PREVALENCE, DRUG PRESCRIPTION PATTERNS, CLINICAL FINDINGS IN CANINE GASTROENTERITIS AND GENETIC ANALYSIS OF CANINE PARVOVIRUS ISOLATES IN NIGERIA

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ABSTRACT

Gastroenteritis is a frequent presentation in canine practice with challenges in its diagnosis and management. Canine Parvovirus (CPV) is a common cause of gastroenteritis, mortality, and economic losses with recurrent vaccination failure. Due to limited documented information on the condition in Nigeria, this study was designed to investigate the prevalence, aetiologies, clinical presentation, and management of canine gastroenteritis, and characterise CPV isolates in Nigeria.

Retrospective data of 3,882 dogs presented to ten veterinary clinics from seven locations (Abeokuta, Abuja, Ibadan, Jos, Makurdi, Onitsha and Warri) in Nigeria from January to December 2016 were analysed for prevalence and drug prescription patterns for gastroenteritis. Also, 157 cases of gastroenteritis were prospectively evaluated for their aetiologies and usefulness of clinical pathology in prognostication using standard procedures. Electrocardiograms of 40 dogs with confirmed Canine Parvovirus Enteritis (CPE) using rapid in-clinic assay kit and polymerase chain reaction were evaluated for cardiac involvements. Protocols of CPV vaccination failure (94/157) were examined for appropriateness. Polymerase chain reaction was done on the vaccine and positive clinical samples using primers specific for parvoviral DNA and subjected to sequencing and phylogenetic analysis. Data were analysed using descriptive statistics, Chi-square, and logistic regression at $\alpha_{0.05}$.

Prevalence of gastroenteritis was 41.2% and was influenced by dog breed (α=0.014), vaccination status (α=0.047) and period of the year (α=0.03). Polypharmacy was high with an average of 5.4 drugs prescribed in each treatment regimen. Antibacterials (48.3%) and antiparasitics (23.8%) were extensively prescribed. Canine parvovirus (92.9%), gastrointestinal parasites (12.1%), coronavirus (2.6%), liver disease (0.6%) and undetermined causes (1.9%) were identified as the aetiologies of the clinical cases. Colic (Odds Ratios [OR]=0.01; α=0.001), leukopaenia (OR=3.5, α=0.01), hypoalbuminaemia (OR=7.1; α=0.006) and pancytopaenia (OR=0.2; α=0.002) at initial time of presentation were prognostic for prolonged duration of management and poor outcomes. Electrocardiographic changes comprising ST-depression (7.5%), tall T-wave (27.5%), S-wave deepening (20.0%), prolonged QT-duration (25.0%), prolonged P-wave duration (17.5%), and tachycardia (15.0%) were seen in 70.0% of confirmed CPE cases. Vaccination failure was associated with the protocol adopted, with one-, two-, three- and four-dose protocols having failure rates of 51.1%, 28.7%, 19.1% and 1.1%, respectively. Sequence and phylogenetic analysis of 11 clinical samples showed that CPV-2c (63.6%) and CPV-2a (36.4%) were the predominant strains and were genetically closely related to Asian and European strains. Amino acid changes (T301S, D305Y, Y323I, Q370R, T440A, Y444S and I447M) were observed in the VP2 protein of the clinical isolates. The vaccines that were sequenced contained CPV-2a only.

Canine parvovirus and gastrointestinal parasites were the leading causes of canine gastroenteritis in Nigeria. Prescription patterns used in managing several cases were injudicious. Presentation with colic, hypoalbuminaemia and leukopaenia are useful indicators for poor prognosis and prolonged management. Assessment of cardiac functions in canine parvovirus enteritis is recommended. Inappropriate vaccination protocols, viral mutations, and incorporation of only CPV-2a strain in vaccines licensed for vaccinating dogs against canine parvovirus portends risk of vaccination failure.

Keywords: Canine parvovirus, Prognosis, Electrocardiogram, Polypharmacy, Vaccination failure

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CERTIFICATION

We certify that this work was carried out by Dr F. K. Shima in the Department of Veterinary Medicine, University of Ibadan.

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GLOSSARY OF ABBREVIATIONS

ACTH	Adrenocorticotropic hormone
ADOI	Advertised duration of immunity
AdST	Advertised start time
AFT	Advertised finish time
AHD	Acute haemorrhagic diarrhoea
AIEC	Adherent and invasive *Escherichia coli*
ALB	Albumin
ALP	Alkaline phosphatase
ALT	Alanine transaminase
AST	Aspartate transaminase
ARD	Antibiotic-responsive diarrhoea
AVI	Advertised vaccination interval
BID	Twice a day (drug prescription)
bp	Base pair
BUN	Blood urea nitrogen
cAMP	Cyclic adenosine monophosphate
CAV	Canine Adenovirus
CBC	Complete blood count
CCoV	Canine coronavirus
CDV	Canine distemper virus
CFU	Colony-forming unit
cGMP	Cyclic guanosine monophosphate
CNS	Central nervous system
COP	Colloid osmotic pressure
CPE	Canine parvovirus enteritis
CPV	Canine parvovirus
CRTZ	Chemoreceptor trigger zone
CSF	Cerebrospinal fluid
CVD	Concomitant vomiting with diarrhoea
CYP-450	Cytochrome P-450
DHLPPi	Distemper, hepatitis, leptospirosis, and para-influenza polyvalent vaccine

DNA	Deoxynucleic acid
DOH	Duration of hospitalisation
DOM	Duration of management
DOI	Duration of immunity
ECG	Electrocardiography
ELISA	Enzyme-linked immunosorbent assay
EPI	Exocrine pancreatic insufficiency
FISH	Fluorescence in-situ hybridisation
FPLV	Feline panleukopaenia virus
FMT	Faecal microbiota transplantation
FPV	Feline parvovirus
FRE	Food-responsive enteropathy
GDV	Gastric dilatation volvulus
GI	Gastrointestinal
HAI	Haemagglutination inhibition
HAU	Haemagglutination units
HGE	Haemorrhagic gastroenteritis
IBD	Inflammatory bowel disease
IFA	Immunofluorescence assay
IgG	Immunoglobulin G
IL-2	Interleukin-2
IM	Intramuscularly
ISH	In-situ hybridisation
IV	Intravenously
LVH	Left ventricular hypertrophy
MCH	Mean corpuscular volume
MCHC	Mean corpuscular haemoglobin
MCV	Mean corpuscular haemoglobin
MDA	Maternally derived antibodies
MEV	Mink enteritis virus
MLV	Modified-live vaccines
NK-1	Neurokinin-1
NPO	*Non per os* (nothing by mouth)

NLR	Negative likelihood ratio
NSAIDs	Nonsteroidal anti-inflammatory drugs
NTS	*Nucleus tractus solitarius*
PCR	Polymerase chain reaction
PCV	Packed cell volume
PLE	Protein-losing enteropathy
PLI	Pancreatic lipase immunoreactivity
PLR	Positive likelihood ratio
PO	*per os* (by mouth)
PPIs	Proton pump inhibitors
Q12H	Every 12 hours, etc.
RaPV	Racoon parvovirus
RBC	Red blood cells
RVH	Right ventricular hypertrophy
SC	Subcutaneously
SEM	The standard error of mean
SIBO	Small intestine bacterial overgrowth
SID	Once a day
SIRS	Systemic inflammatory response syndrome
T-cells	Thymus-derived cells
TCID	Tissue culture infective dose
TEC	Total erythrocyte counts
TLC	Total leukocyte counts
TID	Three times a day
VTH	Veterinary Teaching Hospital
VTM	Virus transport medium
WBC	White blood cells
WHO	World Health Organisation
ZnSO4	Zinc sulphate

DEFINITION OF TERMS

Antibiotic-responsive diarrhoea	A syndrome whereby an animal with chronic diarrhoea of unknown cause improves with antibiotic therapy but relapses upon its withdrawal.
Biomarkers	Qualities or clinical signs estimated and assessed empirically as pointers of ordinary biological or malady processes, or pharmacologic impacts of therapeutic intervention.
Cardiac arrhythmias	An arrhythmia is a problem with the rate or rhythm of the heartbeat.
Cardiomyopathy	Chronic disease of the heart characterised by hypertrophy of the myocardial and fibrosis.
Gastroenteritis	Inflammation of the stomach and intestinal mucosal characterised by nausea, vomiting, diarrhoea, and abdominal discomfort.
Diarrhoea	An increase in faecal fluidity, defaecation frequency and volume.
Duration of management	The number of days treatment was given calculated as date treatment stops minus date started.
Dysbiosis	A quantitative and/or qualitative term used to describe alterations usually decreases in intestinal microbes or faecal microbiome.
Enteropathy	Any clinical condition or disease of the intestines, such as enteritis.
Firmicutes	Non-pathogenic bacteria of the intestinal microbiota, most of which are Gram-positive. They account for 30% to 95% of the normal microbiome and afford maintenance health benefits to their host.
Food-responsive enteropathy	A condition when a cat or dog has a gastrointestinal disorder that resolves with dietary modification.
Normosol-R	A balanced isotonic electrolytes solution for parenteral fluid and electrolyte replenishment of acute losses of extracellular fluid.

