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Assessment of Haematological and Serum Biochemical Parameters of Vaccinated and Non–Vaccinated Dogs Presented with Canine Parvoviral Enteritis

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Certification

I certify that this research work was carried out by Dr. Funmilayo Toyin Enitan Doherty-Odueko under my supervision at the Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria.

Dedication

This project is dedicated to the Glory and honor of Almighty GOD

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Abstract

Canine Parvoviral Enteritis (CPE) is a very fatal and highly contagious viral enteritis of young susceptible dogs, in 2006 it was expected that CPE was cause of over 75% of puppies that died of viral gastroenteritis worldwide.

Even though this disease can be control and prevented through properly vaccination, there have been reports of properly vaccinated puppies coming down with CPE

And some pet having no record of ever been vaccinated with CPE showing resistant to the disease.

This study aims to find out if vaccinating a pet against CPE makes any significant different in prognosis of infected puppies by statistically analysis methods using computer ANOVA methods in analyzing variation in vaccinated and non-vaccinated infected CPE puppies hematological and serum biochemical parameters , 20 animals were enrolled in this studies , 17 males and 3 female ages from 7 weeks to 6months , confirmatory diagnosis of CPE with commercially available ELISA test kits using sterile fecal sample from each research animals.

5mls of blood were taken for both hematology and serum biochemical analyzed using standard laboratory procedures

Data were statistically analyzed with social science statistically analysis package using histogram, Bar chart and linear graphs to illustrated varying in various analytics and results are taken as statistically at P values of.> 0.005

This study concluded that there is significant different in serum and hematological parameters of vaccinated pet compared to unvaccinated pet during and after illness

With pet with previous history vaccination show more favorable blood picture when compare to unvaccinated pets which seem to be more severely affected by CPE challenges

All analyzed pet parameter varies within groups and between groups ,total white blood cells counts and other differential white blood cells were within normal values and agreed with previous done in this area except lymphocytes counts with deviated slightly from expected outcome of lymphocytopenia but show abnormal deviation of increases in lymphocytes , there is lymphocytosis in non-vaccinated dogs that are resistant to CPE infection , same increases in lymphocytes counts was observed in vaccinated puppies that did not have CPE , and vaccinated puppies that came down with CPE infection but recovered fully when the blood sample was recollected for analysis two week later after their convalescent and discharges from veterinary, same lymphocytosis were equally noticed in non-vaccinated puppies that came down with CPE infection but recovered, so this study established that increase lymphocytosis can be used as definitive good prognosis indicator for CPE infection in both vaccinated and non-vaccinated animals.

List of Abbreviations

ALB: Albumin; ALT: Alanine transaminase; ALA: Alanine; AST: Aspartate Aminotransferase; CPE: Canine Parvoviral Enteritis; CPV-1: Canine Parvovirus type 1; CPV -2: Canine Parvovirus type 2; CPV 2a: Canine Parvovirus type 2a; CPV-2b: Canine Parvovirus type 2b; CPV 2c: Canine Parvovirus type 2c; Cu: Copper; Cl: Chlorine; GIT: Gastrointestinal tracts; Glob: Globulin; Na: Sodium; Neutro: Neutrophil; Mono: Monocyte; Lym: Lymphocyte; K: Potassium; TP: Total protein; Cl+: Chloride; Se: Selenium; Fe: Iron; RBC: Red Blood Cell; WBC: White Blood Cell; ELISA: Enzyme Linked Immunosorbent Assay; GIT: Gastrointestinal tracts; Std: Standard; Norm: Normal; FPV: Feline Panleucopaenia Virus; DNA: Deoxyribose nucleic acid; Met: Methionine; Glu: Glutamine; Zn: Zinc

Chapter One Introduction

Introduction

Canine Parvoviral Enteritis (CPE) is a viral disease that affects young un-vaccinated susceptible puppies worldwide since its discovery in 1978.

Cause by single stranded non enveloped DNA virus named Canine Parvovirus type 2 strains/variants (CPV 2a, CPV 2b and CPV 2c).

Despite advances made in veterinary medical diagnosis, prevention and management of CPE, it has continued to rank as major

causes of fatal viral gastro-enteritis among susceptible young and immune deficiency animals with no proper history of vaccinations worldwide (Goddard 2010).

It was estimated in 2006 that over fifty thousand susceptible young puppies were reported to have died of CPE or its complication worldwide which were estimated to represent roughly seventy percentage of gastroenteritis animal sampled. This disease shares great similarity with other diseases that present with similar clinical signs associated with gastro-enteric diseases such as lethargy, depression, in appetite, with or without fever, vomiting diarrhea that ranges from watery to mucous and bloody foul smelling (Goddard and Leisewitz, 2010)

This virus has great affinity for rapidly dividing cells like bone marrows, lymphoid tissues and intestinal crypts cells.

Common clinical signs include but not limited to diarrhea, vomiting, depression, dehydration, lack of appetite and temperature are variables

Distortion of cellular elements is a common histopathological finding in Canine Parvoviral Enteritis (CPE) infection and this can be used as important diagnostic and prognostic aid.

For example, anemia is a common clinical syndrome observed in CPE infection, this is assumed to due to oxidative stress and not related to virus suppression of Erythropoiesis [Panda et al 2009], while distortion and variation in differential white blood cells count is reported to be due to viral suppression of bone marrow activities [Goddard et al 2008]

Analysis of variation in various hematological and serum biochemical parameters such as total and differential white blood cells count like (Neutrophils, Eosinophils, Basophil, Lymphocytes) and red blood cells, total proteins, albumin, globulin etc. has been used as important diagnostics and prognostic tools in management of CPE.

Matured cellular elements such as red blood cells and white blood cells such as Neutrophils, Eosinophils, lymphocytes, Monocytes and Basophils has definite range of normal production and finite life span.

Their entire lifecycle (production, maturation, release into systemic circulation, destruction of senile or damaged one and their subsequent elimination from systemic circulation) are carefully balance and properly regulated.

These processes their production, subsequent release into systemic circulation, maturation, break down and recycling of old or damaged or infected one plus re circling of reusable materials from destroyed or metabolized cellular elements and subsequent excretion from kidney.

All these processes go on within the body in a constant regulated precise protocol.

Deviation from their normal range can result into sickness or can be as a result of ill health or indication of ongoing illness

It is a known fact that observed major clinical pathological phenomena associated with CPE were also observed in other gastro enteric disease, so analysis of clinical signs and Hematological parameters might not be enough to serve as confirmatory diagnosis but these analyses when combine with other clinical variations in serum biochemical parameters can also serve as important adjunct that can aid in accurate deduction of clinical diagnosis and management of Canine Parvoviral Enteritis (CPE).

Research Problem

Despite recent advances made in veterinary diagnosis and management, there have been documented cases of properly vaccinated animal coming with CPE infection and on the other hands there have been report of dog having no history of been vaccinated against CPE not having any disease during endemic periods within susceptible age group

Research Objective

To assessed if vaccinating puppies against CPE raised under normal Nigeria homes conditions as any significant different in combating the spread and control of CPE infection

To determine if vaccinating against CPE aid infected puppies' chances of survival in case of field challenge to compared using statistical analysis methods such as average means value, standard deviation, P – value, histogram, Bar charts and line graphs if there is any significant different in variation in hematological and serum biochemical results of vaccinated and non-vaccinated puppies raised naturally under typical Nigeria homes

To comparatively assessed data generated from the study using statistical analysis methods mean, standard deviation, P-values and social science ANOVA packages if there is any significant different in vaccinated and non-vaccinated puppies hematological parameters values.

To determine if vaccinating against CPE using common commercially available DHLPP brand has effects on puppies' abilities to survival CPE infection in case of field challenge by comparing the variation in hematological and serum chemical parameters of vaccinated and non-vaccinated puppies.

Research Aim

This study aim to analyze deviations observed in hematological and serum biochemical parameters by measuring variations in normal healthy dogs with no history of vaccination against CPE or coming down with the disease serving as positive control animals against those animals that had no records of vaccinating against CPE that comes down with the infection serving as negative control group , with healthy puppies that has been vaccinated and had no record of CPE illness were not sick with CPE and those of puppies that are vaccinated against CPE that had either suspected vaccine break or vaccine failure and came down with the disease if there any significant different in their recovery rate after veterinary medical management.

Justication for This Study

Canine Parvoviral Enteritis is an acute often fatal acute gastroenteritis of young puppies less than 6weeks to 7 months of age.

Can be serious social economic illness and it management involve intense veterinary hospitalization with high veterinary medical cost, time and expertise needed for it successful clinical management because of the importance of these affected pets to their owner some of whom have adopted these pets as part of their family, that losing them is like losing a beloved child, these pets were seen as part of family social life with their owner sometimes developed physical, social ,emotional and psychological attachment to these pets

So been able to say scientifically if vaccinating these pets be better protected against this disease and if they are challenge by new field strain, they have better chances of surviving it.

Null Hypothesis

There are no significant different in hematological and serum biochemical parameters of dogs vaccinated and non-vaccinated against CPE infections

There are significant different in hematological and serum biochemical parameters of dogs vaccinated and non-vaccinated against CPE infection

Chapter Two Literature Review

Introduction

Canine Parvoviral Enteritis is an acute, highly contagious and often fatal, common cause of infectious viral enteritis of young puppies between the ages of four weeks to seven months old and immunocompromised whelping bitches caused by Canine Parvoviral Type -2 variants [CPV-2].

It was designated Canine Parvoviral Type 2 because another virus known as Canine Parvoviral Type 1[CPV-1] or Minute Canine Parvovirus has been isolated and identify earlier in 1967 but this one was found to cause a more fatal disease in young puppies, although it is Canine Parvovirus, it was found to be antigenically difference from Canine Parvovirus Type -1[CPV-1) a virus belonging to canine parvovirus previously isolated from military dog in Germany in 1967. which is not as pathogenic nor virulent as CPV-2, also CPV-1 can be found in feces of apparently normal dogs.

CPV-2 is highly virulent and more pathogenic than CPV-1 and show more antigenic similarity to Feline Pan Leucopenia Virus (FPV), Mink Enteritis Parvovirus (MEP), Raccoon and Fox Parvoviruses than CPV-1, in fact CPV-2 differs from [FPV] by two amino acids in it viral capsid VP2, it is also cause mild diseases in Mink. Canine Parvovirus Type 2 is believed to has been a product of direct mutation from Feline Pan leucopenia as a result of keeping dog and cat as companion animal together in the same house (Truyen 2006)

CPV-2 was first officially recognized as the cause of highly contagious new endemic fatal dog disease in North America in 1978 and later in Japan, Europe and Australia but subsequent serological retrospective studies of sick dog's serum indicates that this virus began infecting dogs in early 70s, this was due to finding viral specific antibody for CPV 2in stored serum of ill dog' in Greece in1 974, Netherland 1976 and Belgium in 1977 Serological studies of stored dogs' serum carried out on dogs in Japan, New Zealand, Australia and United State of America in 1978 confirmed the present of this virus in those countries.

Canine Parvoviral Enteritis was first reported in Nigeria in Zaria in 1984 (Adeyanju et al 1984) and later in southern part of the country in 1985 (Kamolu,1985).

This virus has strong affinity for rapidly dividing cells, it first replicates in lymphoid tissues of Oropharynx, thymus, bone marrow, mes-enteric lymph nodes before it is disseminated into small intestinal crypts epithelial cells.

CPV-2 by infecting the lymphoid tissues causes immune suppression directly through lyses of lymphocytes and indirectly through bone marrow depletion of lymphocytes progenitor stem cells inside the bone marrow.

Viral replication in lymphoid tissues leads to marked atrophy of lymphoid tissues in thymus cortex, splenic follicles, lymph nodes of Peyer patches, the same viral replication activities in epithelial cells of intestinal crypts lead to necrosis and sloughing off of intestinal crypts epithelial cells in gastrointestinal tract.

Virology of Canine Parvoviruses

They are two distinct canine parvoviruses.
Canine Minute virus or Canine Parvovirus type 1
Canine Parvovirus Enteritis or Canine Parvovirus type 2

Canine Parvovirus Type 1

This is mildly pathogenic Canine Adeno associated virus also known as Canine Parvovirus Type 1 or Canine Minute Virus, it belongs to Genus Boca virus or Boca parvovirus this virus has been isolated from feces of apparently normal dogs. It is very common and widespread but not as pathogenic as any of CPV-2 viral strains and it is antigenically different from Canine Parvovirus type 2 but similar to Bovine and human Boca viruses genetically, it was first isolated in German military dogs with mild illness in 1967, it was transmitted through fecal oral routes and via trans placental from infected dam to fetus in uterus. CPV1 causes mild diarrhea, due to sub clinical enteritis, CPV-1 has causes pneumonia, myocarditis and lymphadenitis in puppies of 5 days to 21 days old. Most affected puppies have a mild disease but few might have a serious clinical form known as fading puppy syndrome

CPV-1 causes infertility, stillbirth or abortion in affected bitch because of its similarity with CPV- 2 and CHV a thorough diagnostic work up is needed to confirmed CPV-1, use of PCR or immune electron microscopy are needed to diagnose CPV-1.

At present no commercial vaccine is available for CPV-1, it is can only be prevented by maintaining cleaning environment in whelping bitch and avoiding overcrowding in shelter animal kennels.

Canine Parvovirus Type -2

Canine Parvovirus Type 2 (CPV2), also known simply as PARVO, it highly pathogenic parvovirus that affect domesticated and wild canines it is a single stranded, non-enveloped DNA virus, that is extremely resistant to various disinfectant agents and it is very hardy can survival under various adverse conditions in the environments but it is susceptible too and easily destroy by 10% solution sodium hypo chloride, it is highly mutagenic and still believe to be evolving, as at now it exact origin is still unknown, however three popular theories are postulated as source of it emergence.

1. It is believed to evolved from mutation from Feline Pan leucopenia virus or other carnivores' parvoviruses because it differs from Feline Pan leucopenia virus (FPV) and Mink Enteritis Virus by only few DNA bases in its viral capsid (J W Black, M.A Holscher et al 1979, McMaster et al 1981, Tratschin et al 1982, R.V.H Pollock and L.E Carmicheal 1982)
2. This might probably due to laboratory tissue culture contamination (Johnson and Spradbrow 1979) this assumption has not been proven yet and it worldwide fast distribution it assumed aided by vaccine contamination, however this too has not been proven yet
3. Or due to cross infection from cat housed together with dog as household companion pet (Truyen U: 2006) or from domesticated dog coming in contact with wild canines because of its similarity to Mink, fox and Raccoon parvovirus and also because some variants of CPV -2 readily infect minks.

CPV -2 viral particle are small particles, spherical in shape, approximately 20nm in diameter, and naked non-enveloped (Euguster and Nairai 1977, Appel M J et al 1979).

The CPV-2 was first isolated in1978 and by1980 it has become pandemic with new strains been isolated in 1979 designated CPV2a, this new strain has replaced the original CPV-2 virus in most infected dog isolates and it is found to be more virulent to cat than original CPV-2 strain.

There were only small antigenic variations between strains of CPV-2 (CPV-2a, CPV-2b, CPV-2c) detectable only by monoclonal antibodies and genetic analysis.

CPV-2a was discovered in 1979([Parrish C.R and Connell 1985), (Parrish C.R, Have P, Foreyt W et al 1988) and by 1980 has replace the original CPV2 in circulation another new strain was discovered in 1984 designated CPV 2b this differs from CPV2a by one or two amino acids substitution in its viral capsids 11 [VP 2].

