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# Evaluation of Antibody Titre in Dogs Vaccinated against Canine Parvovirus in Jos, Plateau State, Nigeria

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## ABSTRACT

*Canine parvoviral enteritis is a highly contagious disease with fatal outcomes, in most cases populations with high morbidity and mortality rate. The study was conducted to determine the immune status of dogs vaccinated against Canine Parvovirus (CPV) using enzyme linked immunosorbent assay (ELISA). Sixty randomly selected dogs were used for the study, out of which four had no level of determined antibody titre (1-2 S - unit), fifty six had protective immunity (3-6 S-unit) which comprised 24 (40%) males and 36(60%) females. Among the males, 23(95.83%) were protective while 33(91.67%) among the females were protective. A total of 17 dogs were puppies and all had protective immunity (3-6 S-unit) while 43 were adults out of which 39 (90.70%) had protective immunity (3-6 S-unit). A total of 30 dogs were sampled from each local government area out of which 28(93.33%) had protective immunity (3-6 S unit) in each local government. The breeds included in the study were Caucasian, Mongrel, Rottweiler, Lhasa, Bull mastiff and German Shepard out of which all had protective immunity 56(93.33%) (3-6 S-unit) except 4(6.67%) for Caucasian that were unprotected. Based on the number of primary vaccinations, 3 dogs had single, 4 dogs had double and 52 dogs had triple primary vaccination out of which 56 dogs had protective antibody titre (3-6 S-unit). There was no significant difference (P>0.05) between sex, age, location, breeds and level of immunity while there was a significant difference (P< 0.05) between the number of primary vaccination and immune titre of the dogs sampled.*

**Keywords:** Canine Parvovirus, Antibody titre, Dog, Evaluation, Jos

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## INTRODUCTION

In Nigeria, roaming and scavenging as well as uncontrolled importation of dogs are some of the factors that favour the occurrence of diseases among dog populations (Adejoke, 2005; Ogbu *et al.,* 2016). Animal diseases such as Canine parvovirus which cause diarrhoea in dogs have become important problem globally (Mosallanejad *et al.,* 2008).

Canine parvovirus (CPV) is a deadly virus affecting the Canid family, causing virus-induced destruction of rapidly dividing haemopoietic and lymphopoetic precursor cells such as crypts of intestinal epithelial cells, thymus, lymph nodes, bone marrow precursor cells, blood cells and cardiac cells leading to multi-organ dysfunctions (Decaro *et al*., 2005; McCaw & Hoskins*,* 2006; Ogbu *et al.,* 2022). Canine parvoviral disease is a highly contagious, multi-systemic, potentially, serious fatal viral disease mainly affecting dogs of all age and sex. Dogs are known to be one of the most susceptible hosts of this disease; it also affects wolves, foxes, and other canids. Canine parvovirus enteritis was first reported in the USA (Eugster and Nain, 1977), but the identification of the causative virus was first documented as CPV-2 in Canada in June 1978 (Appel *et al.,* 1979 and Ford, 1988) and has since then been reported in many other countries including Nigeria (Kamalu, 1985). Canine parvovirus is an important pathogen of dogs and is responsible for significant morbidity and mortality despite the availability of safe and effective vaccines (Waner *et al*., 2004; Decaro *et al*., 2006, Ezeibe *et al*., 2010; Chollom *et al.,* 2013). It is caused by a virus that affects the gastrointestinal tract of puppies and adult and was once recognized as the leading cause of death in dogs (Harder *et al.,* 1996). It is characterized by vomiting, bloody diarrhoea, myocarditis and leucopenia (Streck *et al*., 2009). Its transmission from infected to susceptible dogs takes place mainly by the faeco-oral route, but dogs can also become infected from fomites such as shoes, clothing, the hands of human, food bowls and other utensils (Decaro *et al*., 2005). Risk factors associated with the development of clinical disease, include stressors (such as early weaning, overcrowding and parasite load), insufficient passive or active immunity, geographical region, and the presence of other pathogens (Kalli *et al*., 2010).