PlasmaLyte	A balanced crystalloid solution with varying electrolyte formulations and composition mimicking the physiological plasma electrolyte concentrations, osmolality, and pH.
Polypharmacy	The practice of inappropriate and/or concurrent prescription of multiple drugs to an individual patient to be taken at a time.
Potency	Is the drug concentration that can produce 50% of the maximum response or activity.
Prognosis	An evaluation of the outcomes to be achieved from any medical treatment.
Puppy primary vaccine series	Vaccines that a puppy must take within the first three months of life, in a series of three or four doses 2-4 weeks apart, e.g. DHLPP vaccine; to protect them from infectious diseases.
Rational drug prescription	A prescription written appropriately by a clinician in accordance with the clinical needs of the patient, in correct dose regimen and a reasonable financial cost to the client.
Small Intestinal Bacterial Overgrowth (SIBO)	Refers to an upsurge in the population of small intestinal bacteria.
Vomiting	Forceful repulsion of the stomach contents through the mouth.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Canine gastroenteritis

The digestive system is essential in the normal functioning of the body. It supplies energy by way of digesting and absorbing nutrients. It also neutralises toxins and eliminates wastes from the body. The main clinical presentations of gastrointestinal (GI) diseases are diarrhoea, vomiting, dehydration, weight loss, constipation, change in appetite, flatulence, blood or mucus in the faeces, and abdominal cramps. The term gastroenteritis is used when the mucosae of the digestive tract are inflamed or irritated. Gastroenteritis is the regular reason for acute onset of vomiting, anorexia, diarrhoea, and abdominal discomfort in small companion animals with vomiting and diarrhoea as the most frequent presentation in small companion animal medicine (Tams, 2003; Elwood *et al*., 2010). Diarrhoea is an increase in faecal fluidity, defecation frequency and volume; typically resulting from diseases of the small intestine with resultant loss of fluid and electrolyte (Armstrong, 2013a). Vomiting is the expulsion by force the contents of the upper stomach, resulting from gastric, gastrointestinal, or systemic nongastrointestinal diseases. A greater percentage of gastroenteritis is mild and resolves without treatment but mild therapy may be required in some cases. Life-threatening cases require a thorough diagnostic approach and/or intensive care (Elwood *et al*., 2010; Armstrong, 2013a).

There are myriad causes of gastroenteritis, such as, dietary (e.g. indiscretion, food allergy and tolerance), endoparasites and infectious agents (like bacteria, viruses, and fungi) among others documented for small animals (Willard, 2009). Intercurrent infection is possible in canine gastroenteritis and may exacerbate the severity, as well as, influence the prognostic outcomes in affected patients. Due to multiple aetiologies involvement, arriving at a definitive diagnosis and management of some cases becomes very challenging thereby increasing costs of treatment. Also, frustration and dissatisfaction for the owner, potential unresolved clinical signs are associated with

suffering for the animal as well as injudicious use of drugs or polypharmacy. Antibiotics remain the predominant therapy of choice in the management of canine gastroenteritis in many clinical practices (German *et al*., 2010; Marks, 2013; Gberindyer, 2016). Rational drug prescription reduces drug failures, adverse drug events, treatment cost (Pramil *et al*., 2012), as well as drug resistance unlike the irrational use of drugs.

The clinical consequences of gastroenteritis are determined by the aetiologies involved. Mild vomiting and diarrhoea cause few metabolic disorders. However, moderate to severe diarrhoea with persistent vomiting can result in loss of gastrointestinal fluid and subsequently dehydration, hypovolemic shock, electrolyte changes (e.g. hypokalaemia, hypochloraemia, and hyponatraemia) and metabolic acid-base changes - acidosis and alkalosis (Boag *et al*., 2005; Armstrong, 2013a). Loss of bicarbonate from the GI tract and dehydration predispose to metabolic acidosis. This culminates in hypovolaemia, tissue anaerobic metabolism, and lactic acid production which are lethal (Armstrong, 2013a).

Limited reports are available on canine gastroenteritis especially in developing nations like Nigeria. Some available reports show that gastroenteritis is prevalent and associated with high morbidity in the canine population. Reports have emerged that gastroenteritis and colitis are among the top diseases in small companion animals in the United States (Banfield, 2013). Also, in Nigeria where such data are extremely limited, gastroenteritis and CPE were ranked in the top 10 disorders diagnosed in owned-dogs presented for veterinary consultation (Shima *et al*., 2015a).

Accurate diagnosis of any disease and its treatment are an integral part of patient management. To definitively diagnose and manage canine gastroenteritis successfully, a holistic diagnostic plan and a balanced approach based on an adequate understanding of the pathophysiology as well as logical decision-making are required. About 5% of dogs with vomiting and 10% with diarrhoea presented to veterinarians are acute but self-limiting. In most cases, the aetiology was never determined; rather dietary factors, infectious agents and toxins are presumed (Hubbard *et al*., 2007). A presumptive diagnosis of acute self-limiting gastroenteritis was deemed correct, if the clinical signs resolve in one or two days following supportive treatment (Hubbard *et al*., 2007).

Parvovirus enteritis is basically an exceptionally transmissible illness of puppies and young dog. It is transmitted directly or indirectly through contact with contaminated faeces and fomites (Kahn, 2010). Typically, its clinical manifestations include vomiting, bloody-foul-smelling diarrhoea, lethargy, hypoglycaemia, leukopaenia and myocarditis in the neonates (Streck *et al*., 2009). The disease remains an important and greatest challenge to the health of dogs worldwide even in this era of cheap, safe, and potent vaccines (Carmichael, 2005; Decaro *et al*., 2006). It causes high morbidity and death in the affected dogs. Some vaccinated dogs can become clinically infected (Shima *et al*., 2015b; Babalola *et al*., 2016). The disease is incriminated in most cases of acute gastroenteritis in Nigeria (Shima *et al*., 2014; 2015a, 2015b). Its morbidity rate has been reported to be about 100%. Its treatment success is influenced by how early and aggressive treatment is initiated. The survival rate varies between 9% in untreated to between 64–90% in treated animals (Otto and Drobatz, 1997; Kalli *et al*., 2010). Notwithstanding, many dogs may die from CPV-related complications even with adequate treatment (Kocatürk *et al*., 2010).

Predisposing factors include age, breed, inappropriate vaccination protocols, unhygienic environment or overcrowding, environmental stress, the season of the year, intercurrent intestinal parasitism or enteric infections, and weakened immunity (Hoskins, 1997; Shakespeare, 1999; Prittie, 2004; Shima *et al*., 2015b). Vaccination is practised worldwide to control and prevent CPV infection in dogs. Even in the contemporary era of efficacious vaccines, CPV remains a problem in Nigeria with concerns over the high rate of vaccinated dogs coming down with infection (Shima *et al*., 2015b; Apaa *et al*., 2016). This owes probably to single-dose vaccine regimen or inappropriate vaccinations (Shima *et al*., 2015b; Babalola *et al*., 2016). These regimens cannot provide the required optimum immunity in juvenile animals against CPV. Evidence-based reports of the disease in fully vaccinated dogs in Nigeria have created doubts and myths amongst veterinarians and dog owners on whether the current CPV vaccines in use are efficacious and do confer the desired protection against CPV infection (Shima *et al*., 2015b; Apaa *et al*., 2016). Investigations are yet to ascertain the causes and as to whether vaccine-virus has a connection with the disease in fully vaccinated dogs.

The CPE causes enormous loss of body fluid and nutrients, dehydration and hypovolaemic shock (Prittie, 2004). Damage caused to the GIT by viral involvement

heightens the chances of bacteria translocation, coliform septicaemia, systemic inflammatory response stimulation, septic shock, as well as death (Kahn, 2010). Bacteria, particularly *Escherichia coli* were isolated from extra-gastrointestinal organs [particularly liver and lungs] of puppies diagnosed with parvovirus (Turk *et al*., 1990). Haemorrhagic diarrhoea often seen in CPE has been associated with endotoxaemia and cytokine production rather than the direct effect of the virus (Isogai *et al*., 1989).

The diagnosis of parvovirus enteritis is established using the following case definitions: complete history, such as age, exposure to sick dogs, previous vaccinations or natural infection, presence of depression, anorexia, diarrhoea, and vomiting in an unvaccinated or inappropriately vaccinated puppies (Mylonakis *et al*., 2016). Definitive diagnosis involves detecting antigens in the faeces of CPV suspects, isolation of the virus by cell culture, serology, molecular detection (PCR), necropsy and histopathology (Costa *et al*., 2005). Haplotypes of parvovirus currently spreading in canine populations globally successively substituted the wildtype CPV; are designated as CPV-2a, CPV-2b, and CPV-2c. Among African countries, all haplotypes are circulating in Morocco, Tunisia, and Nigeria in varying proportions (Touihri *et al*., 2009; Amrani *et al*., 2016; Fagbohun & Omobowale, 2018), whereas in South Africa, CPV-2a and CPV-2b dominate (Dogonyaro *et al*., 2013).

Treatment of CPE is mostly supportive; targeted at correcting disturbances in the metabolic acid-base and electrolyte imbalance, fluid deficit following gastroenteritis, and to prevent microorganism-associated bacteraemia, such as *Clostridium*, *Campylobacter*, *E. coli* and *Salmonella* species. The cornerstone management includes intravenous administration of balanced isotonic fluids and electrolytes supplemented with glucose, antibiotics, antiemetics and colloids in severely hypoproteinaemic patients (Prittie, 2004; Dudley and Johnny, 2006; Crawford and Sellon, 2010). The most common antibiotics used are gentamicin usually combined with a β-lactam such as amoxicillin for control of aerobic Gram-negative and Gram-positive bacteria organisms (Prittie, 2004). Control and prevention could be achieved through appropriate vaccination protocols, usually three-dose regimen at 6–8, 10–12 and 14–16 weeks of age, and environmental hygiene (Kahn, 2010).

1.2 Problem statement

Gastroenteritis exhibited by vomiting and/or diarrhoea is the most treated disorder in canine practice. Its aetiologies are multiple, consisting of primary GI or systemic extra-gastrointestinal diseases with secondary effects on the gastrointestinal tract. Hence, gastroenteritis is among the disorders some clinicians find difficult and so exasperating to diagnose and treat successfully (Marks, 2015). Consequently, antibiotics are injudiciously administered to such patients, with recovery often wrongly ascribed to the elimination of the suspected pathogens (Marks, 2015). Similarly, reports showed that antibiotics are abused in the management of gastroenteritis in dogs in many veterinary practices (German *et al*., 2010; Gberindyer, 2016); but only 5% to 10% of the cases presented to veterinarians are diagnosed or their aetiologies are determined. Most often, they are presumptively attributed to diet-related factors, infectious agents, and toxins upon resolution of clinical signs (Hubbard *et al*., 2007).