CPV 2a differs from original CPV-2 by having amino acids substitution within its viral capsid 2 (VP2) at position Met87leu, Ile101Ther, Ala300Gly, Tyr324 Ile, Gln 370Arg, Thr44oAla and Asp305Tyr.

Another viral strain was discovered to have had another changes as position Asn 426 Asp and at position 297 (serine was substituted for amino acid Alanine at 297) this new strain was named CPV 2b in 1984 (Martella V, Decaro N, et al 2005, Decaro N, Desaro C, et al 2006) and another strain novel new isolate was made in German in 1996 with antigenic distinct changes at position 426, it was later discover in Italy in 2000 with distinctive antigenic characteristic and amino acid sequence changes at 426 with glutamine been substituted for Asparagine thus altering the viral antigenic site epitome A , this strain was named CPV2c, it has only one amino acid altering at position 426 I.e. GLU 426. (Asp 426 Glu).

These new strains have a wider host range than original CPV2 and can infect cat more readily than Feline Pan leucopenia (Greene C.E 2012)

Antigenicity

CPV-2 is closely related antigenically to FPV and MEV (Appel et al 1982) but it has no antigenic relationship with Canine Minute Virus or CPV-1 (Carmicheal et al 1980) or with Depend virus associated with Canine Adenovirus

It has minor serological cross reactivity with Swine Parvovirus (Managling et al 1983), CPV 2 affect all members of canine's family.

Also, it two clinical manifestation enteritis and myocarditis are diseases not previously seen in dog, Retrospective serological survey of stored sick dog's serum shows that CPV-2 is new viral infection [Pollock 1984], the earliest known antibodies associated with CPV-2 is the one found in stored dog serum in Greece in 1974, Netherland and Belgium in 1976 (Schwer et al 1979)

CPV 2 has high rate of nucleotides substitution rate similar to RNA viruses CPV 2a and CPV2b are antigenically similar to original CPV 2 even though there are some amino acid substitution in their amino acids sequence of their viral capsid VP 2 proteins.

CPV 2a show several amino acids substitution changes in its viral capsids, that give it distinct antigenic characteristic at viral capsid position Met87Leu, Ile101Thr, Ala300Gly, Tyr324Ile, Gln 370Arg, Thr 440Ala and Ala305 Tyr different from original CPV2 viral isolates.

CPV2b also contain these aforementioned changes plus two additional substitutional changes at viral capsid position 426 and 297 (Asn426Asp and Ser 297 Ala), (Ohshima T, Hisaka M, Et al 2008).

Some isolates and strains of CPV 2a are limited in their distribution to some geographical area for example CPV 2A Ile 324 is only found in Uruguayan and Asian countries.

This variants of CPV 2a Ile324 was found to be limited to Asian countries i.e. Thailand, Japan, China, India and Korea, this Asian strain of CPV2a has amino acid sequence substitution at position 324 which is adjacent to position 323 viral capsid, this viral capsid epitome site is important in virus virulent characteristic and host range specificity together with viral capsid position 93, CPV viral capsid position 323 and 93 play an important role in host range specificity and tropism for canine transferrin receptor binding sites (Huefer K Parrish CR 2003)

Another distinct strain was isolated in Germany in 1996 (DeCaro N, Desario C, Addie et al 2007) this strain was later discovered in Italy in year 2000 and was discovered to possess distinct antigenic characteristic different from CPV 2a and CPV2b and slight variation in it viral capsid at position 426 known as Glu 426 mutant because glutamic acid was substituted for Asparagine /Aspartic acid at position 426, it is more virulent, spread more rapidly and can infect cat more readily than FPV (C E Greene, Decaro N, 2012), (Buonavoglia C, Martella V et al 2001)

Also, similar and unique antigenic changes were found in isolates of CPV 2c in China and Taiwan, in this CPV2c China and Taiwan isolates had alteration of amino acid sequence at position 370, Gln370 Arg, these changes were unique and similar to the one found in China Panda parvovirus population (Guo L, Yang SL, Chen SJ et al 2013).

VP2 position 370 is adjacent to viral capsids sites 359 and 375 which make it flexible and unique surface loop of capsid proteins,

also viral capsid sites 359 and 375 are adjacent to viral capsid calcium ion [Ca++] binding site which is very important in the determination of viral infectivity

Any changes in these sites affect virus ability to hemagglutinate red blood cells (Simpson AA, Chandrascker V et al 2000) because is affect virus calcium binding and utilization abilities of this Isolates.

The changes in CPV2c occur at distinct and important antigenic determinant variation sites this make it to have a different antigenic property from CPV2a and CPV2b

Therefore, most commercial vaccines prepared with CPV2a and CPV2b antigenic strains isolates might not confer immunity against infection in cases of field challenge with CPV2c viral isolates.

Mutagenicity

CPV-2 was first discovered in dog in North America and Europe in 1978 as a new viral disease suspected to have mutated from Feline Pan Leucopenia Virus or Mink Enteritis Virus by 1979 this virus had attained a pandemic status. shortly after it was reported worldwide in 1979 and by 1980 a new viral isolates strain has evolved from it, designated as CPV2.

CPV2a evolved from the initial viral strain of CPV 2 with few genetic re assortment of few viral capsid protein bases that changes it virus antigenic characteristic detectable only by detailed genetic analysis and monoclonal antibodies tests.

Further minor antigenic shift occurred in new viral isolates in suspected outbreak of CPE in 1984 this newly isolated strain was designated CPV2b.

These new viral strains have almost completely replaced the old initial isolate i.e., the original CPV2(Parrish et al. 1985)

A few years later, another mutant isolates of CPV 2b with viral amino acid re-assortment atVP2position 426 with amino acid Aspartamine replaced by amino acid glutamine in viral capsid proteins loop arrangement, this position is important in determining viral antigenic properties was reported in Italy in 2000 (Buonavogelia et al. 2001). This new mutant named CPV2c or CPV 2\GLU426 Mutant] was isolated in Italy 2000 (Martella et al 2004) and later in Spain (DeCaro et al 2006) and United Kingdom (DeCaro et al 2007).

The virus has high rate of adaptation to adverse environmental conditions and high mutagenic potentials, these abilities helps in virus high rate of pathogenicity, it has shown remarkable ability to survival in the environment under adverse condition couple with viral high rate of nucleotides substitution rate only comparably to RNA viruses, this abilities has help CPV2 to mutate into a new more virulent , more pathogenic ,more resistant and more environmental stable with increase host range infecting abilities. CPV 2 is believe to still be in it evolving stage, these abilities had continued to account for persistent parvovirus enteritis infection seen today (Goddard and A L Leisewitz 2010)

We now have three dominant strains that are mutants of original CPV 2 that causes disease in dog worldwide designated CPV 2a, CPV 2b, CPV2c.

Pathophysiology Of CPE in Susceptible Puppies

After susceptible puppy has been expose to CPV-2 through oral nasal routes the virus infects lymphoid tissue and induces viremia within first 1-5 days of infection CPV- 2 preferred rapidly dividing cells of multiple tissue including thymus, bone marrows, crypts of epithelia cells in the intestines, villus of these cells. Necrosis and sloughing off of these cells led to blunting and decrease nutrient absorption which increases the risk of bacterial trans location, necrosis allows for trans location of enteric flora and further development of systemic inflammatory reactions.

Endotoxins released by bacteria lead to endotoxemia this triggered inflammatory responses released of pro inflammatory cytokines which are potent mediators of inflammatory responses seen (Goddard and Leisewitz, 2010, otto, Drobatz and Soter 1997)

Pathogenesis of CPE

after ingestion of minimal amount of infectious Parvovirus particles, CPV-2 virus migrate to oropharynx and then spread via blood stream to lymphatic tissues, bone marrow and intestinal crypts epithelia cells.

Between day 3 to day 5 of initial infection marked viremia is noticed but without clinical enteric disturbances. There is massive shedding of viral particle but this began to decline from day 6 to day 10 post infection. If puppy is tested around this time, it might give false negative ELISA result because of decline in viral shedding no trace of viral particle in blood of infected animal by day 12. Clinical sign began to manifest by day 4 to day 10 after initial exposure, some dog can continue to shed virus particle up to 3 weeks after onset of clinical signs in some rare case.

There is possibility of shedding noninfectious parvoviral particles

Breed Susceptibility

Although all breeds of dogs are susceptible to Canine Parvoviral Enteritis (CPE) infection but Rottweiler, Doberman pinscher, America pit bullterrier, English springer spaniel and German shepherd dogs are believed to has a higher risk of coming down with the disease than other breed of dog

This may be due to the fact that these are popular exotic breeds that are usually raised by elites as security and companion animals so they are more common among breeds of dogs presented to clinical floor.

Parvoviruses Morphology

Parvoviruses are small, non-enveloped single stranded DNA viruses that are sometime species specific in causing disease in mammalian animals

Canine Parvovirus belong to the family Parvoviridae, genus Parvovirus, these are small viruses with DNA genome of about 5000 amino acids /bases with a hair pin morphological structure.

Using X-ray crystallography, it viral capsid have been found to consist of sixty copies of combination amino acid making up it three viral capsids designated as VP1, VP2 and VP3

VP1 has full sequence with additional N terminal domain, VP2 account for 90% of the viral capsid and it is the major determinant of host range infectivity and pathogenicity it cleaves to VP3 using host protease enzyme.

Parvoviruses has exceptional ability to evolve into a more stable, more virulent strain with increasing host range infecting ability , this has help greatly in their ability to persist in the environment couple with the fact that they can survival in the environment under unfavorable condition with large amount of viral particle shed in feces by infected animal (McCandish 1981) billions of viral particles are excreted by infected dog, this active shedding can last up to 2weeks .This virus has affinity for rapidly dividing cells , this account for it tropism for lymphoid tissue , myocardium cells of puppies under three weeks , bone marrow and intestinal crypts epithelium cells of dogs.(M Appel and CR Parrish 1987) since 1981 most countries of the world has report presence of CPV 2 in their dog population but the most dominant strains isolated in Nigeria using SNAP parvo antigen test are CPV 2a and CPV2b (Dongonyaro et al 2013) and dominant CPV 2 strains isolates from South Africa are CPV 2a and CPV2c. It is possible we have undocumented CPV 2c strain in Nigeria because of large number illegally imported exotic dog breed from South Africa to Nigeria without undergoing adequate and proper quarantine procedure.

Most adult dogs are now resistant to this disease because they must have acquired immunity against it either by surviving natural subclinical infection or through proper vaccination against it.

Most breeding bitches are now immune against CPV2 strains and can pass maternal antibodies to their neonate via colostrum or via the placenta in the uterus. This help greatly in reducing myocardium form of the disease that is prevalent in susceptible puppies below the age of three weeks as the neonate has active maternal immunity for the first weeks of live when infection with CPV 2 can result in myocarditis (Meunier et al 1984) this make myocardium form of the disease to be rare occurrence, occurring only exclusively in pup of individual non immunized pet bitch that comes in in contact with the disease at about the time of whelping , one way this can occur is when such bitches has dystocia and they are presented for cesarean section (McCandlish, 1984)

Although severe clinical enteritis disease occurs in dog younger than six month of age, adult dog with insufficient immunity may be at risk of infection too, if they come in contact with the disease at any age (Marcovich J.E, Stucker K.M et al 2012, Kalli I, Leontides L S et al 2010)

Predisposing Factors to CPE

Breeds: certain breeds of dog show higher risk of coming down with CPE diseases than others , reason for this increase infectivity within breed is unclear but it has been suggested that German Shepherd, Doberman pinscher and Rottweiler breeds of dog has high risk of coming down with CPV 2[Ling et al 2012] because these breeds share common ancestor, they have higher prevalence of Wille brands disease couple with the fact that Rottweiler breed are predispose to genetic immunodeficiency , also these breed are more popular and common among the elites pet owners than other breeds of dog, inadequate vaccine protection due to owner not following strict vaccination protocol. Also, America Pit-bullterrier, Labrador retriever are also among dog breed with high risk of been susceptible to Canine Parvoviral Enteritis [CPE]

Stressful Environment

Animal kept under stressful, poorly sanitized, overcrowded animal shelter area, with poor ventilation or poor cross ventilated area like live animal market are more susceptible to coming down with CPE.

Immunity of the Dog

Puppies from bitch that are properly vaccinated against CPV -2are more resistant than puppies from bitch without CPV-2 vaccination.

Also, puppy that are properly vaccinated has higher resistant to the disease than un vaccinated puppies or poorly vaccinated puppies with incomplete vaccination schedule for CPE, puppies are only immune after two weeks of taking second shots vaccine against CPE

SEX

Intact male older than six months are more likely to come down with CPE than intact bitch because intact male has tendency to stray from their environment in search of estrus bitch and more likely to come in contact with excreted infected feces.

Seasonal Variability

CPE is more common in summer than in winter and in Nigeria there is high prevalence of the disease from January to August, its peak occurrences it in February to April but no CPE case was recorded in September to December (F. Doherty- odueko 2002).

Age Incidence

Dog of any age can be infected but the incident of clinical disease is more in puppies of weaning age, between the ages of six weeks to six month of age, puppies younger than six weeks are protected by maternal antibodies.

Most adult dogs are already immune due to vaccination or sero conversion immunity from sub clinical infection in the environment, after six weeks' maternal antibodies concentration in the serum start dropping below protective concentration in the serum, until about 20 weeks when it would have been depleted to such a low serum concentration that it cannot offer protection to the puppy from any infection. CPE can infect stray unvaccinated dog adult dog up to one year of age.

Shelter Animal

Due to exposure to many animals in close proximity under unsanitary confinement, puppies from shelter animal and adoption centers are more likely to come down with the disease.

Malnutrition

Malnourished animal has low immunity and therefore have high risk of easily succumbing to any environmental challenge including CPE infection.

Poor sanitation

Infected puppies can shed viral particles that are infectious for up to two weeks, these viral particles can survive in unsanitary favorable environmental condition and remain infectious up to eighteen months if there is no proper viral environmental decontamination, so animal kept in poor sanitary environmental condition were at high risk of coming down with the disease.

Animal with preexisting infection

Animal with pre-existing infection like bacterial, viral or parasitic infecting has higher chances of coming down with the disease

Transmission

CPE is transmitted directly by fecal oral route and indirectly through contact with contaminated fomites , during illness sick animal continue to shed massive amount of viral antigen in feces these virus particles can survival in the environment for long time and retain their capability to be infectious even long after cessation of clinical signs of disease, ingestion of contaminated fomite's from environmental contamination play a major role in transmission of CPE (McCartney, McCandlish and Thompson H, 1984) one gram of contaminated feces from actively shedding acute infected puppy is sufficient to infect at least one million susceptible puppies by oral route[Appel et al 1987].

Incubation Period

Signs of enteric disease appear in 4-14 days after exposure to viral particle.

Pathogenesis

After infection by ingesting viral particles through fecal oral route or through inhalation of viral particle from contaminated fomites , viral replication begins in lymphoid tissues specifically lymphoid tissue of the Oro pharynx, mes enteric lymph nodes and thymus and it is disseminated through hematogenous route to rapidly dividing cells of intestinal crypts epithelium cells, this last for three to five days after infection ,marked viremia developed in the plasma and it is noticed up to five days after infection.

After plasma viremia, the virus is found in many rapidly dividing epithelium cells for example epithelial lining of the tongue, esophagus, oral cavity, small intestinal crypts epithelium cells, bone marrow, spleen, thymus and various lymph nodes.