The existence of CPV in Nigeria was first reported by Adeyanju *et al.* (1984) and was confirmed later by Ezeokoli *et al.* (1985) and Kamalu (1985). CPV was thought to be new to the environment having been introduced probably via imported dogs. In Nigeria, there have been several records of mortalities and morbidities in dogs due to parvoviral infection. CPV prevalence in North-Central States (NCS) and Federal Capital Territory (FCT), Abuja was 45% (Ogbu *et al*., 2020); (37.7%) in South East Nigeria Ukwueze *et al.* (2019); (75%) among dogs showing clinical signs of foul smell and haemorrhagic diarrhoeain Magma and Mokola Veterinary clinic in Ibadan, Vet-world Clinic in Abuja and the University of Agriculture Veterinary Teaching Hospital in Makurdi (Apaa *et al.,* 2016); (13.4%) in Delta State (Shima *et al.* (2015).

According to Walsh and Ames (2004), control of canine parvoviral enteritis is mainly by adoption of vaccination and hygienic measures. Sporadic cases do occur particularly in young dogs due to vaccination failures (Waner *et al.,* 2004). Both monovalent and multivalent vaccines are available for control. Interference by maternally derived antibodies is regarded as a major cause of canine parvovirus vaccination failures in young dogs (Pollock and Carmichael, 1982; Buonavoglia *et al.,* 1992). Companion animal viral vaccines represent a significant share of the global veterinary vaccines market for which several manufacturers offer products. Some vaccines however, are formulated using viral strains not currently circulating in the environment and which may be in need of updating in view of the possible emergence of new virulent strains in the field such as canine parvovirus. Veterinarians and researchers have come to 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 serum (Naveh *et al*., 1995; Waner *et al*., 1996; Waner *et al*., 1998; Truyen, 2001). A range of interactions have also been shown in hosts with co-infections which might have implications for successful vaccination (Christensen *et al*., 1987; Helmby *et al*., 1998).

Vaccines can prevent this infection, but mortality can reach 91% in unvaccinated cases. Management often involves veterinary hospitalization. Prevention is the only way to ensure that a puppy or dog remain healthy because the disease is extremely virulent and contagious. Appropriate vaccination should be performed starting at 6-8 weeks of age, with a booster given every 2–4 weeks until at least 16 weeks of age. Likewise, pregnant mothers should be vaccinated early to pass on maternal antibody to puppies (Naveh *et al.,* 1995).

This study aims to evaluate the antibody titre level Immunoglobulin G (IgG) of dogs vaccinated against parvovirus and to determine the level of IgG antibody in dogs vaccinated against CPV.

## MATERIALS AND METHOD

### Study Area

The research was conducted in Jos North and South Local Government Areas within Jos metropolis located on *9° 53' 47.4972" N and 8° 51' 29.9916" E.* of Plateau State (NPC, 2006). Six veterinary clinics were randomly selected from the study area which were: Federal College of Animal Health and Production Technology, National Veterinary Research Institute Vom, ECWA veterinary clinic Bukuru, Nanang Veterinary services Zaramaganda, University of Jos Veterinary Teaching Hospital Jos, HATS Veterinary clinic Jos and MAARS veterinary clinic Jos.

### Study Design

The sampled dogs were randomly selected from the four veterinary clinics purposefully selected based on convenience. Verbal approvals were sought from the managers/owners of the clinics and consent were also sought from the owners of the dogs.

### Sample Size and Sample Collection

A total of 60 samples were collected (ten (10) from each clinic). Blood samples were aseptically randomly collected from the presenting dogs through their cephalic vein. The samples were transferred into a sample bottle which does not contain anti-coagulant. The blood samples were allowed to clot and sera separated immediately by centrifugation at 1500×g for 5 minutes. Information on sex, breed, vaccination and location were obtained using pretested structured questionnaires.

### Laboratory Analysis

The sera were analysed using the enzyme linked immunosorbent assay (ELISA) (Truyen, 2001). The ImmunoComb test is a modified ELISA kit that is designed to determine antibody titre to canine parvovirus antibody in the serum.

The ImmunoComb Kit procedure contains 2 main components, a comb shaped plastic card, hereafter referred to as the Comb and a multi compartment developing plate. The Comb has 12 teeth sufficient for 12 tests. Eeach tooth will be developed in a corresponding column of wells in the developing plate. Test spots of CPV are attached to each tooth on the Comb. The upper most spot is a Positive Reference (positive control). Purified CPV antigen is attached at the lower middle.