Polypharmacy and injudicious drug prescriptions are common in clinical practice globally (Kirsten *et al*., 2010; German *et al*., 2010). Injudicious drugs usage represents a potential hazard to the patients as well as increases the cost of treatment (Sanz and Boada, 1988). Rational drug prescription reduces drug failures, adverse drug events, treatment cost (Pramil *et al*., 2012), and drug resistance. Studies assessing drug prescription patterns are lacking in veterinary medicine in Nigeria and Africa at large.

In Nigeria, the prevalence, aetiologies, and whether there are breed predisposition or seasonal variations regarding the occurrence of gastroenteritis in dogs have been understudied. Also, the application of laboratory diagnosis in many veterinary practices in Nigeria is relatively low, hence, in many acute cases of canine gastroenteritis, CPV infection is incriminated. Canine parvovirus infection is a life-threatening illness which is preventable by vaccination but has not been eradicated or reduced in the canine population globally despite the availability of potent vaccines. DNA sequences from some parts of Nigeria only CPV–2a haplotype (Apaa *et al*., 2016), while CPV-2c and CPV-2b are detected in smaller proportions (Fagbohun and Omobowale, 2018).

The vaccines used for vaccinating dogs are reported to cross-protect dogs against the other biotypes. However, there remain uncertainties about the efficacy of the various brands of the vaccines available in Nigeria. Whether they share similar genetic characteristics with the field-strains circulating in the dog populations in Nigeria has not

been adequately investigated. Ultimately, there are both anecdotal and confirmed reports of CPV disease in vaccinated dogs (Shima *et al*., 2015b; Apaa *et al*., 2016; Babalola *et al*., 2016). Investigating the vaccine usage patterns in Nigeria and molecular characterisation of the vaccine and field-strains may help determine the cause of CPV vaccination failure.

Lastly, owing to the widespread vaccination of dogs, some people believe that parvovirus cardiac involvement is rare in dogs (Meunier *et al*., 1984; Martyn *et al*., 1990; Maxie and Jubb, 2007). However, recent reports demonstrated that parvovirus myocardial damage is still common and has extended window (Ford *et al*., 2017). Cardiac involvement in CPV disease is rarely detected on auscultation. Therefore, electrocardiography may be an alternative in detecting such cardiac changes.

1.3 Study aim

The research goal is to clinically investigate canine gastroenteritis in order to:

1. Quantify gastroenteritis and assess drug prescription patterns for dogs diagnosed with gastroenteritis in Nigeria using retrospective data.
2. Identify the aetiologies and evaluate clinicopathologic profiles of canine gastroenteritis patients in Nigeria.
3. Determine prognostic biomarkers influencing the DOM and clinical outcomes in dogs presenting with gastroenteritis.
4. Evaluate the electrocardiogram of dogs diagnosed with CPE.
5. Assess vaccination protocols of vaccinated dogs diagnosed with CPE in Nigeria.
6. Characterise CPV strains in vaccines licenced for vaccinating dogs in Nigeria and clinical samples.

1.4 Justification

Gastroenteritis remains the most frequent presenting disorder in canines with challenges in its diagnosis and management but its peculiarities are understudied in Nigeria. Its potential causes are not comprehensively investigated compared to other clinical conditions of dogs, such that several cases of haemorrhagic gastroenteritis are presumptively diagnosed as CPV infection. Gastroenteritis is among the most challenging disorders many veterinarians find very frustrating to diagnose and treat. Hence, antibiotics are often administered imprudently to diarrhoeic patients, with a view

of eradicating pathogens presumed to be the cause (Marks, 2015). Establishing relevant information on the prevalence, coexisting infections, clinical presentation, management practices and prognostic biomarkers will be valuable for the successful management of canine gastroenteritis.

Parvovirus is a key cause of canine gastroenteritis, mortality, financial losses, and repeated vaccination failure in Nigeria but is largely under-studied. Many brands of vaccines are licenced for vaccinating dogs against CPV infection in Nigeria, but whether the strains used in developing them are the same as the field-virus circulating in Nigeria remain elusive. There are reported cases of CPV disease and low antibody titre in fully vaccinated dogs necessitating further study (Apaa *et al*., 2016; Babalola *et al*., 2016). Available data on CPV covered only a small region in Nigeria; warranting a large-scale investigation to have a clearer data on the distribution of the strains, CPV vaccine usage patterns to determine the reasons for parvovirus vaccination failure; and suggest measures to reduce CPV infection in Nigeria. Furthermore, CPV appears to be persistently involved in myocardial damage but it is in most cases under-recognised (Ford *et al*., 2017). This study will demonstrate the usefulness of ECG in detecting cardiac abnormalities, as well as help optimise monitoring and treatment strategies for patients diagnosed with CPV infection.

Furthermore, gastroenteritis posed risk of transmitting zoonotic diseases. Considering the zoonotic potential of *Ancylostomosis*, *Giardiosis,* and *Toxocariosis* and their frequent association with gastroenteritis, it is imperative to investigate their prevalence in dogs presenting with gastroenteritis in Nigeria. This will broaden our knowledge on their prevalence and help veterinarians take precautionary measures when handling dogs with gastroenteritis.

Lastly, periodic assessment of drug prescription patterns is paramount to identifying drug prescription snags, sensitisation of veterinarians on judicious use of drugs. Provision of policies regarding drug prescription will ensures the appropriate use of drugs, checkmate drug resistance, as well as, adverse drug events arising from inappropriate drug usage. Above all, drug utilisation evaluation is an ongoing and systemic quality improvement process, which helps to evaluate prescription patterns and/or audit drug use. Assessing drug prescription patterns for canine patients with

gastroenteritis in order to find loopholes, if any, will help suggest corrective measures for addressing prescription issues in veterinary practice in Nigeria.

1.5 Research questions

1. How prevalent is canine gastroenteritis in Nigeria?
2. Are prescription practices for dogs with gastroenteritis in Nigeria appropriate?
3. What are the causes and clinicopathological picture of dogs with GE in Nigeria? Are there indicators of DOM and clinical outcomes in dogs with gastroenteritis?
4. Is there cardiac involvement in dogs with CPE detectable on electrocardiogram (ECG)?
5. Are CPV vaccination protocols used in Nigeria appropriate?
6. What are the strains of CPV currently circulating in dogs in Nigeria? And are there differences between field-strains and those of vaccines licenced for the vaccinating dogs in Nigeria?

CHAPTER TWO

LITERATURE REVIEW

2.1 Canine gastroenteritis

Dog is the most common small companion animals found in almost every human settlement around the globe (Shima *et al*., 2015a). Like any other domesticated animals, they are susceptible to several diseases or conditions requiring veterinary attention. Gastrointestinal (GI) disorders are frequently presented in canine practice. Basically, GI disorders manifest clinically as anorexia or change in appetite, diarrhoea, vomiting, flatulence, blood or mucus in the faeces, constipation, dehydration, emaciation, and colic (Elwood *et al*., 2010). The term gastroenteritis denotes the inflammation of the stomach and intestinal mucosa, characterised by either vomiting or diarrhoea or both. In canine practice, vomiting and diarrhoea are considered the most common presenting complaints that are sometimes very difficult to diagnose and manage judiciously (Tams, 2003; Marks, 2013). An American study ranked gastroenteritis and colitis among the top 19 diagnoses for dogs (Banfield, 2013). In Nigeria where such data are extremely limited, gastroenteritis due to several agents including CPE were among the ten topmost clinical disorders diagnosed in dogs (Shima *et al*., 2015a). Although gastroenteritis can be frustrating, most cases are diagnosed and treated judiciously without difficulties. It should be known that neither diarrhoea nor vomiting constitutes a diagnosis itself. They are merely clinical signs of disease processes affecting certain body systems. Their aetiologies are numerous with vomiting alone ascribed to over 400 potential causes according to a recent report from an American diagnostic registry service (Tams, 2014).

The incidence and prevalence of diarrhoea and vomiting are either underestimated or underreported in clinical practice even though they are frequent presenting complaints. There are relatively inadequate reports on the prevalence of canine gastroenteritis in developing nations like Nigeria despite being an age-long issue in canine practice. Maybe, cases presented to veterinarians are poorly diagnosed and improperly documented. This could also be attributed to the absence of research interest in

ostensibly common clinical conditions like gastroenteritis and/or the complexity of this disorder.

A web search on this subject returned very few documented literature. A study from Nigeria reported a clinic-based prevalence of parvovirus enteritis and other unconfirmed gastroenteritis cases in 8.3% and 7.0%, respectively in 571 dogs presented for veterinary consultation (Shima *et al*., 2015a). In Britain, the estimate was about 19.0% vomiting and 15.0% diarrhoea (Hubbard *et al*., 2007). In Ireland, the frequency was reported to be 35.1% diarrhoea and 21.1% vomiting (Wells and Hepper, 1999). The prevalence of diarrhoea was 36.15% of canine conditions surveyed in India (Dutta *et al.*, 2013), which corroborated the report by Wells and Hepper (1999) from Northern Ireland. An England study rated diarrhoea in the top eight common disorders in dogs, and accounted for 6.4% of the 148,741 dogs surveyed, without breed-association (O'Neill *et al*., 2014). The lowest rates reported is 2.2% for both vomiting and diarrhoea (Edwards *et al*. 2004) and 2.2% for diarrhoea and 2.1% for vomiting in dogs presented for veterinary consultation in the US (Lund *et al*. 1999). Dogs seem to be more predisposed to gastroenteritis than cats (Armstrong, 2013a). In another study involving nearly 2.2 million dogs and 460,000 cats in 2012, gastroenteritis and colitis were found more prevalent in dogs compared to cats (Banfield, 2013).