The severity of the disease is determined by cells turnover rate at these epithelial cells, higher cells turnover rate in lymphoid tissues and intestinal crypts epithelium means higher viral replication rate and more destruction of cells at these sites and more tissue necrosis observed.

During four to six weeks of age enterocytes of the intestinal crypts has higher mitotic index and higher cell dividing and replication rate, this is due to the fact that around this time there is change in puppies' diet due to weaning and change in intestinal microflora, this makes puppies more susceptible to infection around this time.

Parvovirus infects the germinal epithelium of the intestinal crypts causing destruction of the epithelium and villous damage and collapsed thus leading to characteristic pathological lesion of shortened and atrophic villi of the intestines, this altered the absorptive properties of the intestine's epithelium cells in the gastro intestinal tract.

There is extensive lymph cytolysis in the germinal Centre and cortex of thymus because of higher mitotic index in the thymus, this is responsible for the lymphopenia found in infected puppy. Early lymphoid tissue infection with overt clinical signs accompany by temperature raise and lymphopenia initiates the disease in all cases of clinical manifestation, there after myocardium cells and intestinal crypts epithelial cells are affected.

In neonatal puppies' rapid myocytes replication occurs during the first 2 weeks of life [Bishop and Hine 1975], while intestinal epithelium cells turnover rate is slow during this time [Meunier 1983] these situations reverse itself in the following weeks, when intestinal crypts epithelial cells start replicating actively at four weeks of age, cardiac growth continue as hypertrophy not as replication although DNA synthesis and nuclear kinesis continue until at least 8weeks of age [Bishop 1972] infection of susceptible neonatal puppies any time as from four weeks of age result in enteritis

However, infection in susceptible bitch at various stages of pregnancy does not cause intra uterine infection in fetus (Meunier et al 1984), also Parvoviruses infection does not cause stillbirth or affect conception rate, Parvoviruses infection does not have any effect on reproduction as it does not affect incident rate of stillbirth, average litter size does not increase or reduces in an experiment conducted on two thousand brooding bitches (Meunier 1981).

Clinical Forms

There are two major clinical form/manifestation of the disease that is

Cardiac Form Enteric Form

Cardiac Form

Seen in young neonatal puppies less than three week of age and immune compromised bitches, it manifests as sudden death in apparently normal puppy after exposure to sudden stress, excitement or exercise.

Affected puppy gasp, mucous membrane become cyanotic with death occurring under two hours of initial clinical manifestation due to non suppurative myocarditis, mortality may be up to seventy percent in affected litter. Surviving puppy from infected litter are susceptible to heart disease later in life.

By eight to twelve weeks of age surviving puppy show sign of acute heart failure (cardiomyopathy) which include dyspnea, tachycardia, tachypnea with ascites and hepatomegaly (Fisher et al 1980, McCandlish 1984) sudden death is due to irregular heartbeats and delay onset of chronic congestive heart failure.

Most affected puppies were infected immediately after whelping but because most bitch are now immune to CPE through natural field challenge or through proper vaccination protocol, there is passive transfer of maternal antibodies to puppies thus this form of the disease is rare [Appel and Parrish 1987].

Enteric Form

Enteric form of Canine Parvoviral Enteritis (CPE) is the commonest form of the disease, Canine Parvovirus [CPV] is a small non enveloped single stranded deoxynucleic acid virus [DNA] that has high tropism for cells and tissues in their differentiation stage or has rapid turnover rate which include GIT epithelia cells and bone marrow, (Parrish 1995, Pollock and Coyne 199) viral activities in the intestinal epithelia cells leads to cells damage, necrosis and sloughing off this in turn is associated with bacterial translocation within intestinal peritoneum and lumen and the accompanied systemic inflammatory reactions (Otto, Drobatz and Soter 199)

CPV -2 is the commonest cause of viral enteritis in young puppies of six weeks to six months, the disease starts as nonspecific gastrointestinal tract disturbances. Affected animals are withdrawn, lethargy, vomiting, diarrhea, decrease skin turgor, dehydration, cold extremities, weigh loss due to lack of food intake, vomiting and diarrhea and as the disease progresses the diarrhea turn from mucoid to become blood tinged or bloody diarrhea, (Goddard and Leisewitz 2010, Lamm and Rezabek 2008) foul smelling, intractable fluidly, these signs are not limited to Parvoviral enteritis induces diarrhea, animal become dehydrated, hypothermia is due to diarrhea and vomiting, jaundices and hemorrhagic diathesis [Disseminated intravascular coagulopathy] may develop terminally(Otto C M. et al 2000). Secondary bacterial infection may lead to bacteremia and endotoxemia.

Bacteremia and endotoxemia may lead to systemic inflammatory responses [SIR], endotoxins and pro inflammatory cytokines released by secondary bacterial infection into systemic circulation are integral part of observed patho physiology of oxidative stress reactions in CPE, these cytokines were mediators of inflammatory reaction observed and subsequent hemostasis. (Otto et al 1997, Weiss and Rashid 1998). Although there is suppression of bone marrow activities and erythropoiesis this does not lead to significant reduction in RBC, the anemia observed in CPE is assumed to be as a result of ongoing oxidative stress and has nothing to do with viral suppression of erythropoiesis (Panda et al 2009)

Death is due to dehydration and loss of vital electrolyte imbalance, leucopenia further acerbates immune system with may lead to endotoxic shock and comma. (Shantz1987, Sherding 1983)

Diagnosis

Suspect canine parvoviral enteritis in young puppy of six weeks to six months with no history of proper vaccination record again CPV-2 and shows the following clinical signs, active animal suddenly withdrawn to itself, stopping eating for about two to three days, vomiting, lethargy, diarrhea. Depression and fever, this clinical signs are not specific for CPE and cannot serve as confirmatory diagnosis.

Use of commercially available fecal enzyme immunoassay test (ELISA) can be used to performed rapid confirmatory diagnosis of CPE on the clinic floor.

Laboratory confirmatory diagnosis of CPE can be made with haemagglutination of pig, cat and Rhesus monkey RBC at PH of 6.5 at 4°C with viral antigen from sick puppy fecal extract.

The specificity of haemagglutional is determined by titration of the sample in parallel presence of normal and immunized dog serum.

Freely infected dog fecal sample contains many thousands of haemagglutinating units of viral antigens electron microscopy can be used to performed confirmatory diagnosis viral isolation and identification from suspected sick animal fecal sample viral amplification of viral DNA using PCR assay of suspected fecal sample (D K Macinitire et al 1997) serology can be used for retrospective confirmatory diagnosis of suspected case or use of IgM or IgG capture enzyme linked immunosorbent assay on a pair sera or use of probe based real (Z Yilmaz and S Senturk 2007) post mortem lesion and histopathology studies of these lesion can also aid in definitive diagnosis of CPE. (ZYilmaz and S Senturk 2007).

There are slide agglutination test and slide inhibition test can detest all strains and genotype of CPV are commercially available using Porcine erythrocytes (S Y Marulappa et al 2009)

Radiography

Contrast radiographic image of the gastrointestinal tract can detest pathological lesions in the abdominal lumen, although these changes are not specific to enteritis caused by CPE but they can aid in arrival at definitive confirmatory diagnosis, radiographic changes observed include fluid and thinning of intestinal mucosa lining coupled with low intestinal motility.

Ultrasonography

Ultrasound examination of abdomen can detest abdominal and peritoneal effusion and intussusception of intestinal lumen.

Clinical Pathology

Prominent histological examination finding of complete blood cells in CPE cases include leucopenia due to neutropenia and lymphopenia observed was due to destruction of bone marrow precursor stem cells for lymphocytes due to viral replication activities in lymphoid tissues. CPV activities in lymphoid tissues causes depletion of stem cells, destruction of lymphoid tissue parenchyma and lysis of lymphocytes.

Leucopenia is so severe that leucocytes count could be as low as 500-2000 leucocyte per microliter or less, ml leucocytes count or rebound neutrophilia is useful indicator of recovery in sick animal.

Haematocrit

It can be variable, not specific good indicator, it can be low due to intestinal hemorrhage or high due to dehydration from fluid loss as result of vomiting and diarrhea

It has been established that various anemia is seen in CPE infection due to oxidative stress going, the aim of this study is to see effect of CPE on pet a hematocrit, 'can we use hematocrit values as an indicator of good prognosis'

Serum Chemistry

Analysis of serum chemistry is a very good indicator of oxidative stress in animal body although it cannot be use as good specific indicator of CPE infection because result obtained can be seen in other enteritis cases but it results can give us prognostic guide.

Noticeable hypokalemia is due to anorexia ,vomiting and diarrhea, hypocalcemia is due to hypoalbuminemia with may be relative hypoalbuminemia or absolute hypoalbuminemia with might be due to reduction in plasma protein concentration due to intestinal hemorrhage or as a result of hemodilution due to over re hydration therapy, there is noticeable increase in alpha 2 globuline concentration despise reduction in plasma protein this can be due to hepatic synthesis of acute phase protein [APP] stimulated by endogenous leukocytes mediator that are produced as a result of tissue damage and inflammatory process , production of acute phase protein lead to reduction in albumin synthesis.

Increase production of Alkaline phosphatase and Alanine transaminase enzymes observed were as a result of reduce oxygen concentration in the liver due to low circulating blood delivered to the liver or due to many circulating bacteria endotoxin as a result of compromised intestinal epithelial absorption capacity due to destruction of GIT lumen, in CPE infection PH can be acidic or alkaline depending or predominant ion loss due to vomiting or diarrhea, vomiting lead to loss of hydrogen ion and chloride ion loss, while electrolytes ion depletion observed in CPE can be variable and ion loss depend on the origin of the diarrhea it is small intestine or large intestine] majority of CPE cases show metabolic acidosis due to excessive loss of bicarbonate ion[HCO3], unlike in human total ionized magnesium concentration cannot be use as a good indicator good prognosis.

Serology

Determination of positive antibodies against CPV can be misleading because 95% of dog population now have seroconversion due to previous exposure to CPV in sub clinical infection in the environment or through vaccination so they may give false positives test result.

However, specific serology test for IgM analysis by indirect fluorescent antibody [IFA] or Mecaptoethanol procedure provide more definitive serological evident of recent infection because IgM is only found in first week of clinical infection.

Positive definitive confirmatory diagnosis of Canine Parvoviral Enteritis required demonstration of active secretion of viral antigen in feces which can be done on site by [ITE-parvotest, IDEX, assure parvovirus symbiotic] all these are commercially available ELISA test kits and easily to conduct and give reliable positive result which indicate active fecal excretion however recent vaccination with attenuated live vaccine may give similar result too.

Management

Chances of survival for clinically infected puppies increases if such puppies are place on intense veterinary medical hospitalization and clinical aberration signs and symptoms are managed as soon as they are observed.

Although CPE start with nonspecific enteric disease clinical signs, CPE should be suspected in any young puppies of six weeks to 6months with or without proper vaccination history coming down with any signs of enteritis disease in endemic area or during sudden seasonal changes or transitory period between wet and dry seasons , the treatment should commence as soon as possible , infected puppies has better chances of survival if they were placed on appropriate par enteral fluid therapy to manage electrolytes imbalance and dehydration due to vomiting and diarrhea couple with bactericidal broad spectrum antibiotic par enteral injections.

Fluid Therapy

One of the major noticeable clinical signs of CPE is intractable vomiting and projectile foul smelling diarrhea, these clinical signs cause rapid electrolytes in-balance and depletion of electrolyte ion distorting the animal normal acid based balance and normal body internal homeostasis.

Replacement and maintenance loss electrolytes ion is one the major cardinal point of successful CPE management. Determination of appropriate crystalloids to use in replacement of loss electrolytes is very important because in CPE both metabolic acidosis and metabolic alkalosis can be observed, determination of body PH should

be done or use of isotonic crystalloids like normal saline and lactated Ringer, (Ringer lactate solution should be use with cautions in cases of CPE with severe metabolic acidosis or metabolic alkalosis or if there is any indication extensive hepatic damage that might affect lactate metabolism or when administering ceftriaxone as systemic antibiotic in CPE management.) any crystalloid solution that contain Calcium ion should not be with Ceftriaxone injection.

Also, any puppies with noticeable hypersensitive reactions to cereal product should not be given any crystalloid that contains dextrose, metronidazole infusion should not be given with lactate ringer infusion over a long period of time.

Intravenous routes are most preferred route of fluid administration because severe dehydration impaired absorption of fluid from subcutaneous routes, intravenous routes also help to rapidly replace and correct electrolytes in balance in circulation in puppies with hypovolemic shock

Colloidal fluid can also be administered with 50% isotonic crystalloid fluid to help improve and balanced circulation oncotic pressure loss observed as result of high depletion of serum protein observed in CPE.

Potassium chloride at dose rate of 20 mEq/l is administered with fluid to help correct hypokalemia normally observe in CPE. Fluid replaced at a dose rate of 40-60ml /kg body weight multiple by percentage deficit.

Antibiotic

Par enteral administration of broad-spectrum antibiotic that is bactericidal is essential in CPE management because of disruption of intestinal mucosal integrity with adversely affect intestinal normal micro flora population aminoglycosides group of antibiotics are very effective and well tolerated in hydrated puppy.

Cephalosporin are very good but concomitant administration of ceftriaxone with lactate Ringers solution, Ringer solution or injection or any fluid that has calcium ion should be avoided to avoid calcium precipitation

Antiemetics

Anti-emetics are very important set of drug use in CPE management because of frequent vomiting, metoclopramide is a dopaminergic antagonist that block chemo receptor trigger zone and also has pro kinetic effect on GIT very effective but strongly contraindicated in CPE puppies with accompany intussusception.

Ondansetron and Dolasetron both serotonin receptor antagonist can also be use in case of frequent uncontrollable vomiting metoclopramide, Ondansetron and Dolasetron are antiemetic that can act centrally and peripherally to stop nausea and vomiting caused by central and peripheral stimulation of vomiting pathway.

Nutritional Support

Introduction of bland enteral feeding early has improved chances of puppies' survival and improve earlier restoration of mucosal integrity faster [Prittis I 2000, Veir J.K 2014]

Antiviral Treatment

Since CPV-2 strains share similar antigenic characteristic with Feline Panleucopenia Virus, use of Feline Recombinant interferon w[rFeIFN-w] has been recommended and has given a promising result in tested case involving ninety-four dogs with naturally occurring CPE infection there are noticeable drastic improvement in clinical conditions and severity of disease, dogs are treated for three days at dose rate of 2.5 micro gram per kilogram body weight of rFeIFN-w intravenously for three days

Osteltamivir a neuraminidase inhibitor has been use to successfully improve affected CPE puppies' hematological parameter and body weight when administer for five days at dose rate of 2mg/kg orally.

It does not seem to have any effect on reducing mortality rate in affected puppies. Also human recombinant granulocyte colony stimulating factor [G-CSF] has been employed in the past in management of CPE with no documented benefit on treat outcome on overall management of CPE.