The first step of the test was done by depositing 5µl serum specimen in a well in row A of the multi-compartment developing plate by using a capillary tube.

The Comb was then inserted into the well(s) in row A (printed side facing you) and incubated for 5 minutes. To improve mixing, the comb was dipped up and down at the start of each incubation (each row). This motion was repeated at least twice in all of the remaining rows. The well in row B was pierced and the comb was inserted for 2 minutes. Before transferring Comb from one well to the next, the foil was pierced off the next well. Excess liquid from Comb teeth was shaking off onto a tissue. Comb was inserted into the next well

(row C) for 5 minutes; comb was inserted into the remaining wells (row D &E) for 2 minutes each and the last well (row F) for 5 minutes. Upon completion of the colour development in row F, the Comb was moved back to row E for 2 minutes for colour fixation. The Comb was taken out and let to dry for 1-10 minutes. Specific IgG antibodies from the specimen, if present, bind to the antigen at the test spots and will be labelled in row C, which contains an enzyme labelled anti-dog IgG antibody (Biogal, 2007).

The Immunoblot Enzyme-linked Immunosorbent Assay (ELISA) method (Truyen, 2001), is a semi quantitative procedure based on colour comparison between a standard and a test sample result usually expressed in "S" units on a scale of 0 – 6 (Biogal, 2007).

The score of 0 means that the dog has no detectable antibodies against the disease, and scores of 1 - 2 means a low level of antibodies not considered to be protective.

Scores of 3 - 4, however, are consistent with a protective level of antibodies where 3 S-unit mean low protective immunity and 4 S-unit mild protective immunity, and the score of 5 - 6 reflects high level of immunity, where 5 S-unit means moderate protective immunity and 6 S-unit high protective immunity. Thus, for dogs with scores of 3 or higher, revaccination is not needed.

As per the manufacturer's information, S3 represents a positive response of 1:80 titers (H.I. test for anti CPV antibodies).

**Statistical Analysis**

Data generated were analyzed using descriptive statistics and the results were presented in tables. The level of significance was accepted at $P< 0.05$.

**RESULTS**

Based on the manufacturer's instruction on interpretation of the results, the following is the key:

1-2 S-units denotes no level of immunity, 3-4 S-units denotes low protective immunity and 5-6 S-units is high.

From the overall results, a total of 4 (6.67%) had no level of immunity, 21 (35.00%) had protective immunity while 35 (58.33%) had high level of immunity

**Relationship between Immunity and Sex**

Based on the sex (Table 1), 24 dogs were males while 36 dogs were females. The percentage of males protected were 23(95.83%) while that of the females were 33(91.67%).

There was no significant association ($P>0.05$) between sex and the antibody titre of dog against parvovirus,

**Influence of Age**

A total of 17 dogs were puppies (1-6 months) while 43 dogs were adults (above 6 months). Among the puppies, those with protective immunity were 17 (100%) while for adult, 39 (90.70%) had protective immunity (Table 2).

The influence of age on the antibody titre of dogs against parvovirus was not significant at ($P> 0.05$).

**Influence of Location**

**Jos-South**

A total of 30 dogs were sampled in Jos South and Jos North LGAs respectively (Table 3). Equal number of dogs revealed protective immunity in both Jos South and Jos North LGAs which were 28 (93.33%)

The influence of location of dogs on their antibody titre against parvovirus is not ignificant at ($P> 0.05$).

**Relationship among Breeds**

Six breeds were sampled which included: Caucasian (44), Mongrel (1), Rottweiler (4), Lhasa apso (3), Bull mastiff (3) German shepherd (5). The results from these dogs based on the immunity status showed that except Caucasian which had 40 (90.91%) of the sampled dog with protective level of antibody titre, other breeds had100% of the breeds with protective titre of the antibody (Table 4).

The influence of breed on the antibody titre of dogs against parvovirus is not significant at (P> 0.05).