Risk factors associated with gastroenteritis are well documented for dogs. Gastroenteritis affects dogs without regards to age but with some levels of variations in the episodes of vomiting and diarrhoea. Diarrhoea and vomiting are less common in adults compared with young dogs and puppies. Secondary immunity acquired in life over time by matured dogs could afford protection against infectious agents associated with gastroenteritis (Sævik *et al*., 2012). In a Norwegian study, 585 large breed dogs were monitored for the incidence and prevalence of gastroenteritis from birth to two years, vomiting or diarrhoea episodes or both occurred only once. A relatively long interval between episodes was observed in those that had several episodes. There existed an association between vomiting and diarrhoea occurring in the same dog, with much lower frequency in adults compared to young puppies that were fully vaccinated and dewormed, while the risk for developing diarrhoea decreased as the dog grows older (Sævik *et al*., 2012). These findings validated the reports of several other authors that also reported that incidences of vomiting and diarrhoea decrease with advancing age

(Hoskins, 1997; Wells and Hepper 1999; Tupler *et al*., 2012). Ultimately, a peak incidence of diarrhoea-related diseases in the temperate regions occurs mostly during the summer compared to the winter months (Houston, 1996; Shakespeare, 1999).

2.2 Diarrhoea in dogs

Many risk factors and pathways induce diarrhoea in dogs. Understanding its aetiologies and pathophysiology in addition to comprehensive history-taking and diagnostic workups are crucial in the successful management of this condition in dogs.

2.2.1 Aetiologies of canine diarrhoea

The aetiologies associated with gastroenteritis in small companion animals are multifactorial. A variety of disease processes and conditions can predispose to diarrhoea. Diarrhoea can be caused either by GI or a manifestation of systemic nongastrointestinal diseases or both. Canine GI diseases associated with acute diarrhoea include a sudden change in diet, overeating, food allergies, food intolerance and dietary indiscretions. Equally important causes of diarrhoea are developmental and physical obstructions, infections, systemic (e.g., pancreatitis), metabolic (e.g., Addison's disease), and hepatic diseases (Twedt, 2000; Tams, 2000). Dietary indiscretions, pathogens (e.g., bacteria and viruses), and endoparasites are the leading causes of gastroenteritis in small animals (Willard, 2009; Armstrong, 2013a). Intercurrent infections with any of the agents are common and often exacerbate the severity of diarrhoea and influence its prognosis.

2.2.1.1 Viral diseases

The most frequent viral pathogens associated with gastroenteritis in dogs are canine coronavirus (CCoV), CPV and rotavirus (Salem, 2014; Grellet, 2016). The CCoV is the second cause of diarrhoea in puppies in the temperate regions with CPV taking a lead (Kempf *et al*., 2010). Both viruses have now evolved into new strains (Hoelzer and Parrish, 2010). Infection with the coronaviruses is more common in temperate than in the tropic region. CCoV infection is mild, self-limiting and is associated with a low mortality rate (Decaro and Buonavoglia, 2008). Nonetheless, reported outbreaks of fatal systemic enteric disease with pantropic strains of the virus with clinical signs mimicking CPV infection are common (Zicola *et al*., 2012; Decaro *et al*., 2013a). Furthermore, CCoV–CPV co-infection with a severe consequence is common in dogs in recent times (Decaro *et al*., 2008a). Both viral infections have age predisposition and high mortality. Puppies within the age bracket of three months are more susceptible (Grellet *et al*., 2014;

Grellet, 2016). Mixed CCoV–CPV infection is more severe and fatal (Evermann *et al*., 2005). It is associated with poor prognosis in puppies, but adult dogs have higher chances of recovery (Pratelli *et al*., 1999; Zicola *et al*., 2012).

Another important viral disease of dogs is the canine distemper disease. It is a multi-systemic fatal illness, characterised by respiratory, GI signs such as diarrhoea, and central nervous signs (Greene and Appel., 2006; Pratelli, 2011; Salem, 2014). The disease alongside rabies is a notable cause of fatality in canines (Deem *et al*., 2000).

A study involving 935 cases of acute haemorrhagic canine gastroenteritis showed that viruses were detected in 44.2%, comprising CPV (19.9%), CCoV (17.3%) and paramyxovirus (13.9%). Furthermore, co-infection was recorded in 6.5% of the dogs examined. Younger dogs significantly had higher rate of detection of these viruses compared to mature ones (Kempf *et al*., 2010). Canine viral diarrhoea is common in Nigeria but there are limited reports regarding their occurrences. The most commonly investigated/reported is CPV. Studies in Nigeria indicated that CPE is a more common problem among dogs presenting with gastroenteritis in canine practice (Chollom *et al*., 2013; Shima *et al*., 2014).

2.2.1.2 Bacterial infections

Bacterial enteritis of dogs is associated with a variety of infectious agents, such as *Clostridium*, *Yersinia*, *Campylobacter*, enterotoxigenic *Escherichia coli* and *Salmonella* species (Weese, 2011; Armstrong, 2013a; Marks, 2015). These organisms are a part of the normal microflora hence associating them with the clinical cases of diarrhoea is very challenging (Armstrong, 2013a). Thus, most clinicians treat diarrhoeic animals empirically and injudiciously with antibiotics with the intention of eliminating alleged pathogens (Marks, 2015).

Bacterial involvement in gastroenteritis can occur as secondary to other infections and is detected as coliform septicaemia. The destruction of the epithelium of the intestinal tract by viral disease assaults causes the translocation of bacterial, septicaemia, systemic inflammatory response syndrome or death in an affected animal (Turk *et al*., 1990). *Escherichia coli* were cultured from extra-gastrointestinal organs (liver and lungs) of dead puppies that suffered severe parvovirus enteritis (Otto and Drobatz, 1997). Other secondary bacteria isolated from diarrhoeic patients manifesting severe septicaemia

and/or endotoxaemia, as well as a fatal disease, were *Salmonella*, C*lostridia*, and *Campylobacter* organisms (Turk *et al*., 1992; Marks and Kather, 2003). Ultimately, haemorrhagic diarrhoea is caused by endotoxaemia and cytokines production rather than the direct consequences of viral infections (Isogai *et al*., 1989).

2.2.1.3 Haemorrhagic gastroenteritis

Haemorrhagic gastroenteritis (HGE) denotes a syndrome manifesting as acute vomiting and bloody diarrhoea with marked haemoconcentration (Spielman and Garvey, 1993). The packed cell volume (PCV) of the affected dog ranges from 55% to more than 60%. Haemorrhagic gastroenteritis is a life-threatening condition in young dogs because there may be shock following excessive loss of circulatory volume and electrolytes. The precise aetiology and pathogenesis are not well understood. Nonetheless, infection with or hypersensitivity to *Clostridium perfringens*, type–1 hypersensitivity reaction to food ingredients or CPE are incriminated. Small dog breeds than the toy and miniature breeds as well as large breed dogs are the most frequently affected. There is no report regarding sex and ages predisposition. Haemorrhagic gastroenteritis manifest as acute bloody diarrhoea, resembling raspberry jam (Armstrong, 2013a). Although it appears to be non-contagious, dogs living together occasionally develop clinical signs at the same time. Outbreaks involving several cases were reported in some countries (Puotinen, 2009). Studies have rarely associated *Clostridium perfringens* enterotoxins with HGE in dogs. Intravenous fluid administration and gastroprotectants remarkably improve this condition within one or two days (Armstrong, 2013a). Antibiotics are usually prescribed but may not certainly improve the condition as observed with treatment using amoxicillin/clavulanic acid combination (Unterer *et al*., 2011). No one treatment fit all cases of HGE.

Although most GI pathogens cause mild, self-limiting diarrhoea, some acute bacterial diarrhoea can be life-threatening, requiring prompt intensive care. Injudicious use of antimicrobials in acute uncomplicated diarrhoea may do more harm than good. Therefore, antimicrobials should be administered or may be necessary only in those patients manifesting systemic signs of illness (Armstrong, 2013a; Marks, 2015).

2.2.1.4 Parasitic diseases

Most parasitic enteritis is caused by *Ancylostoma*, *Coccidia*, *Giardia*, *Trichuris vulpis* and *Uncinaria* species (Armstrong, 2013a; Marks, 2015). *Giardia* is common, yet a

frequently overlooked parasite in patients presenting with diarrhoea, vomiting and anorexia. In the United Kingdom, *Giardia* and *Toxocara* remain the predominant parasites recovered from dogs with GI signs (Batchelor *et al*., 2008). Young dogs and those that had housing location changed were more likely to be positive to endoparasites (Berset-Istratescu *et al*., 2014). Endoparasites prevalence rates in Swiss dogs was 15.6% (Berset-Istratescu *et al*., 2014) and lower than prevalence reports in different dog populations (household dogs [20.4%]; kennel dogs [63.0%]; Belgian canines with GI illnesses [32.5%]) (Claerebout *et al*., 2009). Pooled worldwide *Giardia* prevalence of 12.0% and 15.2% have been documented for cats and dogs, respectively. The prevalence differs substantially between studies and locations (Ballweber *et al*., 2010; Bouzid *et al*., 2015). Concurrent endoparasites burden such as verminosis, giardiosis or coccidiosis act as an added stress factor that potentially worsens the severity of CPE (Prittie, 2004). These intestinal parasites increase the rate of intestinal epithelial cells destruction (Humm and Hughes, 2009). Developing a diagnostic plan that includes endoparasites screening is imperative in identifying and eliminating parasites-associated GI diseases. One of the protozoan infections usually associated with CPE is Giardia. Metronidazole remains a drug of choice for treating it (Vesy and Peterson, 1999; Tams, 2014). Parasitic diarrhoea is generally self-limiting but can cause debility in affected patients.