Equine endotoxin antiserum has been used in the past with significant impact on treatment outcome. Bactericidal permeability increasing protein [rBPI21] has been used in the past and it does not reduce seem to have any effect on endotoxin concentration in the abdominal lumen but intestinal mucosa protestant coating agent like Sucralfate and H2 blocker can be use

Pain Therapy

Colic as a result of hemorrhagic enteritis and intestinal intussusception is a common sign in CPE use of analgesic like Butorphenol or Buprenorphine are beneficial or Hyoscinebutylbromide [Buscopan] an anticholinergic drug is also very good in reduce abdominal pain observed in CPE

Prevention

Canine Parvoviral Enteritis can be effectively prevented by following strict vaccination protocol in susceptible pet population by using polyvalent vaccines containing antigens for Canine Distemper, Canine Hepatitis, Leptospirosis, CPV-2 and Canine Parainfluenza. These vaccines contain modified live strains of CPV-2 and can be administer at 6-8weeks, follow by first booster shot at 10- 12 weeks and second booster shot can be given at 14 -16 weeks old they can repeat at 6 months to 12 months later. This schedule was endorsed by world small animal veterinary association.

Most commercially available vaccines contain modified live vaccines that can be used to prevent infection in susceptible animal or protect already infected puppy, some of these vaccine can provide immunity cover that can last up to 5-7years.

Any puppy that succumbs to CPE infection after completing the initial vaccination protocol at 16 weeks should be re vaccination twice again at four-week interval.

In shelter environment or overcrowding population puppy can start receiving CPV-2 Vaccine at four weeks old and repeat after 3-4 weeks later, good hygiene and strict bio security sanitary protocol are very important in limiting outbreak of CPE infection in susceptible population. Wash all contaminate hard surface and fomite with 10% sodium hypochloride as disinfectant very effective in killing the virus

Vaccine

What is vaccine?

vaccines are biologically active pharmaceutical preparation that stimulate the body immune response systems to produce active or passive acquire resistant to a particular infectious agent it meant to protects or produce from.

It could be non-virulent strain of the disease-causing agent or another similar strains or similar organism that has the same antigenic characteristic or proteins part of the organism membrane surface that is big enough to have antigenic property and can be recognize as foreign object by the animal immune system and can stimulate antibody production again the organism or denature form of toxin produced by the organism or killed or attenuated form of the disease-causing agents.

What is vaccination?

Vaccination is the act of administering vaccine so that the animal immune system can produce antibodies against that particular biological agent (antigen/immunogenic protein part of biological organic or cloned) used in vaccine preparation.

Vaccination is a form of prophylaxis treatment by pre inoculating a susceptible man or animal with attenuated or less virulent form of the pathogen or killed form of the virulent pathogen with regimented doses to stimulate production of protective antibodies or administration of hyper immune serum from previously exposed and sensitized man/animal to a susceptible man or animal before or after exposure to virulent disease causing agent to prevent them coming down with a more serious clinical disease.

Vaccination is preventive therapy, not curative so you don't vaccinate an already exposed man or animal withactual clinical disease with killed or attenuated form of the disease-causing agent but you can use the hyper immune serum from recovery or convalescent patient as form of vaccination.

Advantages of vaccination

Vaccination helps in eradication of endemic virulent disease e.g., Rinderpest and small pox were successfully eradicated by vaccination

Prevention of disease
Improve animal health and productivity
Increase profit from improve animal health and productivity
Reduce production cost by eliminating veterinary and human medical bills as a result of animal or man ill- health
Reduce mortality from fatal disease
It is cheaper to vaccinate than to treat sick animal/man
Eliminate farmer psychological torment as a result animal ill health or loss

Disadvantage of vaccination
Hyper sensitive reaction
Vaccine failure
Vaccine break

Types of Vaccines

Lives vaccines;
This vaccine preparation contains attenuated form of the microbes e.g., Chicken pox and Measles vaccines
Killed vaccines;

They are made from infectious agent's surface protein on it outer coats e.g., Whooping cough vaccine
Toxoid vaccines;
Made from denature toxin produced by the bacteria or virus e.g., Tetanus toxoid and Diphtheria toxoid
Biosynthetic vaccine or Recombinant vaccine;
These are manmade synthetic substances that are similar to microbe molecular antigenic characteristic e.g. Hepatitis B.

Mechanism of Action of Vaccines

Vaccination involves priming animal immune system to recognize a particular infectious agent or part of membrane, or denatured form of toxin by introducing small, safe and standardize quantity of killed or attenuate form of the vaccine to it so the animal immune system, so the animal immune cells can easily recognize the offending microbe as foreign and resist any infection from it. They prepare the body soldier cell or immune system to fight or resist a particular infectious agent without them coming down with clinical disease.

Reasons For Vaccine Failure

We say a vaccine has failed when the body cannot produce enough protective antibody titer to protect the vaccinated man or animal from coming down with clinical disease when faced with field challenge after been vaccinated again the organism causing the clinical disease

Pathogens may have mutated developing different serotype from the one use in making vaccine so man or animal body do not have antibody for the new serotype

Vaccinating too early, may lead to maternal antibody neutralizing the vaccine.
Poor storage or breaking of vaccine cold chain, denature vaccine.
Vaccine antigens may have different immunologic responses in vivo and in vitro responses
Poorly manufactured vaccine process.
Wrong vaccine dosage and wrong vaccine dosing regiment
Wrong route of vaccine administration
Age at first vaccination
Interval between subsequent vaccine too early or too late
Immune compromised animal
Strong field challenge
New strains of pathogens
Vaccinated man or animal been exposed to stronger field challenge before administered vaccine antigen has produce enough protective antibody titer in the system
Poor nutrition

Control

Proper vaccination schedule
Intense hospitalization of sick dog
Good nutrition
Good hygiene
Reduce overcrowding

Chapter Three

Materials and Methods

Experimental Animals

A total of 20 dogs, were randomly enrolled into this study from animals presented to veterinary clinics as experimental animals. These animals ranged between 7 weeks to 5 months old, with body weight from 4.3 to 12 kg. Seventeen (17) of these dogs were patients of Diamond Veterinary Clinic, Lagos, two were from Paw and Pup Small animal hospital, Lagos state and one was from Federal University of Agriculture Abeokuta, Veterinary Teaching Hospital, Abeokuta. All animals had fully documented patient profile and sufficient information on history, feeding, housing and other management practices which were carefully evaluated before being enrolled into this studies. The dogs recruited into the study were further grouped into four.

Group A

Dogs that are clinically healthy animal, they had no record of any CPE vaccination; consisting of five dogs presented with noclassical CPE or any other gastroenteritis clinical signs of vomiting, lethargy, passage of foul-smelling diarrhea. All dogs were gotten from Diamond Veterinary Centre. Confirmatory diagnosis of absent of CPE antigen in the patients were done with commercial CPE ELISA test kit [APTECH). The animals in this group were used as control group

Group B

This group also comprises five animals, recruited from patient managed at DIAMOND VETERINARY CENTRE, LAGOS ages 22 weeks to 24 weeks, three males and two females, vaccinated with popular brand of commercially available DHLPP vaccine at 7- 8 weeks, 11-12 weeks and 15-16 weeks, all three-vaccine shot were given at 4weeks intervals, blood samples was collected from this

group 2 week after the third DHLPP shot, never came down with down with CPE, this group served as second control group

Group C

Never Vaccinated Against CPE but Came Down with Classical CPE

Dogs that were clinically sick with CPE; consisting of five dogs presented with classical CPE clinical signs of vomiting, lethargy, passage of foul-smelling diarrhea. Two of these dogs were from Paw and Pup Veterinary clinic in Ikotun area of Lagos state, while the three others were from Diamond Veterinary Centre. Confirmatory diagnosis of CPE in the patients were done with fecal samples using commercial CPE ELISA test kit [APTECH). None had history of accurate CPE vaccination record.

Group D

Vaccinated, sick and recovered animals. This group also comprised five animals, recruited from patient managed at DIAMOND VETERINARY CENTRE, LAGOS ages 6 weeks to 24 weeks, three males and two females, two of the animals in this group has record of receiving two shots of DHLPP vaccines at four weeks' interval with popular brand of commercially available DHLPP vaccine both came down with classical CPE under one week of taking the second shot of DHLPP.

Two animals in this group has records of receiving three shots of DHLPP by veterinary technician in another establishment only referred to DIAMOND VETERINARY CENTRE for veterinary medical management when they came down with signs suspected of classical CPE, three shot were given at two weeks' interval and one animal in the group has record of receiving only one shot of DHLPP at six weeks but the puppy came down with classical CPE at 11 weeks going to 12 weeks. All animals 'fecal samples were collected using sterile swabs and all tested positive to CPE antigen with commercially available CPE ELISA TEST KITS, all tested positive and all presented with classical CPE gastro enteritis signs, hospitalized for minimum of ten days, some hospitalization lasted for 17 days, they all fully recovered and blood samples are taken from each animal under this group after 10-14 days of being discharged from hospitals

Blood Sampling

5ml of blood samples was collected from each study animal from cephalic veins of all animals with 2.5 ml place in vacutainer containing EDTA for hematological study as anticoagulant; the samples were rock gently to thoroughly mix with anticoagulant. The remaining 2.5ml was put into plain vacutainer without anticoagulant, allowed and to settled and clot, centrifuge and used for serum and biochemical analysis

Disposables

Sterile gloves
5ml syringes and needles
EDTA vacutainer
Plain vacutainer
Swabs
Fecal sample
ELISA test kits

Research Procedures

Fecal samples were taken from all test animal for CPE test, using commercially available CPV test kits the sample were taken from pet rectum using sterile swab that accompanied the test kits swab was collected the collected specimen fecal samples was inserted into one ml assay diluents and allowed to mix well, settle down for 10 minutes a few drop are taken using the accompanying pipette titration tube, few drops were dropped gently into kit test cup, color changes are observed to indicate positive or negatives according to kit manufacturer indicated interpretations

Determination of hematological parameters.

Hematology.
Hematological Analyses
PACKED CELL VOLUME

The packed cell volume (PCV, %) and hemoglobin concentration (g/dl) were determined using the micro hematocrit and cyanomethemoglobin methods as described by (Lepherd et al., 2009)and (Srivastava et al., 2014) respectively. While the total white blood cells (WBC) and differential white blood cell counts (Neutrophils, lymphocytes, eosinophils, monocytes and basophils) ($\times 10^9$/L), erythrocyte ($\times 10^{12}$/L) and platelet ($\times 10^{12}$/L) counts were evaluated by hemocytometry(Bain et al., 2016). Erythrocyte indices including mean cell volume (MCV) (fl), mean corpuscular hemoglobin concentration (MCHC) (g/dl) and mean corpuscular hemoglobin (MCH) (pg) were determined by calculations (Latimer, 2011).

RBC Counts

Isotonic fluid is used to dilute red blood cells samples in ratio 1: 200

Procedure

The diluted blood samples are draw with pipettes to 0.5 mark, sample blood are wipe from pipettes tip and the diluting fluid is draw into 101 mark of RBC pipettes, this give 1 :200 dilutions, few drops of blood samples are discarded at the tips, on the counting chambers

The blood was mixed gently and thoroughly

Allow to settle for few minutes before been counted in hemocytometer under microscope by locating the central square divided into 25 square under microscope which are further subdivided into 16 square count the RBC cells in 25 squares by subdividing into 5 squares under 40X magnification to avoid counting twice

Diluting fluid

Sodium chloride 0:85g

Distilled water 100ml

Calculation

Total counting area =1/5sq min

Dilution =1:200

Depth of fluid filled area =1/10mm

Number of RBC under examination in 1 cube mm= RBC counted in 5medium sq x200x10

RBC/cube mm =RBC counted in 5sq x10000

DETERMINATION OF PACKED CELL VOLUMES

This measurement of proportion of each test sample blood that is made up of cells,

Procedure

Blood is taken from EDTA sample into a plain capillary bottle into about 2/3 of the capillary bottle, outside of capillary were wiped clean, the capillary is sealed by rotating gently and passing over flames

The tube is place in centrifuge machine with open end facing outward

the blood sample were centrifuge at 10000rpm for 5minutes

The PCV were noted using hematocrit reader

Hemoglobin Concentration

Hemoglobin is converted to cyanmethhemoglobin in alkaline medium by adding cyanide, this is gotten by adding ferric cyanide that convert the hemoglobin ferrous iron ion into ferric state form met hemoglobin, this reacts with Potassium cyanide to form cyanmethemoglobin which were measured in spectrophotometer

The hemoglobin concentration in each blood sample is determined using spectrophotometer by diluting 20microlitre of blood sample with prepared DRABKIN SOLUTION using SAHLI pipette the blood is diluted I n 1:251 allow to mix thoroughly for about 10 minutes the optical density of solution is read at 540nm wavelength using DRABKIN solution as blank, the concentration is read I standard curve prepare

Preparation Drab kin Solution

SODIUM BICARBONATES [$NAHCO_3$]	1g
Potassium cyanide [KCN]	50mg
Potassium ferric cyanide [$K_3Fe[CN]_6$]	200mg
Distilled water	1000ml

Determination of Total Leukocytes Count in the Samples

The leukocytes counting is similar to red blood cells count except the pipette has white beads and mixing bulb with 11 marking

Blood is draw up to 0.5ml marks and dilution fluid is draw up to 11 making 1;20 dilution white blood cells are not destroying in acidic fluid medium; stain dye is added into make nuclei visible

WBC Dilution Fluid Composition t

Glacial acetic acid	2ml
Gentian violet [1%]	1ml
Distilled water	100ml
or	
Glacial acetic acid	0.5ml
Distilled water	25ml
or	
Conc Hcl	1ml
Water	100ml

Draw blood sample into white bead pipette above 0. 5ml expel air bubble and excess to 0,5ml draw the diluting fluid into desire marks mixes gently, allow to settle for 1 to 2 minutes

Locate the upper larger chamber of counting divided into 16 chambers

Adjust the counting light in that area.

Determination of Serum Chemistry

Determination of Serum Electrolytes

Sodium

Sodium was determined spectrophotometrically using the Teco® Diagnostic kit. The reagent composition contains the filtrate reagent (2.1mM uranyl acetate and 20mm magnesium acetate in ethyl alcohol), acid reagent (dilutes acetic acid) and color reagent (potassium ferric cyanide, non-reactive stabilizers and fillers).

The test tubes were labeled as blank, standard and tests. 1.0 mL of filtrate reagent was pipette into all tubes. Then 50 ul of samples was added to respective tubes and distil water to blank. All tubes were shaken continuously and mixed for 3 minutes. Subsequently tubes were centrifuged at high speed (1500g) for 10 minutes and supernatant extracted. Another test tube was labeled as before and 1ml of acid reagent pipette into all tubes. 50 ul of supernatant was added to respective tubes and mixed. 50 ul of color reagent was added to all tubes and mixed. Absorbance of solutions in the tubes was read at a wavelength of 550 nm against a blank. Sodium concentration was calculated.

Sodium conc (mEq/l) = (Abs of blank – abs of sample/Abs of blank – abs of Std) X conc. of Std

Potassium

Potassium was determined spectrophotometrically using the Teco® Diagnostic kit as described by the potassium reagent in the kit contains sodium tetraphenylboron (2.1mM), preservatives and thickening agents.

Test tubes were labelled as blank, standard and tests. 1.0mL of potassium reagent was pipetted into all tubes. 0.01mL of samples were added to respective tubes, mixed and left to sit at room temperature for 3 minutes. After 3 minutes, the absorbance of the mixtures was read at a wavelength 500nm, against a reagent blank. Potassium concentration was calculated.

Potassium conc. (mEq/l) = (Abs sample/Abs of Std) x Conc. of Std

Chloride

Serum Chloride was determined spectrophotometrically using the Teco® Diagnostic kit. The reagent composition contains the chloride reagent (0.058mM of mercuric nitrate, 1.75Mm 0f mercuric thiocyanate, 0.74mM of mercuric chloride and 22.3mM of ferric nitrate) with non-reactive ingredients and stabilizers in dilute acid and methanol.