**Relationship between Immunity and Vaccination**

Based on the number of primary vaccination, some dogs took single vaccination, double vaccination or triple vaccination (Table 5). Out of 4 dogs that received single dose of CPV vaccine, none had protective antibody titre. There were 5 dogs that received double doses of the vaccine and 4 (80%) had protective antibody titre. Furthermore, 52 dogs received triple doses of CPV vaccine and all had protective antibody titre (100%).

The influence of vaccination on the antibody titre of dogs against canine parvovirus is significant at (P< 0.05).

**Table1: Relationship between CPV antibody titre and sex of vaccinated dog**

| Sex | Non protective | | Protective immunity% | | | | Protective% | Total% |
|---|---|---|---|---|---|---|---|---|
| Titre Value | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Male | 0(0) | 1(4.17) | 2(8.33) | 6(25) | 9(37.5) | 6(25) | 23(95.83) | 24(40) |
| Female | 0(0) | 3(12.5) | 49(11.11) | 10(27.78) | 12(33.33) | 7(19.44) | 33(91.67) | 36(60) |
| Total | 0(0) | 4(6.67) | 6(10) | 16(26.67) | 21(35) | 13(21.67) | 56(93.33) | 60(100) |

Calculated chi square =0.55 Degree of freedom (df) (2-1) (2-1) =1

**Table 2: Relationship between CPV antibody titre and age of vaccinated dog**

| Age | Non protective | | Protective immunity | | | | Protective % | Total (%) |
|---|---|---|---|---|---|---|---|---|
| Titre valve | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Puppies | 0(0) | 0(0) | 2(11.76) | 6(35.29) | 5(29.41) | 4(23.53) | 17(100) | 17(28.33) |
| Adults | 0(0) | 4(9.30) | 4(9.30) | 10(23.26) | 1626.67) | 9(20.93) | 39(90.70) | 43(71.67) |
| Total | 0(0) | 4(6.67) | 6(10) | 16(26.67) | 21(35) | 13(21.67) | 56(93.33) | 60(100) |

Calculated chi square =1.69 Degree of freedom (df) (2-1) (2-1) =1

**Table 3: Relationship between CPV antibody titre and location of vaccinated dog**

| Location | Non protective | | Protective immunity | | | | Protective % | Total (%) |
|---|---|---|---|---|---|---|---|---|
| Titre valve | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Jos south | 0(0) | 2(6.67) | 4(13.33) | 7(23.33) | 9(30) | 8(26.67) | 28(93.33) | 30(50) |
| Jos north | 0(0) | 2(6.67) | 2(6.67) | 9(30) | 12(40) | 5(16.67) | 28(93.33) | 30(50) |
| Total | 0(0) | 4(6.67) | 6(10) | 16(26.67) | 21(35) | 13(21.67) | 56(93.33) | 60(100) |

Calculated chi square = 0 Degree of freedom (df) (2-1) (2-1) = 1

**Table 4: Relationship between CPV antibody titre and breeds of vaccinated dog**

| Breed | Non protective | | Protective immunity | | | | Protective % | Total (%) |
|---|---|---|---|---|---|---|---|---|
| Titre valve | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Caucasian | 0(0) | 4(9.09) | 4(9.09) | 12(27.27) | 15(34.09) | 9(20.45) | 40(90.91) | 44(73.33) |
| Mongrel | 0(0) | 0(0) | 1(100) | 0(0) | 0(0) | 0(0) | 1(100) | 1(1.67) |
| Rottweiler | 0(0) | 0(0) | 0(0) | 2(50) | 0(0) | 2(50) | 4(100) | 4(6.67) |
| Ger. Shep | 0(0) | 0(0) | 1(20) | 0(0) | 4(80) | 0(0) | 5(100) | 5(8.33) |
| Bull mastiff | 0(0) | 0(0) | 0(0) | 1(33.33) | 1(33.33) | 1(33.33) | 3(100) | 3(5) |
| Lhasa Apso | 0(0) | 0(0) | 0(0) | 1(33.33) | 1(33.33) | 1(33.33) | 3(100) | 3(5) |
| Total | 0(0) | 4(6.67) | 6(10) | 16(26.67) | 21(35) | 14(23.33) | 56(93.33) | 60(100) |