2.2.1.5 Dietary indiscretions

Acute diarrhoea caused by food allergies and dietary indiscretions regularly occur in dogs and cats. Dietary indiscretion involves chemicals and toxins contaminated diets, change in diet, foreign materials, plant intoxication, and garbage or table scraps. Dogs may be exposed to foodborne intoxications or infections following indiscriminate eating habits and exposure to toxigenic substances. Food intoxication associated with gastroenteritis accounted for about nine per cent of poisonings in dogs according to reports from Nigeria (Shima *et al*., 2014; 2015a). Feeding home-made diet, scavenging, and change of diet increase the odds of canine diarrhoea (Stavisky *et al*., 2011). In a similar scenario, the occurrence of gastroenteritis correlated positively with scavenging behaviours in dogs but not with eating table food (Hubbard *et al*., 2007). Also, pica increases the risk of diarrhoea in dogs (Berset-Istratescu *et al*., 2014).

Food-responsive enteropathy (FRE) is associated with chronic diarrhoea in dogs but the response rate is relatively higher (45% to 60%) when fed elimination diets containing

novel, single sources of intact or hydrolysed proteins (Mandigers *et al*., 2010). The implementation of dietary therapy often rapidly resolves FRE in most dogs within three to four days (Marks, 2013). Diarrhoea due to dietary indiscretions is typically self-limiting and resolves particularly when the invasive food source is removed.

2.2.1.6 Intestinal dysbiosis and bacterial overgrowth

The intestinal microbiome comprised bacteria, viruses, fungi, protozoa, and archaea. Bacteria constitute the largest fraction of the intestinal microbiota, with dissimilarities in the individual bacterial composition of each healthy animal (Ziese and Suchodolski, 2019). The Fusobacteria, Proteobacteria, Bacteroidetes and Firmicutes are bacterial phyla that constitute 99% of the gut microbiome in small companion animals (Suchodolski *et al*., 2005; Handl *et al*., 2011).

The intestinal microbiome or microflora supports the maintenance of the GI healthiness and integrity of their host. They aid in metabolism and several other physiologic processes in the body including immune modulation (Bansal *et al*., 2010; Arpaia *et al*., 2013). Furthermore, they protect from pathogen invasions, and aids in food digestion or metabolism and energy harvest from diets to provide nutrients, such as vitamins and amino acids (Cummings *et al*., 1987; Sunvold *et al*., 1995). They as well provide nutritional support for enterocytes (Cranny and McCormick, 2008). The total intestinal microbial load in animals is 10^{12} to 10^{14} bacterial (Armstrong, 2013a). Any alteration in the intestinal microbiota will predispose to chronic enteropathies (Honneffer *et al*., 2014).

Dysbiosis is a term used to describe quantitatively and/or qualitatively the changes involving intestinal microbes. Simply put, dysbiosis is the alterations in the microbial composition and/or diversity. Faecal microbiome alterations provide useful evidence on the development of diarrhoea. The pathogenesis of chronic and acute enteropathies is influenced by changes in microbial load/population (Webb, 2016). Dogs suffering from AHD have marked changes in their faecal microbiota, which differs between chronic and acute disease situations (Suchodolski *et al*., 2012). Large and giant breeds have decreased odds to develop bacterial overgrowth compared to dysbiosis of their microbiota (Westermarck *et al*., 2005). Intestinal dysbiosis can be caused by many GI disturbances such as ARD, FRE, IBD, acute diarrhoea due to stress, infections, or dietary indiscretion; abnormal intestinal motility (blind loops, diverticula, intestinal obstruction,

strictures); exocrine pancreatic insufficiency; and drugs [e.g., Antibiotics, NSAIDs and antacids] (Suchodolski and Whittemore, 2016).

According to German *et al*. (2003) many chronic enteropathies in cats and dogs related to Small Intestinal Bacterial Overgrowth (SIBO). Chronic diarrhoea that improves with antibiotic therapy (e.g., tylosin and metronidazole) but relapses when the antibiotic is discontinued, without any clear assertion for other possible causes is termed antibiotic-responsive diarrhoea (ARD). Both ARD and SIBO are used interchangeably but there is no complete overlap between the two terms. Some dogs with SIBO-associated GI disorders are not always ARD-related (German *et al*., 2003). The ARD is a less common disorder in small breeds compared to large and giant breeds, such as Alsatians, and is a regular finding in small bowel diarrhoea or large bowel diarrhoea without a known cause (Westermarck *et al*., 2005).

2.2.2 Pathophysiology of diarrhoea

Water and electrolyte absorption and secretion within the GI tract are achieved by means of a finely, balanced, dynamic process. Loss in the balance following either a reduction in absorption or increased secretion culminates in diarrhoea (Whyte and Jenkins, 2012). Basically, diarrhoea can occur through one or a combination of four different pathophysiological processes (Rozanski and Rush, 2007). These mechanisms sometimes occur simultaneously, therefore, becomes difficult to determine the predominant one in many diseases of small animals. The most significant mechanisms in cats and dogs are changes in mucosal permeability and osmotic forces (Armstrong, 2013a).

2.2.2.1 Osmotic diarrhoea

This arises when excessively unabsorbed osmotically active solutes are retained in the intestinal lumen and hold water. Diarrhoea, therefore, results following an upsurge in the quantity of osmotic particles aiding the passive movement of fluid along the osmotic gradient of the GI lumen (Rozanski and Rush, 2007; Whyte and Jenkins, 2012). Predisposing factors associated with osmotic diarrhoea include mal-absorption; as in exocrine pancreatic insufficiency (EPI) or Addison's disease where poorly digested nutrients are mal-absorbed and their presence in the GI lumen continues to attract water with it. Secondly, osmotic diarrhoea can arise from overeating and dietary indiscretion of poorly absorbed nutrients after consumption (Armstrong, 2013a), as well as, motility disorders (Whyte and Jenkins, 2012). The retention of poorly absorbed nutrients causes

fermentation of carbohydrates, as well as, dysbiosis of the gut, increasing the amount of osmotically active particles within the GI tract. This form of diarrhoea often resolves after the withdrawal of the poorly absorbable diet or substance (Armstrong, 2013a).

2.2.2.2 Secretory diarrhoea

This occurs when abnormal quantities of electrolytes, fluid, and ions are activated by a specific pathway by a toxin or abnormal enterocytes are inherently secreted into the intestinal lumen. This results in a large amount of fluid being secreted beyond the capacity of the intestine to absorb (Rozanski and Rush, 2007; Whyte and Jenkins, 2012). Intracellular secondary messengers (cAMP and cGMP) stimulate the secretion of chloride while inhibiting the absorption of sodium. This causes the flow of water along its concentration gradient and subsequently diarrhoea (Rozanski and Rush, 2007). Infectious diseases causing diarrhoea through the secretory mechanism are salmonellosis, enteropathogenic *E. coli* and IBD. Similarly, secretory diarrhoea may occur following the stimulation of intestinal secretion by by-products of dysbiosis. Dysbiosis hampers the processing of bile acid result in increased accumulation concentrated primary bile acid in the colon and consequently secretory diarrhoea (Duboc *et al*., 2013; Giaretta *et al*., 2018). Secretory diarrhoea is mostly accompanied by changes in ion transport but not food-related; fasting the patient may not resolve secretory diarrhoea (Armstrong, 2013a).

2.2.2.3 Increased mucosal permeability

Inflammation of the GI epithelia alters the tight intercellular junctions, causing increased permeability which in turn triggers leakage of ions, electrolytes, blood, fluids, and plasma proteins into the intestinal lumen (Rozanski and Rush, 2007). It is commonly seen with intestinal erosion, ulceration, neoplasms (lymphoma), inflammatory bowel disease and ancylostomosis (Armstrong, 2013a; Kelly, 2016).

2.2.2.4 Abnormal motility

Alterations in the intestinal motility cause diarrhoea through reduction or upsurge in transit time (Rozanski and Rush, 2007). This abnormal motility often occurs secondary to disorders-causing diarrhoea. Stimulation of giant aboral contraction and reduced segmental contractions of ileum and colon portions of the small intestine are triggered mostly by platelet-activating factors synthesised and released from inflammatory response mediators- immunocytes (Armstrong, 2013a).

2.2.3 Clinical features and consequences of diarrhoea

The clinical implications of diarrhoea are determined by the causal agents, as well as, its severity i.e. whether mild or chronic. The initial and common clinical manifestations of GI disturbances are vomiting, dehydration, anorexia, colic, fever, lethargy, and diarrhoea, which may be watery, mucoid, and/or bloody. Large quantities of fluid loss normally occur when the integrity of the GI mucosa is compromised. Fewer metabolic alterations are associated with mild diarrhoea unlike in moderate to severe diarrhoea which causes marked dehydration, hypovolaemic shock, electrolyte changes, such as hypokalaemia, hypochloraemia, hyponatraemia, metabolic acid-base disturbances, such as acidosis and alkalosis (Boag *et al*., 2005; Armstrong, 2013a). Loss of bicarbonate from the intestines and dehydration are the major predisposing factors of metabolic acidosis, anaerobic tissue metabolism, hypovolaemia, as well as, the production of lactic acid (Armstrong, 2013a). Patients with life-threatening diarrhoea are regularly dehydrated, depressed, experienced frequent vomiting and abdominal cramps. Also, melaena or haematochezia with attendant abdominal mass or dilated loop of the bowel are commonly seen. Such patients may develop ascites, cough, hepatomegaly, jaundice, lymphadenopathy, oliguria, or anuria, oculonasal discharges, as well as, fever. Puppies with severe diarrhoea experience weight loss, emaciation, or death. To clearly distinguish nonlife-threatening diarrhoea from life-threatening diarrhoea is sometimes difficult. Therefore, aggressive management is cautiously initiated as if the patient is suffering from life-threatening diarrhoea (Armstrong, 2013a).