Test tubes were labeled as blank, standard and tests. 1.5ml of chloride reagent was pipette into all tubes. 10ul of samples was then added to respective tubes and distil water to blank. The mixture was incubated at room temperature for at least 5 minutes. Absorbance of the mixture in the tubes was read at a wavelength of 480nm against a reagent blank. Chloride concentration was calculated.

Chloride conc. (mEq/l) = (Abs of sample/Abs of std) x Conc. of standard

Determination of Serum Total Proteins

Total Protein

Total protein in serum was determined using the Biuret method using Randox® kits as described by (Orhueet al. (2005) according to the manufacturer's instruction. Briefly, three clean test tubes labeled blank (B), standard (S) and Test (T) were arranged in a test tube rack. 0.02 ml of distilled water, standard protein and serum was added to each of the test tube respectively. 1ml of R1 was then added to all the test tubes and the solutions were mixed and incubated for 30 min at room temperature. The absorbance of the samples and the standard were read at 546 nm wave length against the reagent blank.

Protein Concentration (mg/dl) = (Absorbance of test/Absorbance of standard) X Conc. of Standard

Albumin

Albumin concentration was determined Spectrophotometrically using the Albumin Randox® kit as described by Orhue et al. (2005). Reagent 1 (R1) contains BCG concentrate which comprises of succinate buffer (75mmol/L; pH 4.2), Bromocresol green 0.15mmol/L, preservative.

Three clean test tubes labeled blank (B), standard (S) and Test (T) were arranged in a test tube rack. One bottle of R1 was diluted with 87ml of distilled water. 0.01ml of distilled water, standard protein and serum was added to each of the test tube respectively. 3ml of the diluted reagent was then added to all the test tubes and the mixture were mixed and incubated for 5 min at room temperature.

The absorbance of the samples and the standard were read at 630nm wave length against the reagent blank.

Albumin concentration (mg/dl) = (Absorbance of test/Absorbance of standard) X Conc. of Std

Globulin

The total serum globulin was calculated by subtracting the total albumin concentration from the total protein concentration.

Determination of Concentration of Serum Trace Elements

Determination of concentration of serum trace elements (Copper, Zinc, Selenium and Iron)

To determine the serum trace mineral levels, perchloric and nitric acid mixture (in a ratio of 3:7 respectively) was used to digest serum samples and an atomic absorption spectrophotometer (Shimadzu Asc-6100, Japan) was used to determined levels of copper, iron, selenium and zinc. The standard solution consisted of (1000 g/mL) of copper, iron, selenium and zinc. Values were expressed in mol/L of serum as described.

Chapter Four

Animals Hematological Result Presented in Tabular in Comparison with Normal Hematological Reference Ranges

Group A

Table 1 Hematological Table Showing Analyzed Result for Animals in Group A

This groups comprises of 5 animals that had no record of taken any vaccine shots against CPE and never shows any clinical signs associated with classical CPE or any know gastroenteritis

Brief Summary

All tested animal has hematological analyzed parameters within normal references range with lymphocytosis, although 40% shows low monocytes count with

REFENCE RANGES	PCV (%) 35-57	Hgb g/dl 11.9-18.9	RBC $X10^{12}$/L 4.95-7.87	WBC $X10^{9}$/L 4000-11000	Neutro (%) 58-85	LYM (%) 8-21	EOS (%) 0-9	BAS (%) 0 - 1	Mono (%) 2 - 10
ANIMAL A1	44	14.5	8.3	11.4	63	38	2	0	1
	Normal	Normal	Normal	Normal	Normal	High	Normal	nor-mal	low
ANIMAL A2	47	15.1	7.8	14.5	65	32	1	1	1
	Normal	Normal	Normal	high	Normal	high	normal	nor-mal	normal
ANIMAL A3	42	14.8	8.5	15.3	66	30	2	1	1
	Normal	Normal	High	High	Normal	High	Normal	Normal	Normal
ANIMAL A4	48	12.7	6.7	12.6	67	32	1	0	0
	Normal	Normal	Normal	High	Normal	High	Normal	Normal	Normal
ANIMAL A5	50	16.2	7.1	16.4	66	31	1	1	0
	Normal	Normal	Normal	High	Normal	High	Normal	Normal	Low

Group B

Table 2

Animals showing normal PCV values probably due to dehydration all tested animals had lymphocytosis with other differential white blood cell within normal range This group hematological results agrees with works of (Panda et al 2009)

REFENCE RANGES	PCV (%) 35-57	Hb/dl 11.9-18.9	RBC $X10^{12}$/L 4.95-7.87	WBC $X10^{9}$/L 4000-11000	Neutro (%) 58-85	LYM (%) 8-21	EOS (%) 0-9	BAS (%) 0-1	Mono (%) 2-10
ANIMAL B1	48	15.8	7.3	10.9	50	48	0	1	1
	Normal	Normal	Normal	Normal	Low	High	Normal	Normal	Low
ANIMAL B2	63	13.9	9	13	61	37	0	0	2
	High	Normal	High	High	Normal	High	Normal	Normal	Normal
ANIMAL B3	51	17.2	7.9	10.6	64	33	1	1	1
	Normal	Normal	High	Normal	Normal	High	Normal	Normal	Normal

ANIMAL B4	59	19.6	9.6	12.2	62	35	2	0	1
	High	High	High	High	Normal	High	Normal	Normal	Normal
ANIMAL B5	55	18.4	8.5	11.4	60	38	1	1	0
	Normal	High	High	High	Normal	High	Normal	Normal	Low

Table 3

GROUP C Result of Animals That Have No Records of Proper CPE Vaccination but Came with Classical CPE

All animals were anemic with as with low PCV, low hemoglobin concentration results only 20% has hypochromic anemia, all has normal WBC count with lymphocytosis.

This results also agreed with previous done on this area (Panda et al 2009, Goddard et al 2008)

REFENCE RANGES	PCV (%) 35-57	Hb g/dl 11.9-18.9	RBC $X10^{12}$/L 4.95-7.87	WBC $X10^{9}$/L 4 - 11	Neutro (%) 58-85	LYM (%) 8- 21	EOS (%) 0-9	BAS (%) 0 - 1	Mono (%) 2 - 10
ANIMAL C1 RESULT	30	10	5	7.8	62	34	2	-	Normal
	Low	Low	Normal	Normal	Normal	High	Normal		
ANIMAL C2 RESULT	32	9.7	3.9	8.2	64	32	1	2	1
	Low	Low	Low	Normal	Normal	High	Normal	High	Normal
ANIMAL C3 RESULT	28	8.3	4.5	7.4	66	30	2	1	1
	Low	Low	Low	Normal	Normal	High	Normal	Normal	Normal
ANIMAL C4 RESULT	26	10.7	4	6.7	67	32	1	-	-
	Low	Low	Low	Normal	Normal	High	Normal	-	-
ANIMAL C5 RESULT	25	9.3	3.3	9.5	59	38	00	1	2
	Low	Low	Low	Normal	Normal	High	Normal	Normal	Normal

Table 4

GROUP D; RESULTS FOR ANIMALS VACCINATED BUT LATER CAME DOWN WITH CLINICAL CPE

40% of animals in the group has iron deficiency anemia, as seen with analyzed hemogram with PCV values but normal RBC count, 40% has responsive or regenerative anemic All have normal differential white blood count with lymphocytosis this results agreed previous done on this field (Goddard et al 2008)

This agreed with previous experimental results done in this fields (Panda et al, 2009)

REFENCE RANGES	PCV (%) 35-57	Hgb g/dl 11.9-18.9	RBC $X10^{12}$/L 4.95-7.87	WBC $X10^{9}$/L 4000-11000	Neutro (%) 58 - 85	LYM (%) 8-21	EOS (%) 0-9	BAS (%) 0-1	Mono (%) 2-10
GROUP D1	33	11.2	5.5	12.0	63	35	0	0	2
	Low	Low	Normal	High	Normal	High	Normal	Normal	Normal
GROUP D2	58	9.9	4.9	11.4	61	37	0	1	1
	High	Low	Normal	High	Normal	High	Normal	Normal	Normal
GROUP D3	63	10.8	7.1	13.3	63	34	1	1	1
	High	Normal	Normal	High	Normal	High	Normal	Normal	Normal
GROUP D4	31	10.2	5.3	11.8	57	39	1	1	2
	Low	Low	Normal	Normal	Low	High	Normal	Normal	Normal
GROUP D5	50	16.7	6.8	10.5	54	43	1	0	2
	Normal	Normal	Normal	Normal	Low	High	Normal	Normal	Normal

Linear and Histogram Illustration of How Various Hematological Parameters Varies Between Group

