaccination is not required in this scenario. If however, there is low antibody response to vaccination due to vaccination failure as indicated abov, revaccination may be required. In such a case, the use of immunoblot ELISA assay in determining when to vaccinate dogs will be significant.

It was found that dog breeders in Benin City do not vaccinate their puppies particularly with the initial dose of primary vaccination that is usually administered at 6 week. This is to maximize their profits. Prospective puppy buyers are therefore saddled with the responsibility of vaccinating their newly acquired puppies. Also, most pet owners in this area acquire puppies as from 8 - 10 weeks of age when they must have been due for the second dose of primary vaccinations. The data presented indicated high number of dogs 39 (32.5%) that never received any of the booster doses (annual revaccination). This is as a result of the fact that most pet owners whose dogs were due for first booster failed to turn up after the third dose of primary vaccinations. In the same data, 32 (26.7%) of the dogs were not due for annual revaccination as at time of sampling.

All breeds show remarkable levels of IgG titers to both CPV and CDV antigens as seen in Figures 5 and 6. However records in the data of dogs sampled showed that immunizations were more in exotic than in mongrel breeds of dogs. In Benin City, medical records have revealed that exotic breeds of dogs are mostly owned by the elites, high-income earners (affluent) and security companies that utilize dogs in providing their services. These categories of dog owners provide better environments for keeping dogs, provide high quality diets to their pets and can afford to pay veterinary hospital bills. Mongrel breed of dogs however, do not enjoy any of such incentives and their owners are either low-income earners or ignorant of better pet care.

All sexes (Figures 1 and 2) and breeds (Figures 5 and 6) of dogs sampled showed no significant association ($P < 0.05$) with adequate CPV and CDV serum antibody titers but there was a significant association ($P < 0.05$) between CDV and level of immunity using the Chi-Square t-test. Earlier work by Twark and Dodds (2000) reported that sex, age and breed showed no significant association (where $P > 0.05$) with CPV and CDV serum antibody titer. However, in this study there was a significant association between age and CDV serum antibody titer. This may either be due to the fact that most dogs sampled were less than 1 year old or because of the labile and environmentally unstable nature of CDV. The effect of maternally derived antibodies on the young population of dogs mostly sampled in this study and the nature of CDV can result in low or unsustainable immunity over a given period of time. This is also in agreement with what was reported in the literatures (Waner, 2004; Waner et al., 2004; Waner and Keren-Kornblatt, 2006).

Results obtained from this study also revealed that IgG titers were detected from some dogs months to years after vaccinations. This provided the evidence that vaccinations of dogs with a commercially available combination vaccine containing modified-live CPV and CDV antigens confer and maintains adequate immunity for up to 3 or more years as revealed in literature (Tizzard and Yawei Ni, 1998; Schultz, 1999; Schultz, 2000; Twark and Dodds, 2000; Douglas et al., 2004; Gill et al., 2004; Waner, 2004; Waner and Keren-Kornblatt, 2006).

Results of immunocomb assay presented in the data showed that antibody response to CPV was higher than that of CDV (where there was significant S values of 3 –6 in CPV than CDV). CPV is stable in the environment. Natural boosting of immunity and thus sustained protective antibody become possible. CDV on the other hand is labile and susceptible to the environment. Immunity induced tends to be relatively lower than in CPV. This is in conformity with what was reported in literature (Waner et al., 2004; Waner, 2004; Waner and Keren-Kornblatt, 2006). The test of hypothesis at $P < 0.05$ confirmed that there was a high significant association between level of

immunity and vaccination against CPV and CDV antigens ($p < 0.0001$).

Conclusion and recommendation

This present study clearly confirms post-vaccination immunity for canine distemper and parvoviral fractions in a commercial multivalent modified-Live virus vaccine. Routine serological testing has since been successful in determining protection in the swine and poultry industries. It is reasonable to assume, therefore, that same techniques such as the one employed in this study could be applied to companion animals. Serological testing of post-vaccination immunity can allow for the establishment of more cost-effective vaccines and vaccination schedules, elimination of unnecessary revaccinations and clients could be provided with a scientifically based rationale for use of vaccines. The end result therefore would be an improvement in the overall health of animals. In this study, the diagnostic value of using the immunoblot ELISA assay for the rapid detection of CPV and CDV IgG is in total agreement with what was reported in previous studies. Instances where IgG antibody titres are low in dogs previously vaccinated, revaccination becomes necessary. Where IgG levels are low in conditions of natural infections as occurs in CPV infected dogs, accurate early diagnosis and prompt treatment of cases become very easy.

This work has set out to allay the fears on the use of this commercial multivalent modified-live vaccine by veterinarians and other animal health providers in Nigeria. Despite the efficacy of this vaccine in providing post-vaccination immunity to dogs as confirmed in this study, we still recommend that efforts be made by relevant stake-holders like the Universities, Research Institutes, NGOs and other government agencies to fund and intensify research into the development of either local monovalent or multivalent modified-live vaccines for companion animals in Nigeria. This will go a long way in providing better quality vaccines developed from local strains of infectious agents and averting problems usually associated with the maintenance of cold chains such as poor handling, defective storage facilities and inconsistent power supply which are quite phenomenal in less developed countries.

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