2.2.4 Diagnostic strategies in canine diarrhoeic

Diagnosis of life-threatening gastroenteritis demands a holistic approach in order to establish the underlying causes. A combination of techniques may be more beneficial in this regard. Vomiting or diarrhoea that is mild or acute self-limiting are treated symptomatically and may not warrant a thorough diagnostic workout. This is because many of such patients are not seriously ill and clinical signs may resolve without treatment. About 95% of dogs with vomiting and 90% with diarrhoea are acute self-limiting gastroenteritis. Diagnosis in most cases is presumptive (Hubbard *et al*., 2007).

In unresolved cases warranting a further diagnosis, a panel of tests may be required. This is complemented with proper history-taking and physical examination. These could give the clinician a fair idea of the origin of the gastroenteritis (i.e. GI tract or extra-GI

diseases such as pancreatic insufficiency or Addison's disease). The history can reveal information on the duration of the gastroenteritis, faecal characteristic (colour, volume, presence of mucus or occult blood), deworming and vaccination history, defaecation rate and influencing factors among others (Armstrong, 2013a; Marks, 2015). Chronic diarrhoea persisting for about four weeks or longer may require a systematic approach to arriving at a diagnosis and formulating treatment plan different from acute self-limiting diarrhoea, which in most cases, do not warrant a comprehensive workup. In exceptional cases, patients with AHD syndrome, typically lasting for less than one week but suspicious of an array of nonspecific causes (e.g., infectious and non-infectious) may require a comprehensive workup. Whether dietary modification or supplement could alleviate or precipitate GI disorder needs to be considered, as failure to do so can delay diagnosis or improper dietary recommendations (Marks, 2015).

The most important primary screening methods employed in the diagnosis of canine diarrhoea include faecal examination for parasites and bacteria, screening for suspected viruses, haematology and biochemistry evaluation, a test of intestinal function, abdominal imaging, and endoscopy and biopsy. The specific diarrhoea panel tests used are PCR test for confirmation of Giardia, *C. perfringens* enterotoxin 'A' gene, *Salmonella spp*., *Cryptosporidium spp*., CCoV, CPV, as well as, canine distemper virus (Marks, 2015).

2.2.4.1 Screening for suspected viruses

Parvoviruses are the key diseases highly suspected and confirmed in acute diarrhoea in cats and dogs (Dossin, 2011). Presently, several in-practice-rapid assay kits are available for the identification of faecal antigens to parvoviruses. The tests have very high specificity with variable sensitivities. They may produce false-positive results in recently vaccinated dogs (Schmitz *et al*., 2009; Patterson *et al*., 2007). A negative test with high suspicion should not dismiss parvovirus infection but retested using a superior test. In-clinic test kits validated for diagnosing parvovirus in cats have 97% and 100% negative and positive predictive values, respectively (Neuerer *et al*., 2008). The serological test can also be used but interpreting the result is difficult except where very high titres are present or paired samples testing showed increasing titres. Negative test results by in-clinic assay with strong suspicions are confirmed by polymerase chain reaction assay (Dossin, 2011).

2.2.4.2 Faecal examination for bacteria

Characterisation of bacterial diarrhoea is difficult in cats and dogs, as most of the important pathogenic bacterial organisms, such as *C. perfringens*, *C. difficile*, *E. coli*, and *Campylobacter* species are an integral part of normal microbiome of healthy faunae (Marks & Kather., 2003; Weese *et al*., 2001; Rossi *et al*., 2008). Because these organisms can be pathogenic with zoonotic potential, it is equally important screening them in patients with acute gastroenteritis (Dossin, 2011).

Bacterial culture, toxin immunoassays and PCR are diagnostic procedures with relatively low-yield, if performed injudiciously in animals with diarrhoea (Marks *et al*., 2011; Armstrong, 2013a). The procedures may be of value in suspected cases of bacterial enteritis or enterocolitis, faecal culture or PCR when performed only for specific enteric pathogens (e.g. *Salmonella* and *C. jejuni)*. Faecal PCR is superior to culture in the diagnosis of *Campylobacter* species especially in distinguishing the multiple *Campylobacter* species (Queen *et al*., 2012; Marks *et al*., 2011).

Faecal stained smears or cytology is regularly used in the diagnosis of diarrhoea to identify *Campylobacter*-like organisms and spiral-shaped bacteria, WBCs, and *C. perfringens* endospores but is of no diagnostic value in identifying enteric pathogenic bacteria (Marks *et al*., 1999; Marks *et al*., 2002b). These bacteria form part of the normal microflora in healthy animals. Seeing them in smears is not a criterion for discriminating harmful species from harmless commensals (Armstrong, 2013a). No study has correlated the presence or number of spores of *C. perfringens* with signs of disease. However, a correlation was observed between *C. perfringens* enterotoxin and diarrhoea in dogs (Marks *et al*., 2002b).

Published reports on assay of bacterial 16S rDNA using "culture-independent analysis" indicated a change in microflora to Gram-negative bacteria from Gram-positive *Firmicutes*, chiefly *Enterobacteriaceae* in humans and animals with intestinal inflammatory disorders (Janeczko *et al.*, 2008). Increment in *Enterobacteriaceae* numbers correlated with IBD and intestinal mucosal inflammation in cats (Xenoulis *et al*., 2008), GI inflammatory process associated with *E. coli* infection in humans (Baumgart *et al*., 2007) and granulomatous/histiocytic ulcerative colitis in dogs (Simpson *et al*., 2006). These reports did not state explicitly or expressly whether the alterations in microbiomes were the source or aftermath of the inflammations.

The duodenal juice bacterial counts technique for quantifying the bacterial loads in the diagnosis of SIBO is considered as the gold standard (Rutgers *et al*., 1995). Bacterial counts above 1.1×10^9 CFU/mL and 2.7×10^9 CFU/mL in dog and cat respectively, as well as, imbalance in the populations of a specific bacterial species is diagnostic for bacterial overgrowth (Johnston, 1999). The bacterial population in chronic diseases can be presently analysed using cutting-edge molecular methods – fluorescence *in-situ* hybridisation (FISH) and pyrosequencing (Suchodolski *et al*., 2010). Duodenal juice culture may be only valuable when targeting a specific pathogen. In patients with chronic GI diseases, it is considered a very low-yield diagnostic test (Dossin, 2011).

2.2.4.3 Faecal examination for ova and parasites

Faecal examination for intestinal parasites is vital in small animal practice, as they are often associated with chronic debilitating GI disorders in young companion animals (Batchelor *et al*., 2008). Many faecal panel tests are available for diagnosing GI parasites. Faecal floatation is required in the early diagnosis or screening of gastroenteritis against helminths and protozoans, such as *Coccidia* and *Giardia*. A combination of different faecal panel tests is ideal for confirming a true negative result. The presence of parasites on faecal examination does not constitute substantial evidence to incriminate the parasites as the cause of the disease, but the animal can be treated pending an invasive and/or an in-depth assay (Dossin, 2011).

Animals with suspected self-limiting diarrhoea are tested for GI parasites. Centrifugation faecal floatation using zinc sulphate ($ZnSO_4$) is preferred. For the assay of faecal *Giardia* cysts and *Cryptosporidium* oocytes, an indirect fluorescent antibody test can be used. Centrifugation faecal floatation is superior to gravitational floatation (Dryden *et al*., 2006). The floatation solution type and its specific gravity influence the diagnostic yield. Sheather's sugar and sodium nitrate are alternative faecal floatation solutions in lieu of $ZnSO_4$ that give a promising result. A solution of $ZnSO_4$ of low specific gravity, usually 1.2 to 1.3 is preferred. Oher floatation fluids are $MgSO_4$ (Epsom salt), sugar, and sodium nitrate. Fresh faecal specimens that cannot be immediately examined by faecal floatation may be refrigerated to facilitate the preservation of eggs, oocytes, and cysts (Cave *et al*., 2002; Broussard, 2003).

Other useful faecal assay techniques for intestinal parasites are ELISA and IFA test used for *Giardia* and *Cryptosporidium* screening before initiating anthelmintic treatment. The

assay of toxins and faecal culture, toxin assays and PCR are recommended only for animals showing diarrhoea with evidence of sepsis or diarrhoea outbreaks with zoonotic concerns (*Campylobacter*, *Salmonella*), and the possibility of immunocompromised persons having access to the diarrhoeic pet (Carlin *et al*., 2006; Scorza *et al*., 2012).

2.2.4.4 Screening for suspected fungi

Gastroenteritis suspected to be brought about by fungi are screened utilizing rectal scrapping, fine-needle aspiration of stomach related masses and urinalysis for histoplasmosis antigen (http://www. miravistalabs.com).

2.2.4.5 Haematology and biochemical assay

Evaluation of the complete blood count (CBC) of patients with diarrhoea may help rule out many diseases. The presence of peripheral eosinophilia can occur as secondary to intestinal parasites, eosinophilic inflammatory bowel disease (IBD), Addison's diseases, abdominal mast-cell neoplasia, or lymphoma. Severe inflammation, intestinal blood loss, and depression of erythropoiesis are often associated with anaemia (Marks, 2015). Also, additional information relating to the likely cause of vomiting or diarrhoea can be obtained from plasma biochemistry panel tests and can help rule out extra-GI causes. Protein-losing enteropathies, characterised by marked hypoproteinaemia are detectable by biochemical testing. Similarly, hypocholesterolaemia can result from mal-absorption. A discordant blood urea nitrogen-creatinine ratio is a consequence of pre-renal azotaemia, dehydration, haemorrhage, consumption of meal high in protein and emaciation (Marks, 2015). Drainage of bacteria and endotoxins through portal circulation may precipitate reactive hepatopathy characterised by elevated liver enzymes. Hence, increased in liver enzymes in dogs with enteric diseases and pancreatitis should be cautiously interpreted (Marks, 2015).