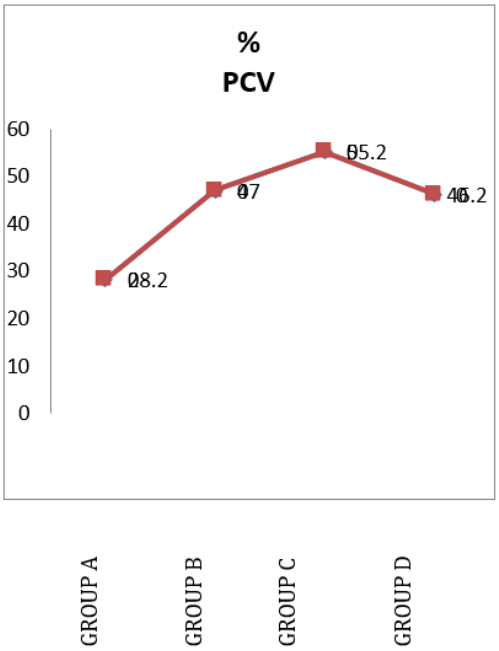

Figure 1A

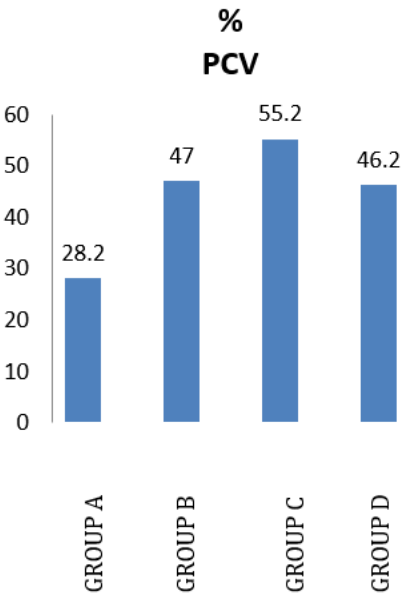

Figure 1B

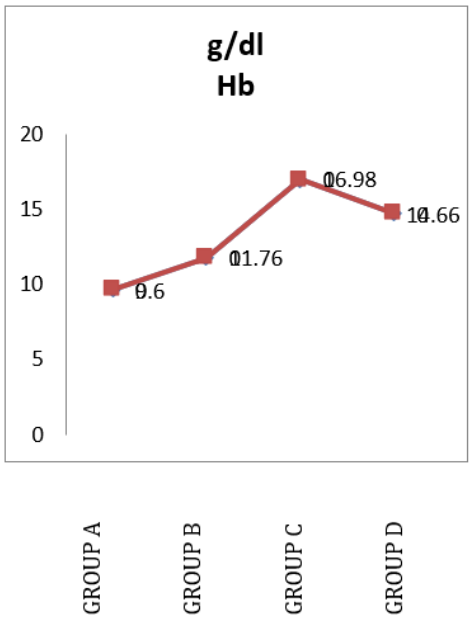

Figure 2A

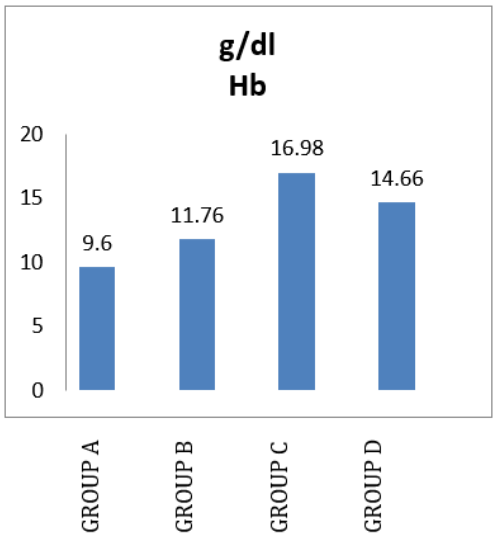

Figure 2B

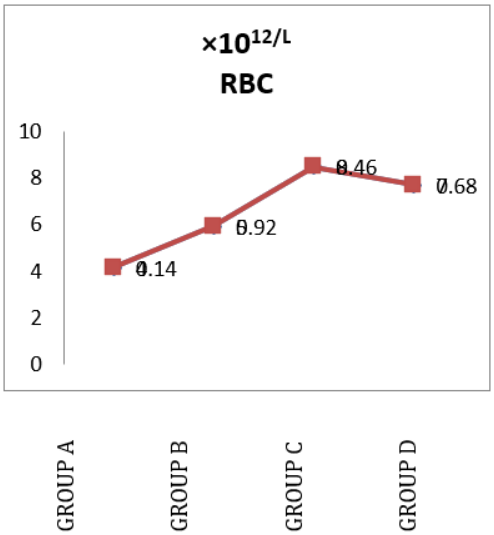

Figure 3A

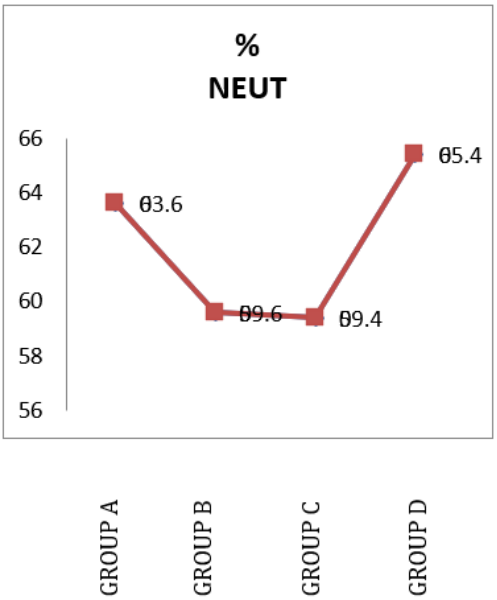

Figure 4A

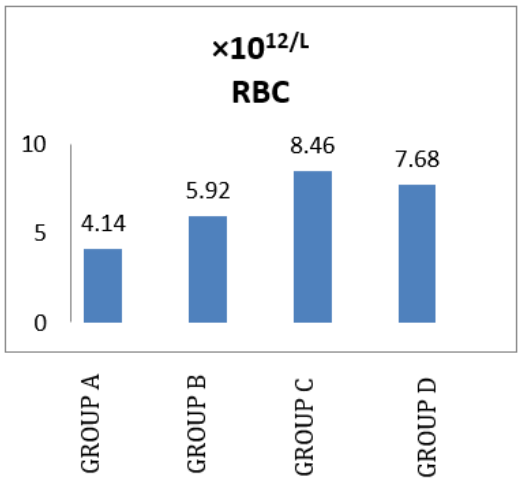

Figure 3B

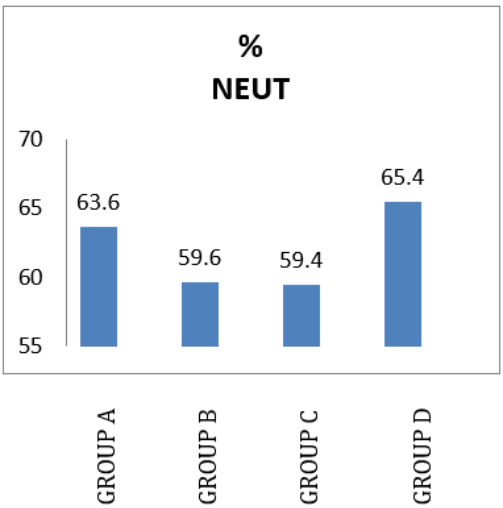

Figure 4B

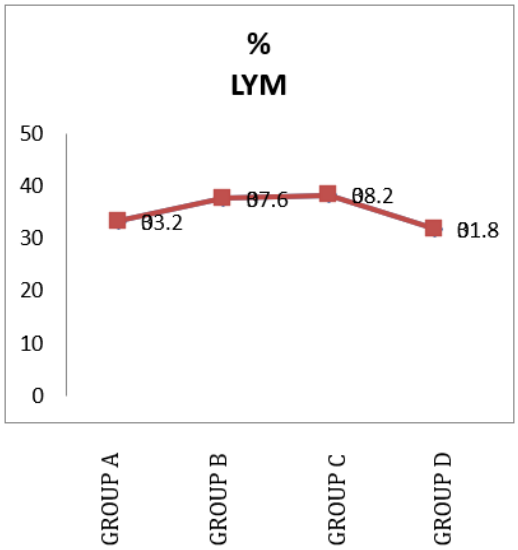

Figure 5A

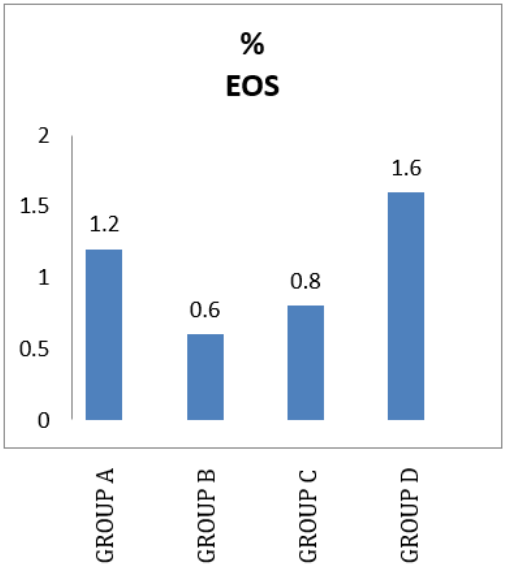

Figure 6A

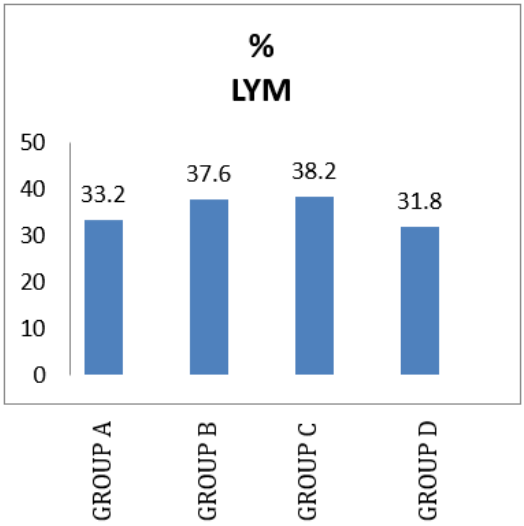

Figure 5B

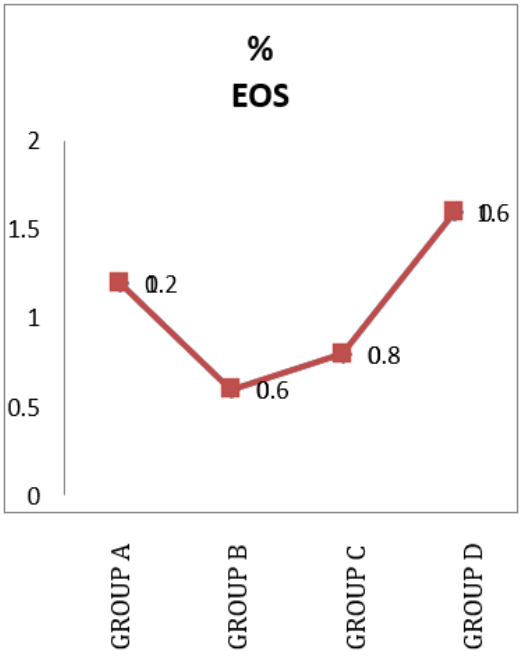

Figure 6B

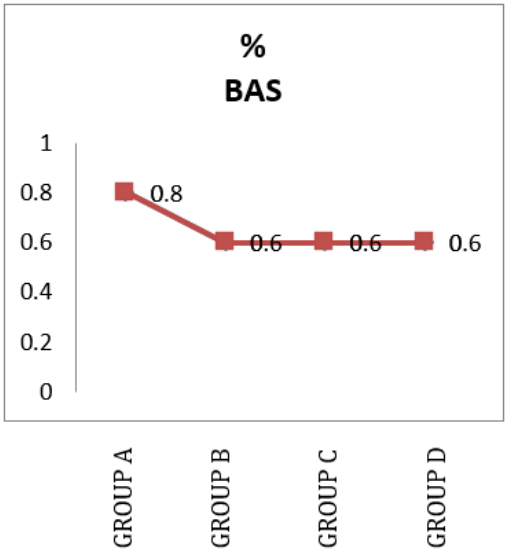

Figure 7A

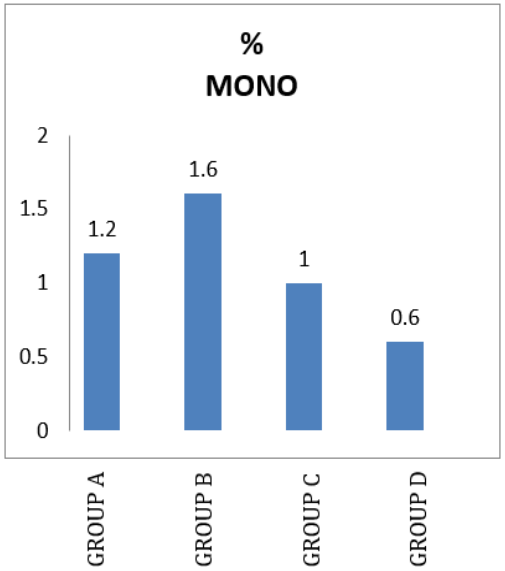

Figure 8A

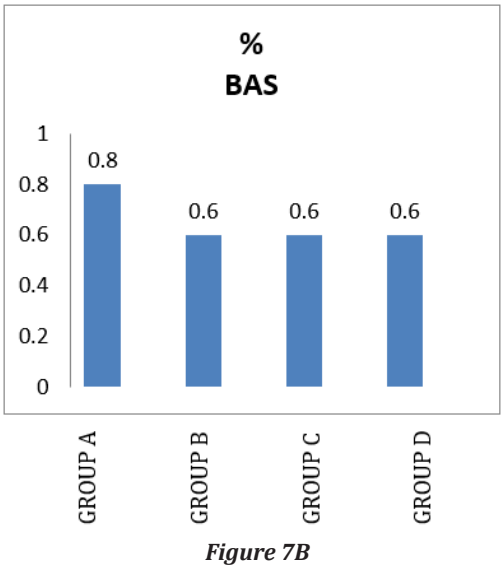

Figure 7B

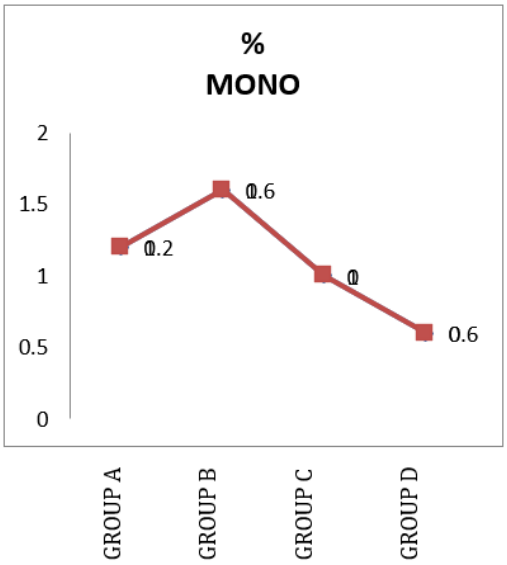

Figure 8B

Serum biochemical results for each group presented in tabular form in comparison with standard reference range.

Serum Chemistry

Table 5

All animal has high serum aspartate aminotransferase which indicates on going liver problem or reduce intracellular oncotic pressure due loss of serum protein disease or malnourishment as seen by 40% hypoproteinemia with low serum electrolytes, this were

expected as all animals were managed under homemade foods, high serum hepatic enzyme seen agreed with previous done in this area.

Group B: This group comprises of animals that are raised under normal Nigeria homes pet environments, they were fed on imported and homemade puppies' food mixed.

First vaccinated them when they were 7weeks, vaccination was repeated four weeks later when they were 11weeks, and final vaccine shots were administered at 15weeks, comprises of 4males and 1 female, blood sample was collected for hematological and serum biochemical analysis using 5ml sterile syringes and needles. At the time of collection of result same for laboratory analysis, none has record of showing any clinical signs associated with classical CPE or any know gastroenteritis, all animal vitals parameters (temperature, pulse rates, respiratory rate) were within normal.

First vaccinated them when they were 7weeks, vaccination was repeated four weeks later when they were 11weeks, and final vaccine shots were administered at 15weeks, comprises of 4males and 1 female, blood sample was collected for hematological and serum biochemical analysis using 5ml sterile syringes and needles. At the time of collection of result same for laboratory analysis, none has record of showing any clinical signs associated with classical CPE or any Know gastroenteritis, all animal vitals parameters (temperature, pulse rates, respiratory rate) were within normal. Hematological results are shown in tabular form in comparison with standard reference range for each analyzed parameters All had hyperproteinemia and hyperalbuminemia, 60% were hypoglobulinemic and 40% has normal serum globulin concentration Animal varying concentration analyzed serum macro elements 80% were hyponatremia and hypochloremia, all were hypocupremia, 20% shows hypokalemia and hyperkalemia

REF. RANGE	T.P g/d 5.4–7.5	ALB g/dl 2.3-3.1	Globulin g/dl 2.7–4.4	AST U/C 13-15	ALT U/C 10-100	UREA mg/dl 8-28	CREAT. mg/dl 0.5-1.7	Na 142-152 meq	K my/d 3.9-5.4	Cl mg/dl 110-124	Cu ug/ml 0.4	Zn ug/ml 0.7-2m
A1	3.6	2.6	1.1	42	35	8.9	1.7	137.9	2.9	60.4	0.06	1.03
	Low	Normal	Low	High	Normal	Normal	Normal	Low	Low	Low	Low	Normal
A2	4.9	3.4	1.5	39	43	10.5	1.4	108.8	3.4	62.5	0.11	0.91
	Low	High	Low	High	Normal	Normal	Normal	Low	Low	Low	Low	Normal
A3	5.4	2.7	2.7	54	47	12.1	2.8	158.1	4.6	74.4	0.05	1.42
	Normal	Normal	Normal	High	Normal	Normal	High	High	Normal	Low	Low	Normal
A4	5.7	2.8	2.9	62	54	12.9	3.7	184.1	4.7	89.2	0.10	1.19
	Normal	Normal	Normal	High	Normal	Normal	High	High	Normal	Low	Low	Normal
A5	5.5	3.5	2.0	43	39	10.2	1.9	134.7	3.5	70.4	0.09	1.11
	Normal	high	low	high	normal	normal	high	low	low	low	low	normal

REF. RANGES	T.P g/d 5.4–7.5	ALB 8/dl 2.3-3.1	Globuling/dl 2.7–4.4	AST U/C 13-15	ALT U/C 10-100	UREA mg/dl 8-28	CREATN mg/dl 05-1.7	Na 142-152	K mg/dl 3.9-5.4	Cl mg/dl 110-124	Cu ug/ml 0.4	Zn ug/ml 0.7-2m
B1	7.7	5.2	2.5	49	23	17.4	3.2	115.8	4.0	99.1	0.10	1.10
	High	High	Low	High	Normal	Normal	High	Low	Normal	Low	Low	Normal
B2	7.9	5.8	2.1	31	24	16.8	2.9	100.9	6.2	102.4	0.13	1.06
	High	High	Low	High	Normal	Normal	High	Low	High	Low	Low	Normal
B3	8.8	4.9	3.5	36	27	18.5	4.2	113	5.6	90.8	0.14	1.04
	High	High	Normal	High	Normal	Normal	High	Low	High	Low	Low	Normal
B4	8.2	4.8	3.4	85	48	20.2	1.2	102.9	3.1	132.7	0.15	0.99
	High	High	Normal	High	Normal	Normal	Normal	Low	Low	High	Low	Normal
B5	6.5	4.6	1.9	77	45	13.9	5.1	145	4.6	85.8	0.12	1.73
	High	High	Low	High	Normal	Normal	High	Normal	Normal	Low	Low	Normal

Group C

Table 7

Comprises of five animals that does not have record of CPE vaccination and came down with the diseases or shows signs associated with gastroenteritis and classical CPE

CPE confirmatory final diagnoses were done using commercially available CPE ELISA test kits. They all tested positive to presence of CPE antigens in their fecal samples

Summary of their tests are show in table labeled figure 7 above All test animals were hypoproteinemia and hypoglobulinemia and varying serum albumin concentration, high serum AST concentration and but normal serum concentration of ALT, Urea and Creatinine concentration and low serum electrolyte concentration all these agreed with previous researcher reports on this works

REF. RANGES	T. P g/d 5.4–7.5	ALB 8/dl 2.3-3.1	Globulin g/dl 2.7–4.4	AST U/C 13-15	ALT U/C 10-100	UREA mg/dl 8-28	CREATN mg/dl 05-1.7	Na 142-152	K my/d 3.9-5.4	Cl my/dl 110-124	Cu ug/ml 0.4	Zn ug/ml 0.7-2m
C1	3.5	2.7	0.8	38	19	9.9	1.7	74.7	2.0	46.8	0.09	1.55
	Low	Normal	Low	High	Normal	Normal	Normal	Low	Low	Low	Low	Normal
C2	3.6	2.9	0.7	30	21	10.5	1.1	93.4	3.1	49.4	0.05	1.61
	Low	Normal	Low	High	Normal	Normal	Normal	Low	Low	Low	Low	Normal
C3	2.9	1.5	1.4	35	18	9.5	2.5	81.2	2.9	44.1	0.15	1.43
	Low	Low	Low	High	Normal	Normal	High	Low	Low	Low	Low	Normal
C4	3.1	1.9	1.2	31	15	8.7	2.3	99.0	1.8	40.6	0.07	1.31
	Low	Low	Low	High	Normal	Normal	High	Low	Low	Low	Low	Normal
C5	2.7	1.3	1.4	42	20	8.9	2.3	110.5	2.5	41.5	0.08	1.34
	Low	Low	Low	High	Normal	Normal	High	Low	Low	Low	Low	Normal

Group D

Table 8

Serum Biochemical Results in Comparison with Normal References Ranges. 80% of tested samples in this group show low serum protein(hypoproteinemia) and hypoglobulinemia with varying concentration of albumin this results agreed with previous work done in this area that CPE infection causes low serum total protein with varying concentration of serum albumin.