Calculated chi square = 1.56 Degree of freedom (df) (6-1) (2-1) =4

**Table 5: Relationship between CPV antibody titre and vaccination status of vaccinated dog**

| vaccination | Non protective | | Protective immunity | | | | Total (%) | Protective % |
|---|---|---|---|---|---|---|---|---|
| Titre valve | 1 | 2 | 3 | 4 | 5 | 6 | | |
| Single | 0(0) | 3(100) | 0(0) | 0(0) | 0(0) | 0(0) | 3(0) | 0(0) |
| Double | 0(0) | 1(20) | 0(0) | 1(20) | 2(40) | 1(20) | 5(8.33) | 4(80) |
| Triple | 0(0) | 0(0) | 6(11.54) | 15(28.85) | 19(36.6) | 12(23.07) | 52(86.67) | 52(100) |
| Total | 0(0) | 4(6.67) | 6(10) | 16(26.67) | 21(35) | 13(21.67) | 60(100) | 56(93.33) |

Calculated chi square = 0.04 Degree of freedom (df) (6-1) (2-1) =4

## DISCUSSION

The results obtained from the study provided evidence that dogs vaccinated against CPV showed and maintained protective antibody titres. In this area of study, CPV is endemic and is a cause of clinically important diseases in dogs associated with high mortality and morbidity. This similar with what was reported in literature (Chollom *et al*., 2013). Investigation of immune status following vaccination using standard procedures like the hemagglutination inhibition (HI), serum neutralization (SN) and immunofluorescent antibody (IFA) has not been commonly practicable in Nigerian due to 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 titres to CPV provides solution to this limitation. This technique has been used to assess antibody response of pups post vaccination and the persistence of serum antibody titres 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 60 dogs of different sexes, ages and breeds so as to give a broader picture of dogs' antibody response to vaccination.

All sexes, ages, locations and breeds of dogs sampled showed no significant association (P>0.05) with adequate CPV and serum antibody titres using the Chi-Square t-test. Earlier work by Twark and Dodds (2000) reported that sex, age and breed showed no significant association (P > 0.05) with CPV serum antibody titre. Vila *et al*., (2018) opined that effective immune protection in primary vaccination depends mainly on the initial titre of maternal antibodies acquired by the neonate. Other factors such as environmental exposure, immunization schedules and immune system activity influence the duration of immunity in adult dogs. The dogs sampled showed significant levels of IgG titres, ranging from 1:80 – 1:640 to CPV. Dogs showing 3 "S" units or greater titre of CPV and/or CDV IgG antibodies were considered immunized and protected. Sera with IgG titres of 4 "S" units were equivalent to 1:160, with 5 "S" units 1:320 and 6 "S" units 1:640, for CPV

Some dogs showed no antibody despite vaccination (single primary vaccination/dose) .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, and genetic inability to respond to certain vaccine antigens, immunosuppression and ineffective lots of vaccine (Tizzard and Yawei, 1998; Schultz, 2000).

Number of primary vaccination did play a significant role in the CPV antibody titre of dogs. This is similar to Tizard and Yawei, (1998) who stated that the level of antibody titre post vaccination is dependent on the level of maternally derived antibody (MDAs) titre at the point of vaccination as high MDAs is capable of neutralizing the vaccinal antibody. Dog that received triple primary vaccination tend to produce protective antibody titre because the last vaccine may be given when the MDAs may have waned down.

**Conclusion and Recommendations**

Findings from this study show that dogs have protective immunity after vaccination against parvovirus. Sex, age, breed and location have no significant difference in respect to immunity status.

Number of primary vaccination (single, double and triple) has significant difference in respect to immunity status. Dogs with single vaccination had a lower immunity compared to those with double. Triple vaccination had a higher level of immunity.

It is therefore recommended that dog breeders/owners should vaccinate their dogs in order to prevent this disease and also for the dogs to acquire immunity against parvovirus. Not only vaccinate, but to as well complete the three primary vaccination needed in other to achieve proper protection.

Awareness should be created by veterinarians to dog owners/breeders on the importance of complete vaccination.

However, further work should be carried out on the relationship between sex, age, breed, location, number of primary vaccination and protective antibody titre (PATs) of dog post vaccinations.

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