2.2.4.6 Test of intestinal function and assimilation

The general intestinal health is evaluated by measuring folate and vitamin B_{12} levels of the patient. A decreased vitamin B_{12} levels indicates a problem with absorption in the ileum, while elevated folate levels are indicative of a shift in the intestinal flora to a less healthy population. Measuring plasma cobalamin and folate concentrations can provide a significant insight into the functional integrity of the ileum and jejunum. Low plasma cobalamin regularly occurs in dogs in many diseases of the GI, such as lymphoma, lymphangiectasia and IBD (Batchelor *et al*., 2007). Cobalamin aids in amino acids and

lipids breakdown as well as in the synthesis of DNA. Deficiency in cobalamin influences highly mitotic cells of the bone marrow and enterocytes. The mucosal repair is impeded when cobalamin is deficient and its absorptive capacity is impaired (Simpson *et al*., 2001). In dogs, hypocobalaminaemia prevalence within the range of 6% to 73% has been described in many chronic enteric disorders (Kathrani *et al*., 2009; Craven *et al*., 2004). This has been linked to hypoalbuminaemia and poor prognosis in canines having chronic enteric disorders (Allenspach *et al*., 2007). Its deficiency was described in breeds, such as the Australian Shepherds, Beagles, Border Collies, German Shepherds, Giant Schnauzers, Shar Pei's, Staffordshire Bull Terriers, etc. (Grutzner *et al*., 2010; Dandrieux *et al*., 2010).

2.2.4.7 Abdominal imaging

Survey radiographic imaging is recommended in suspected cases of abdominal masses, a dilated loop of bowel, ileocolic intussusceptions, foreign bodies, bloating or stomach displacement or GI torsions. Abdominal ultrasonography is usually used to complement abdominal radiography (Penninck *et al*., 1990). In most cases of chronic diarrhoea, the application of abdominal radiography is of little value (Marks, 2015).

2.2.4.8 Dietary trials

Symptomatic therapy with diet manipulation can be employed as a diagnostic approach if dietary indiscretion is suspected. In cases of diarrhoea as a result of dietary indiscretions, clinical signs resolve a few days (3–5 days) when the implicating substance is eliminated or the animal is fed a highly digestible diet or both. If coprological examination reveals the presence of parasites, diarrhoea usually improves two to three days with appropriate treatment. Where no assertion has been made for the aetiology, making a tentative diagnosis of acute idiopathic self-limiting diarrhoea is reasonable (Armstrong, 2013a). Symptomatic treatment improves clinical signs within one to three days. If there is no improvement and clinical signs degenerate or others manifest, it may be indicative of a more serious problem. This may warrant a holistic assessment and rigorous treatment (Armstrong, 2013a). According to Marks (2015), elimination of diet or hydrolysed diet should be tried in patients with a dietary history. The dietary trial helps rule out FRE before obtaining intestinal biopsies in stable animals. Nonetheless, if there is no response to an elimination diet trial in animals with colitis,

feeding of a high-fibre diet can be tried. Ideally, improvement should be observed within seven to ten days of initiating the trial diet (Marks, 2015).

2.2.5 Management of diarrhoea in dogs

Most diarrhoea in dogs resolves rapidly by itself (i.e. self-limiting), typically in less than a week when appropriate supportive therapy is instituted. The prognosis rests on how severe the diarrhoea is aetiology and response to treatment. Ideally, most uncomplicated diarrhoea resolves within two days, with or without therapy. Dogs with uncomplicated diarrhoea will recover fully, while dogs with chronic diarrhoea may require dietary management or medication to keep the condition under control. Dogs with nonspecific diarrhoea can benefit from one or a combination of different therapies such as dietary modification, diarrhoea control, deworming, probiotics, antiemetics, gastroprotectants, antimicrobials or correction of fluid and electrolyte changes (Armstrong, 2013a; Marks, 2015).

2.2.5.1 Fluid and electrolyte replacement and maintenance

Presentation of dogs with gastroenteritis is common in canine practice. Haemorrhagic gastroenteritis poses a threat to life as it causes serious depletion of body fluid, electrolyte, blood, and the general circulatory volume. This increases the mortality rate and sepsis complication in addition to the underlying disorder. Fluid supplementation is the cornerstone therapy in canine gastroenteritis. Fluid administration corrects hypovolaemic, dehydration, acid-base and electrolytes imbalances or anomalies (Brown and Otto, 2008). Fluid deficits due to dehydration are calculated as percentage dehydration estimated multiplied by body weight (kg). Maintenance fluids of 44–66 mL/kg/day and estimated continued fluid losses from diarrhoea are then added to the deficit (Armstrong, 2013a). In managing GI crises in small animals, crystalloids and colloid-crystalloids given intravenously produces good results (Sen *et al*., 2014).

Fluid containing balanced electrolytes, such as Ringer's lactate solution is administered first. The route and rate at which it is administered vary with the state of the dog. Mildly dehydrated dogs can be given fluids subcutaneously, usually a balanced polyionic fluid isotonic to the circulatory volume. However, severely dehydrated patients with lethal acute diarrhoea required fluid intravenously (Mensack, 2008). Dogs with hypovolaemic shock are administered fluid at the rate of 90 mL/kg per hour taking into consideration the physiological outcome or effect of the therapy. Dehydrated animals that are not in

shock are administered fluid at a rate not exceeding 90 mL/kg within six to 24 hours (Prittie, 2004). The maintenance fluid is added in accordance with the plasma concentration of electrolytes and acid-base status of the patient, preferably a balanced isotonic electrolyte (Prittie, 2004).

In puppies with severe gastroenteritis, hypokalaemia, cardiac arrhythmias, myasthenia, paralysis, abnormal peristalsis, and polyuria are common occurrences. Hence, these are preventable by adding fluid containing potassium chloride; usually administered at a rate less than or equal to 0.5 mEq/kg/hr to avoid the adverse effect of this electrolyte on the normal cardiac function. It is essential to add 2.5% to 5.0% dextrose to manage hypoglycaemia (Prittie, 2004).

Patients with haemorrhagic enteritis accompanied with vomiting are hypovolaemic, hypoproteinaemic and hypoalbuminaemic, leading to a reduction in colloid osmotic pressure of the intravascular fluid (Brown and Otto, 2008; Boag, 2013). Administration of crystalloids alone to manage dehydration increases the odds of bacterial translocation, pooling of fluid into the microvasculature of the digestive tract, lowering cardiovascular pressure, as well as, pulmonary and cerebral oedema (Chan *et al*., 2001; Mazzaferro *et al*., 2002; Mazzaferro and Powell, 2013). Patients with hypovolaemia and hypoproteinaemia less than 45 g/L are usually given colloidal infusions (Brown and Otto, 2008). Hypoalbuminaemia less than 20 g/L is associated with poor prognosis (Boag, 2013). The colloidal solution aids vascular colloid osmotic pressure especially in patients with diseases, such as CPV, SIRS, sepsis, among others, as they have the tendency of increasing microvascular permeability (Chan *et al*., 2001; Mensack, 2008; Boag, 2013). It is useful in low volume resuscitation protocols (Mensack, 2008), diseases (e.g. viral enteritis) with a tendency to cause hypercoagulability and prolong coagulation time (Otto *et al*., 2000; Brown and Otto, 2008).

Co-administration of colloid and isotonic crystalloids balances up the therapies (Mensack, 2008; Mazzaferro and Powell, 2013) and reduce crystalloids volume by 25.0% to 60.0%, to compensate for the fluid deficit (Mazzaferro, 2008; Linklater, 2016). Colloidal infusion in modest quantities typically 10–20 mL/kg/day limits the opportunity of negatively affecting blood clotting and kidney function (DiBartola, 2011; Mazzaferro and Powell, 2013; Rudloff and Kirby, 2013).

2.2.5.2 Dietary modification

Enteral feeding has been used for managing GI disturbances but there are controversies about its benefit in canine gastroenteritis. Few studies reported that enteral feeding of dogs with severe GI diseases may improve clinical signs and recovery rate, but the overall benefit should be cautiously interpreted (Will *et al*., 2005). Not feeding the patients for six to 12 hours, and subsequently *ad libitum* feeding of a highly digestible or bland diet, usually three to six meals per day, may improve the clinical signs. Diets low or modest in fats (e.g. low-fat cottage cheese, boiled rice, and chicken, etc.) are preferred. As the diarrhoea resolves, the animal is reintroduced gradually overtime to its usual food. Cases with concurrent vomiting are administered an antiemetic. A diet high in fibre instead of a bland diet reduces tenesmus and aids in the repair of the epithelia of the colon in dogs with signs of acute large bowel diarrhoea (Armstrong, 2013a).

Dogs with FRE (45% to 60%) improve very quickly when fed elimination diets containing novel, single-protein sources. A good percentage of patients showed relatively rapid improvement within a few days, following diet modification. Protein hydrolysate diets reduce exposure of the mucosal immune mechanism to immunogenic epitopes during dysregulation thereby increasing the chances of recovery (Marks *et al*., 2002a; Mandigers *et al*., 2010). Clinical signs resolved with improvement in tissue repair in dogs diagnosed with refractory IBD fed protein hydrolysate diet (Marks *et al*., 2002a). This is superior to the long-term feeding of highly digestible control diet for patients having chronic small bowel enteropathy (Mandigers *et al*., 2010). Nonetheless, other nutritional factors may as well play vital roles in clinical signs improvement or recovery. Dogs with acute vomiting and systemic infection are treated by not feeding them for a day. The relative merits of withholding food, early feeding, and the most suitable characteristics of diets for dogs with acute vomiting are yet to be published in veterinary medicine. Thus, an extremely digestible diet is reasonably suitable (Elwood *et al*., 2010).