All show low serum electrolytes (hyponatremia, hypokalemia, hypocupremia and hypochloremia). High serum AST showing CPE may had affect liver function due to protein loss, reduce oxygenation due to dehydration reducing circulation blood volume or due to hemorrhagic bloodletting in GIT.

REF. RANGES	T. P g/d 5.4–7.5	ALB 8/dl 2.3-3.1	Globulin g/dl 2.7–4.4	AST U/C 13-15	ALT U/C 10-100	UREA mg/dl 8-28	CREA TN mg/dl 05-1.7	Na 142-152	K my/d 3.9-5.4	Cl my/dl 110-124	Cu ug/ml 0.4	Zn ug/ml 0.7-2m
D1	2.7	1.3	1.4	42	20	8.9	2.3	99.2	3.1	65.4	0.07	1.11
	Low	Low	Low	High	Normal	Normal	High	Low	Low	Low	Low	High
D2	4.4	3.1	1.3	40	24	15.2	2.1	121.9	3.5	73.8	0.02	1.26
	Low	Normal	Low	High	Normal	Normal	High	Low	Low	Low	Low	Normal
D3	4.1	2.9	1.2	47	23	13.9	1.3	BY 3	3.3	68.7	0.13	1.17
	Low	Low	Low	High	Normal	Normal	Normal	Low	Low	Low	Low	Normal
D4	4.5	3.9	0.6	73	46	23.5	4.1	110.4	2.8	30.9	0.08	1.26
	Low	High	Low	High	Normal	Normal	High	Low	Low	Low	Low	Normal

D5	5.7	4.1	1.6	50	34	16.7	2.7	101.8	3.1	65.7	0.15	1.28
	Normal	High	Low	High	Normal	Normal	High	Low	Low	Low	Low	Normal

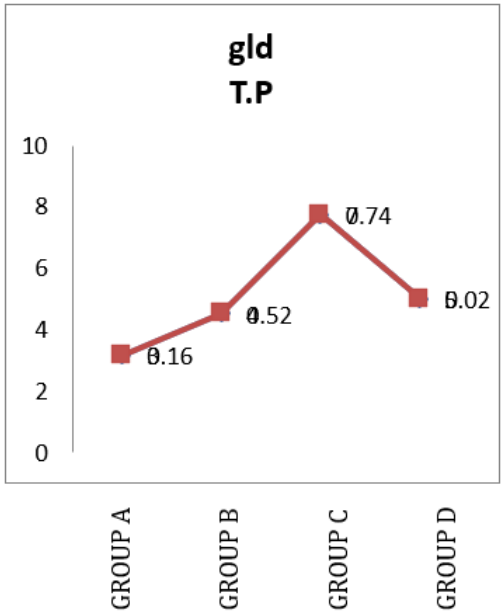

Figure 9A

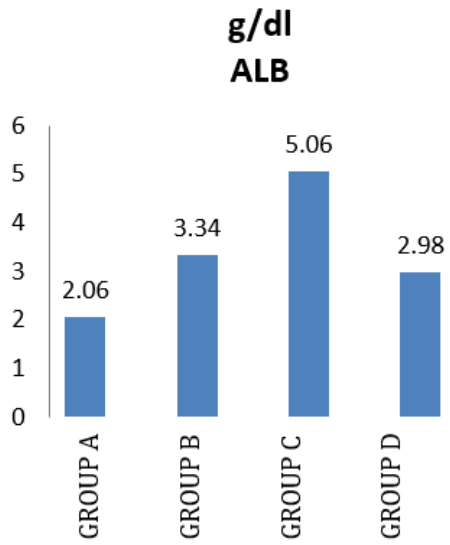

Figure 10A

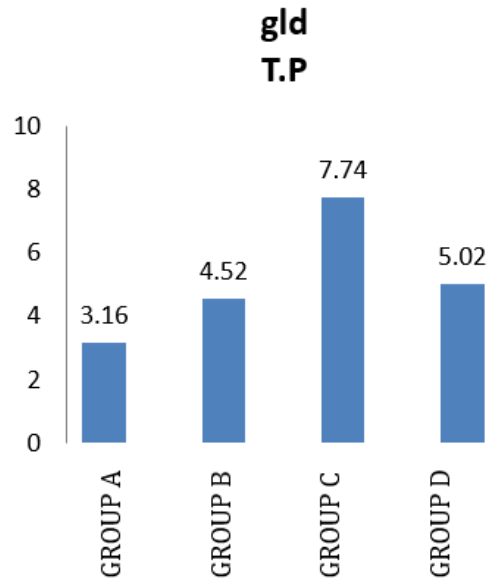

Figure 9B

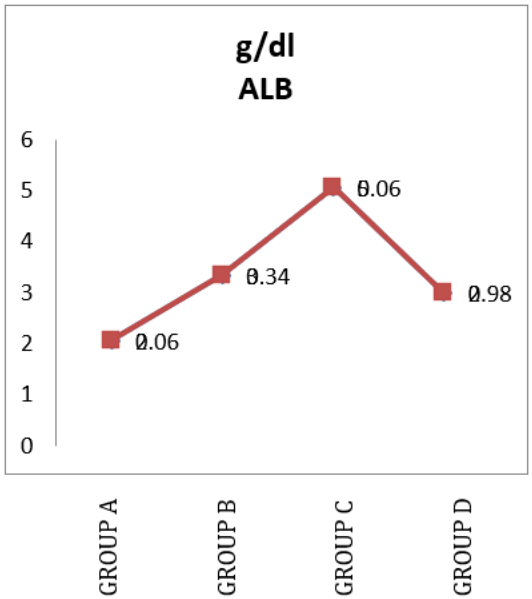

Figure 10B

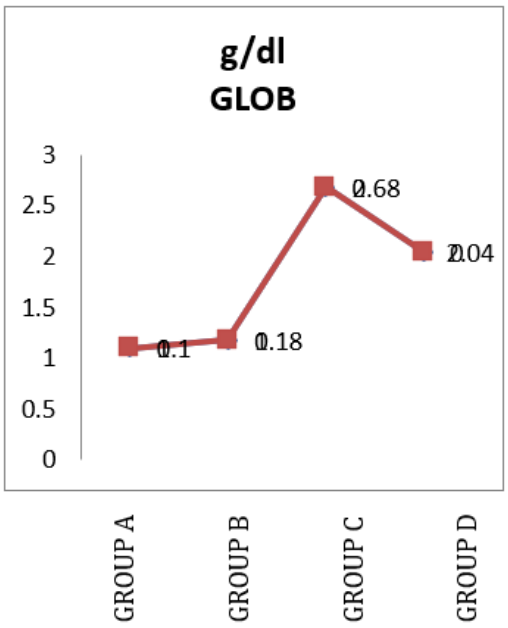

Figure 11A

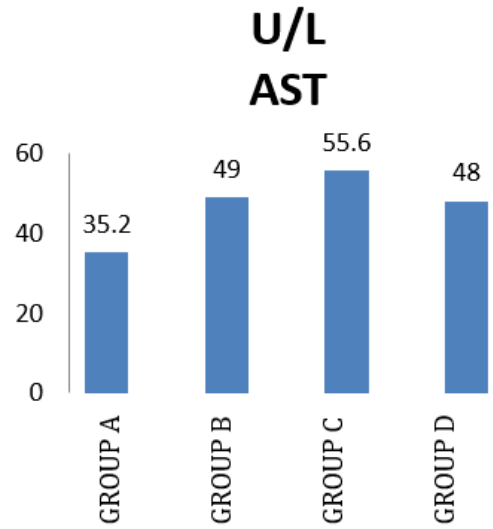

Figure 12A

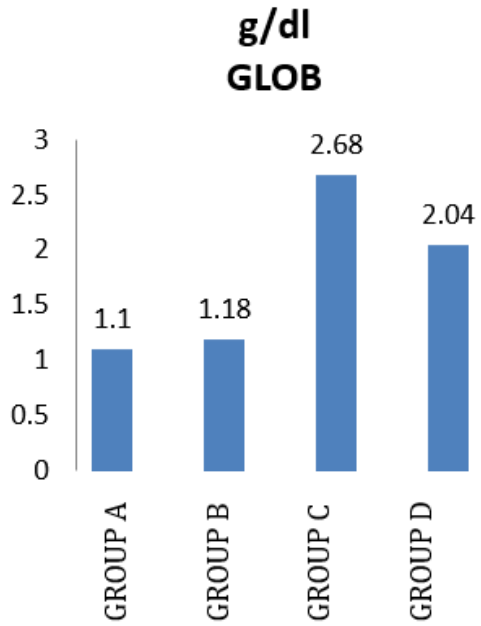

Figure 11B

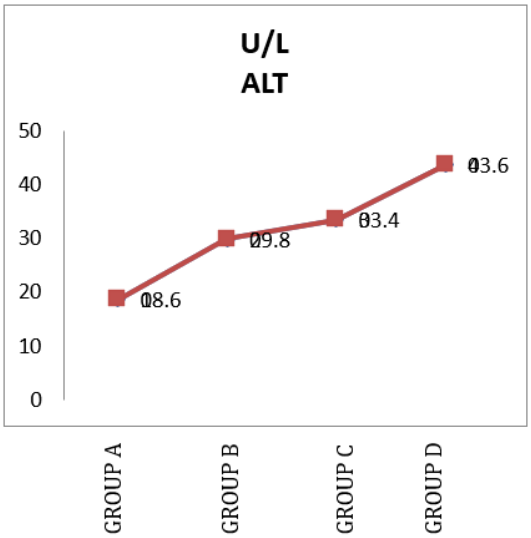

Figure 13A

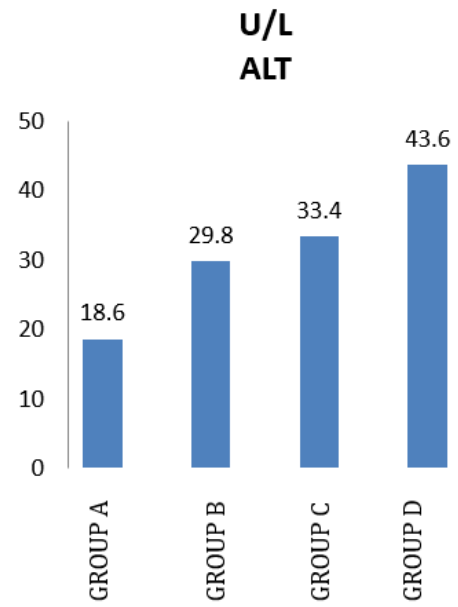

Figure 13B

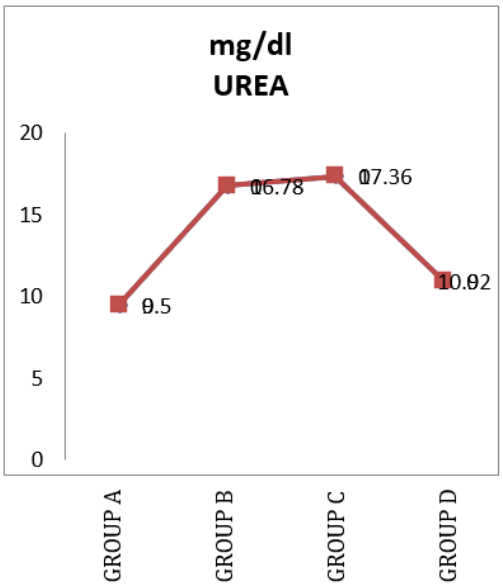

Figure 14B

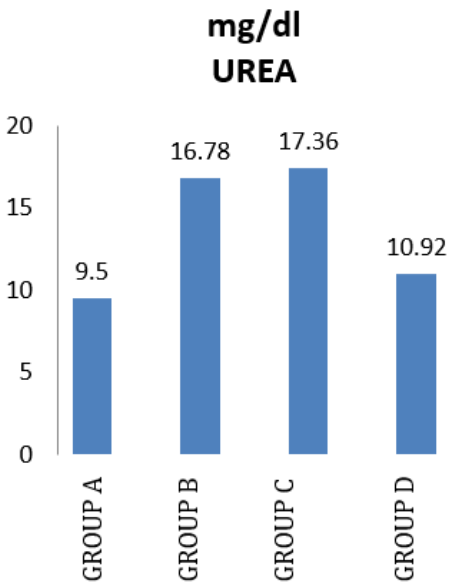

Figure 14A

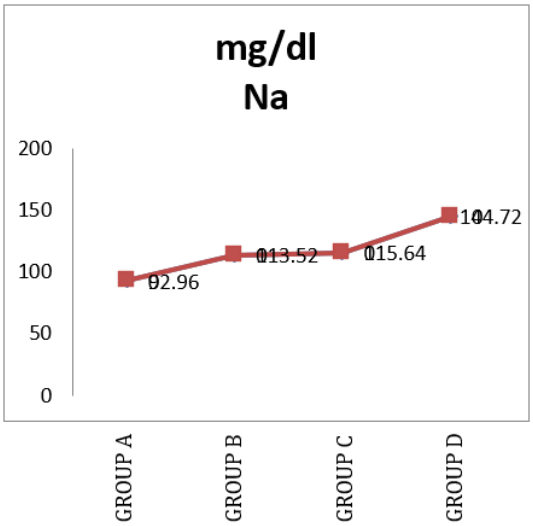

Figure 15A

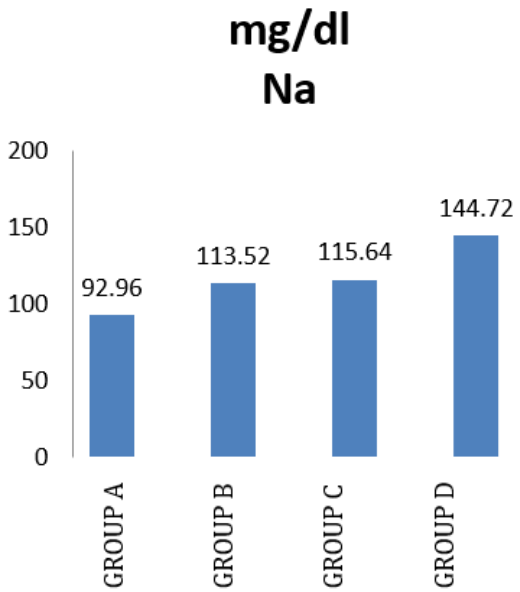

Figure 15B

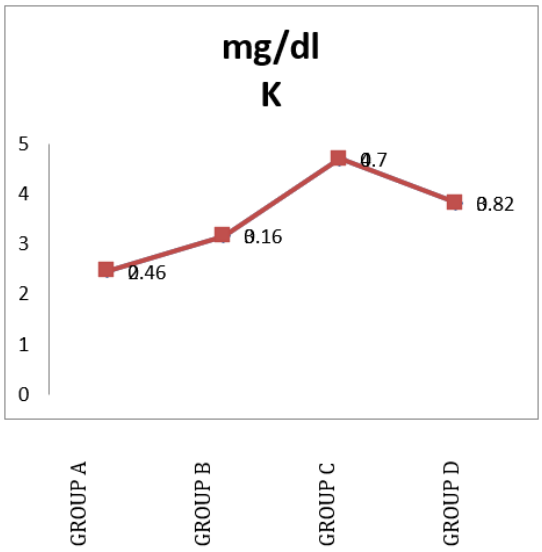

Figure 16B

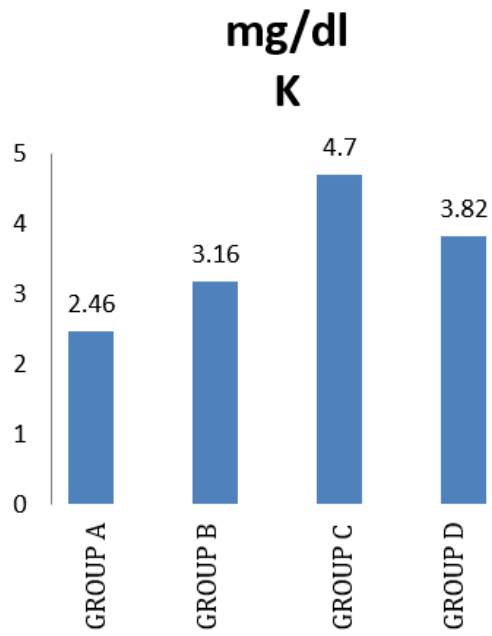

Figure 16A

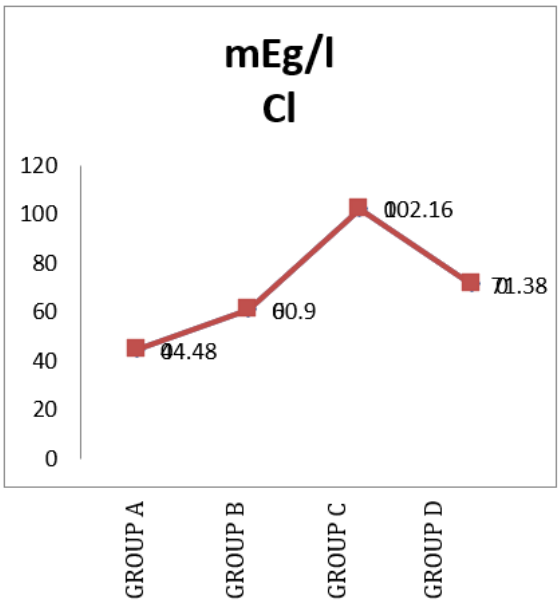

Figure 17A

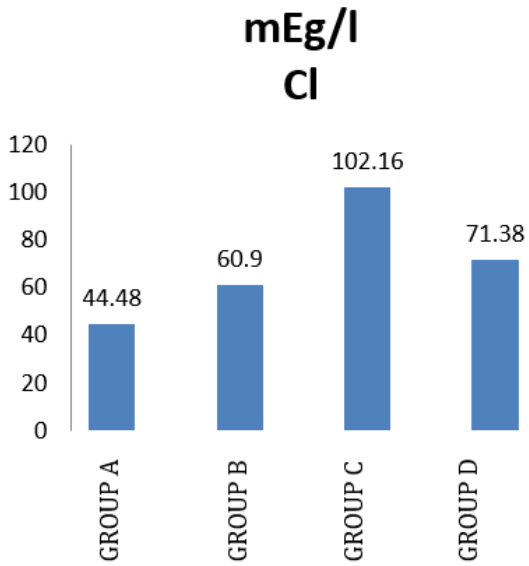

Figure 17B

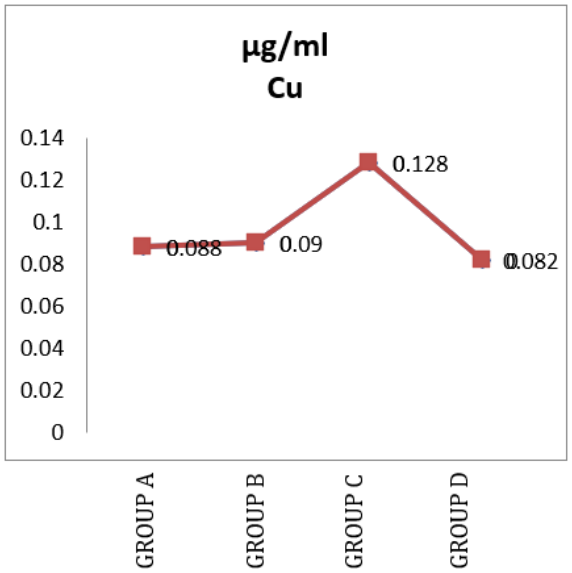

Figure 18B

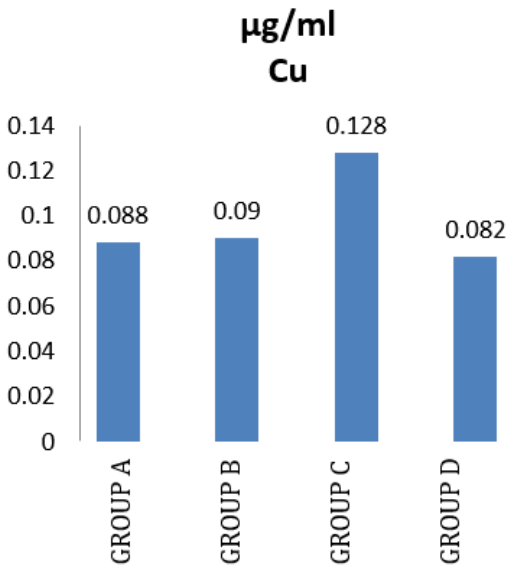

Figure 18A

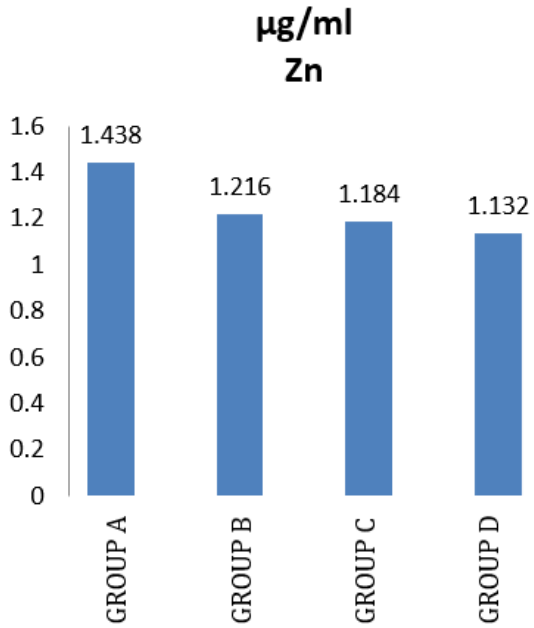

Figure 19A

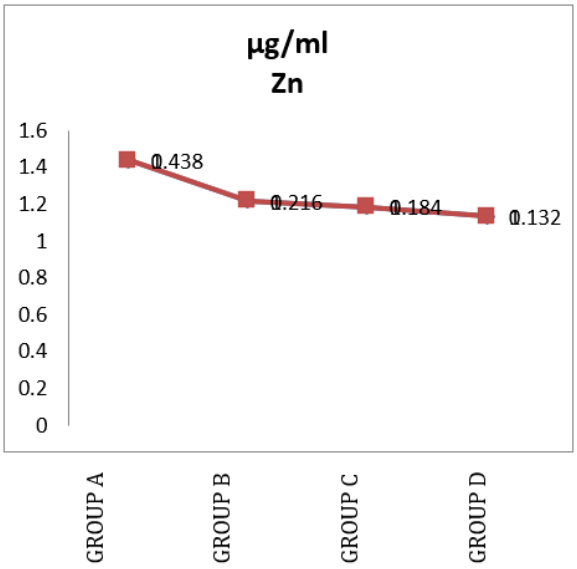

Figure 19B

Chapter 5

Discussion

This research aims to study effected of vaccination of CPE vaccines on vaccinated and non-vaccinated dogs raised under normal Nigeria home environment, to show whether vaccinated against CPE has any significant over those pet that are not vaccinated.

Using social science statistically analysis package, the analyzed parameters were analyzed separately by using statistical methods to find mean average different within group, standard deviation within group and between P Values were gotten and results are deem significant different when p > o,005)

Variation of analyzed parameters is also compare between group histogram and linear graph for proper understanding

Research limitation

No absolute control of pet used for this experiment, owner consent are sought and gotten Laboratory use for this study analysis is far from sample collecting area so we have to use dispatch riders that take sample from Lagos to Abeokuta in Ogun state.

20 animals are enrolled in this study. 17males 3 females
All parents gave consent.
They are divided into 4 four namely GROUP A, B, C, D

Group A

5 Animals are in this groups they are used as negative control group 1, This groups comprises of 5 animals that had no record of been given any vaccine shots against CPE and never shows any clinical signs associated with classical CPE or any know gastroenteritis

All tested animal has hematological analyzed parameters within normal references range with lymphocytosis, although 40% shows low monocytes count, all analyzed serum from this group shows high level of Aspartamine transaminase(AST) enzymes with maybe due to ongoing liver disease or due to low serum total protein concentration problem or reduce intracellular oncotic pressure due loss of serum protein disease or malnourishment as seen by 40% hypoproteinemia with low serum electrolytes, this were expected as all animals were managed under homemade foods, high serum hepatic enzyme seen agreed with previous done in this area

Group B

20% of tested animals has normal hematocrits results that was hyponeutrophilic and hypomonocytosis, while 80% of tested animal having polycythemia half of this polycythemia animals showing normal PCV values probably due to dehydration all tested animals had lymphocytosis with other differential white blood cell within normal range this group hematological results agrees with works of (Panda et al 2009)

All had hyperproteinemia and hyperalbuminemia, 60% were hyperglobulinemic and 40% has normal serum globulin concentration

Animals in this group has varying concentration of analyzed serum macro elements 80% were hyponatremia and hypochloremia, all were hypocupremia, 20% shows hypokalemia and hyperkalemia

Group C

This group consists of five animals

Animals that do not have record of CPE vaccination and came down with the diseases or shows signs associated with gastroenteritis and classical CPE

CPE confirmatory final diagnoses were done using commercially available CPE ELISA test kits.

They all tested positive to presence of CPE antigens in their fecal samples

Summary of their tests are show in table labeled table 3 and 7 above

All animals were anemic with as with low PCV, low hemoglobin concentration results only 20% has hypochromic anemia, all has normal WBC count with lymphocytosis.

This results also agreed with previous done on this area (Panda et al 2009, Goddard et al 2008)

All tested animals were hypoproteinemia and hypoglobulinemia and varying serum albumin concentration, high serum AST concentration and but normal serum concentration of ALT, Urea and Creatinine concentration and low serum electrolyte concentration all these agreed with previous researcher reports on this works

Group D

40% of animals in the group has iron deficiency anemia, as seen with analyzed hemogram with PCV values but normal RBC count, 40% has responsive or regenerative anemic All have normal differential white blood count with lymphocytosis these results agreed previous done on this field (Goddard et al 2008)

This agreed with previous experimental results done in this fields (Panda et al, 2009) 80% of tested samples in this group show low serum protein (hypoproteinemia) and hypoglobulinemia with varying concentration of albumin these results agreed with previous work done in this area that CPE infection causes low serum total protein with varying concentration of serum albumin.

All show low serum electrolytes (hyponatremia, hypokalemia, hypocupremia and hypochloremia). High serum AST showing CPE may have affect liver function due to protein loss, reduce oxygenation due to dehydration reducing circulation blood volume or due to hemorrhagic bloodletting in GIT

Inferences

All data from analyzed parameters are further analyzed by getting their average mean within groups and their standard deviation between group comparison, their p values was also obtained to determine their statistical significant

Variation between group was deemed significant when P-values is > 0.005

For each analyzed parameters PCV; packed cells volume is the ratio of blood made of cells, it is calculated in percentage, PCV is high in dehydrated animal or animal with polycythemia

This study established that there is noticeable significant different in PCV value of vaccinated and non-vaccinated animals

This was also in confirming with statistical analysis of P value > 0.005. see table 10 for this variation is better illustrated with linear and histogram graph representation showing variation in P CV in Figure 1A and 1B on page 49 showing how PCV varies between group.

Hemoglobin; this study confirmed that there is significant different in hemoglobin values of puppies infected with CPE vaccinated and non vaccinated, this can be in see table 10, these variations in hemoglobin values between groups can be better appreciated with linear and Histogram Figure 2 A and 2B on page 50

RBC; this research shows that there is significant different in analyzed RBC values changes between RBC values of vaccinated and non vaccinated dog, see table 10 for this analytical results and see figure 3Aand 3B on page 51 for linear graph and Histogram graph depicting this variation between groups

WBC; there is significant different variation in white blood cells count of vaccinated and non-vaccinated dog infected with CPE. See table 10 for this statistical result

Eosinophil no significant different in analyzed values
BASOPHIL; no significant different
Monocytes; no significant different

Serum biochemical parameters

Total protein, albumin and globulin; this study established that CPE infection caused significant changes in serum biochemical protein content due to protein loss from diarrhea, vomiting, bloodletting from GIT ulceration and sloughing off .this result agreed with previous work done on this subject that CPE infection causes variation in serum albumin and globulin concentration, see table 9 for this analytical result also see Figure 9A, 9B, 10 A, 10B, 11A and 11B on pages 63-65 for linear graph and Histogram graphs show how total serum protein, Albumin and Globulin varies between groups.

Serum electrolytes; this study confirmed that CPE infection cause great variation in serum electrolytes ion such as sodium, potassium and chlorine ions due to electrolyte loss due to vomiting and diarrhea, this confirm with P value great than 0.005 see table 9 for this result also see figure 15A, 15B, 16A, 16B,17A and 17B for linear graph and histogram depicting this variation

Urea; CPE causes significant different in serum urea concentration between vaccinated and non-vaccinated pet AST, Zinc, Copper; no significant different observed in their variations

Chapter 6

Conclusion

The aim of this study is to analysis using statistical method of average mean, standard deviation and P values with social science package 2.0.

If there is any significant different in hematological and serum biochemical parameters of pet infected with CPE with regard to their vaccination status

This study conclude that there is significant different in variations observed in hematological parameters data analyzed between previously vaccinated against CPE infected puppies and non vaccinated infected CPE puppies.

There is significant different in analyzed blood parameters like PCV, RBC, hemoglobin, WBC and lymphocytes of vaccinated and non-vaccinated dogs see table 10 for this. This result agrees with previous study on this virus no significant different were observed for Neutrophil, Basophil, Eosinophil between analyzed data between vaccinated group and no vaccinated group.

Also, significant different were noticed in serum total protein, Albumin, Globulin, serum electrolytes ion like sodium, potassium, chlorine ion.

CPE causes no significant different in serum electrolytes ion like, zinc, copper and AST concentration. This also is in agree with previous on this subject.

Recommendation

This study concluded that there is a great significant different in response to clinical therapy between vaccinated and non-vaccinated pet with vaccinated pet having better hematocrits picture when they succumb to CPE and response better to clinical management than unvaccinated infected pet because of this will recommend all newly pets to be properly vaccinated against CPE.

That all pet owner should be advised to vaccinate their pet properly against CPE.

Contribution to Knowledge

This study was able to determined that all pets managed for CPE in contrast to previous works that report leucopenia due to lymphopenia, that was gradually increase in lymphocytes count as the pet started showing signs of recovery ,which result in lymphocytosis by the time they are discharged as they started responding to therapy there is noticeable increase in their lymphocytes count which continued even after they are discharge from clinic, lymphocytosis was still observed two weeks after they are discharge from clinic.

Increase lymphocytes counts can be use as good prognosis sign in CPE infection.

This report has not been documented before.

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