2.2.5.3 Anthelmintic therapy

Gastrointestinal parasites are often involved in many cases of canine gastroenteritis. A proof of GI parasites involvement warrants the administration of an appropriate anti-parasiticide. Parasites involvement are likely in dogs and cats with acute diarrhoea, even with a negative test as some parasites intermittently shed ova and the diagnostic tests are not 100% sensitive (Armstrong, 2013a). Such cases are treated with broad spectrum

anthelminthics. The use of fenbendazole has extended and excellent coverage against *Giardiosis* and is safer than metronidazole. Fenbendazole is dosed at 7.5 mg/kg IV BID then repeated in three months to eliminate *Trichuriasis* (Armstrong, 2013a). Fenbendazole is very safe with no teratogenic effect reported for it hence it can be used in cats and pregnant animals (Tams, 2014). Administering 50 mg/kg of fenbendazole orally for 5-7 days will eliminate *Giardia*. Drontal Plus® (Bayer Animal Health), a fixed-dose formulation/combination of febantel, praziquantel and pyrantel pamoate is also efficacious in treating *Giardiosis* in small companion animals and is administered once daily not exceeding five days in dogs.

Metronidazole has antibacterial and anti-inflammatory properties, which makes it an excellent drug of choice for treating diarrhoea without assertions of the possible causes (Tams, 2014). To prevent adverse reactions, a maximum dose not exceeding 65 mg/kg per day is recommended. A lower dose (10–20 mg/kg Q12H) is recommended for treating IBD and intestinal bacterial overgrowth (Tams, 2014). In addition to chemotherapy in treating diarrhoea due to *Giardia,* sanitation in the kernel using a quaternary ammonium compound and bathing of the patients to clear *Giardia* cyst from the environment, as well as, on the furs decreases chances of reinfection (Tams, 2014).

To achieve a holistic treatment of other endoparasites, the administration of any good broad-spectrum anthelmintic, singly or in combination is a good strategy. Fenbendazole has a wider activity against roundworms, lungworms and some tapeworms. Pyrantel pamoate is another broad-spectrum anthelminthic that is highly effective against roundworms, such as *Toxocara spp, Uncinaria stenocephala* and hookworm – *Ancylostoma caninum*. Pyrantel acts against non-mature and adult forms of GI helminths but does not act against the migrating evolutional stages in the tissues. Praziquantel is effective against liver flukes and tapeworms.

A combination therapy comprising of fenbendazole and pyrantel has a synergistic antiparasitic effect. Pyrantel in monotherapy achieves maximum efficiency of 75%, while fenbendazole achieves only 45%. But their combination achieves a synergistic effect which is greater than 90%. Therefore, a combination therapy comprising fenbendazole, pyrantel and praziquantel will eliminate roundworms, hookworms, whipworms, and tapeworms respectively (www.biovetapets.cz).

2.2.5.4 Antimotility and antidiarrheal agents

Antidiarrheals are indicated in some cases, such as where diarrhoea is frequent and causes discomfort and loss of an enormous amount of body fluid. Opioids such as a combination of diphenoxylate and atropine sulphate (Lomotil®) or loperamide (Imodium®) may be given to alter intestinal motility and inhibit excessive GI propulsion. They are more effective in reducing the frequency of diarrhoea. The advantage of using these drugs is that they are more potent, act very fast and for an extended duration, as well as, have wider margins of safety with less adverse effects. (Plumb, 2008). Opioids are however contraindicated in suspected toxins or acute diarrhoea of enteric pathogenic bacteria origin because they increase toxin absorption and retention time as well as encourages bacterial proliferation (Plumb, 2008). Opioids act by prolonging GI transit time, increasing fluid absorption, segmentation of the colon and anal tone, as well as, by decreasing diarrhoea frequency, peristalsis, and GI secretions. Opioids are most useful in colitis or large bowel diarrhoea (Armstrong, 2013a). Collies and related breeds may be overly sensitive to loperamide (Plumb, 2008).

a). Loperamide is administered at 0.08 – 0.2 mg/kg PO, Q6–12H or TID for 5 days.

b). Diphenoxylate may be administered a rate of –

i. 0.1 mg/kg, PO, TID in cases with acute colitis and irritable colon syndrome.
ii. 0.05 mg/kg, PO, TID not exceeding 5 days.
iii. 0.05–0.2 mg/kg, PO, Q8–12H.

In chronic or serious cases of gastroenteritis such as CPE, opioids should be used with caution. They delay GI motility, encourage microbial spread and invasion of the mucosa, as well as, increase toxin absorption from the GI tract following inhibition of intestinal contents flow and diarrhoeal elimination (Prittie, 2004).

2.2.5.5 Antibacterial therapy

Treatment of diarrhoea in dogs associated with bacteria appears to be most effective with antibiotics like erythromycin, metronidazole, ampicillin and tylosin. However, antibiotics are cautiously used in dogs having acute uncomplicated diarrhoea. Metronidazole (10–15 mg/kg orally Q12H) and tylosin (10–15 mg/kg orally Q12–24H) are the appropriate antibiotics for nonspecific use (Armstrong, 2013a). Prescription of bactericidal antibiotics with a wider activity may be necessary for dogs with

haemorrhagic diarrhoea, where the intestinal mucosal barrier is deranged; there is translocation of bacteria to other organs, sepsis, endotoxaemia and marked neutropenia. Antibiotics are restricted for use in confirmed cases of bacterial involvement. It is not easy ruling out bacterial involvement and translocation of bacterial is a potentially lethal complication, however, the use of antibiotics should be based on a sound judgement (Armstrong, 2013a).

Antibiotics therapy are beneficial but may worsen endotoxaemia and SIRS (Stockwell *et al*., 1994; Otto and Drobatz, 1997), as well as, cause *C. perfringens* overpopulation and subsequently haemorrhagic diarrhoea (Tsukada *et al*., 1993). Dogs with mild gastroenteritis and normal leucocytes counts are treated with single or without antibiotics. The parenteral route is preferred over oral administration of antibiotics as vomiting, altered microflora and delayed gastric emptying can cause mal-absorption of the oral medications (Armstrong, 2013a; Tams, 2014; Marks, 2015).

A combination of beta-lactamase antibiotics, e.g. ampicillin (22 mg/kg, IV, TID) and an aminoglycoside, (e.g. gentamicin; 6 mg/kg, IV, SID) or Baytril (5 mg/kg, IV, SID) have wider activities against bacteria translocating from the GI tract (Prittie, 2004). Metronidazole (7.5 mg/kg, IV, BID), enrofloxacin (5–10 mg/kg/day, IV), and amoxicillin-clavulanate (12.5–22 mg/kg, orally Q12H) are also excellent for diarrhoea treatment (Kelly, 2016). Administration of metronidazole at a lower dosage, usually 10–20 mg/kg Q12H is adequate to treat SIBO and IBD in dogs (Tams, 2014). Aminoglycosides cause nephrotoxicity hence are contraindicated in dehydrated patients except when well-hydrated. Enrofloxacin is contraindicated in growing dogs due to its ability to destroy growing cartilage (Prittie, 2004).

2.2.5.6 Vitamin B_{12} and folate supplementation

Monitoring and supplementation of Vitamin B_{12} (Cobalamin) and folate are of importance in managing dogs with digestive disorders. Cobalamin deficiency causes ill-thrift and poor appetite. Being high normal to mildly elevated levels of vitamin B_{12} may be beneficial to GI health. Folate deficiency is uncommon in dogs and is not usually supplemented but can be measured to ascertain if there is a need for addition. Hypocobalaminaemia less than 0.2 mg/L in digestive disorders is indicative of a poor prognosis (Allenspach, 2007). Cobalamin may be given at 0.02 mg/kg weekly over four weeks, following monthly or 0.25 to 1.5 mg/kg every week for six weeks and

subsequently at two or three weeks apart for an indefinite period (Marks, 2015). A combination of diets containing only one source of protein e.g. rice and mutton, with cobalamin supplementation provide excellent result in chronic or relapsing enteropathy and hypocobalaminaemia in cat and dog (Craven *et al*., 2011; Toresson *et al*., 2018).

2.2.5.7 Probiotics supplementation

Probiotics are dead or live cells of harmless bacteria which benefit the health of the host when used appropriately in the correct quantity (Suchodolski and Whittemore, 2016). Probiotic organisms include *Lactobacillus*, *Bifidobacter*, *Bacillus*, *E. coli*, *Streptococcus*, *Saccharomyces* and *Enterococcus faecium* species. Their health benefits are direct inhibition of pathogenic microorganism colonisation. They also enhance the immunity of the lymphoid tissues from the GIT. They increase cytokines immune-modulatory activities. Probiotics are used to correct dysbiosis-associated enteropathies in dogs and cats. Probiotics stimulate the gut microflora and immune response and protect the gut from inflammations. Live probiotics have a short lifespan and may disappear in faeces few days following cessation of administration (Armstrong, 2013a; Kyffin, 2014). Their application in canine and feline diarrhoea has not been evaluated on a large-scale. However, few studies and anecdotal evidence support their use. For instance, it was reported that acute diarrhoea in dogs resolved somewhat more rapidly when fed probiotics (Kelley *et al*., 2009; Herstad *et al*., 2010; Bybee *et al*., 2011). Hence, probiotics supplementation in cases of acute self-limiting diarrhoea is regarded as a balanced method for managing diarrhoea (Armstrong, 2013a). Probiotics are only used as an auxiliary treatment in enteropathies. Probiotics are short-lived in the gut hence are regularly fed to the patient. Some are either susceptible or resistant to antibiotics administered to the patient (Webb and Suchodolski, 2014). Variations exist in the type and number of organisms in different probiotic products. Many products do not contain the levels of bacteria or pathogenic bacteria claimed on the label. Hence, care should be exercised with the choice of probiotics as they are not yet a regulated drug (Webb, 2016).

2.2.5.8 Faecal biotherapy

Bacterial dysbiosis is a frequent occurrence dogs suffering from various enteropathies. The changes observed in microflora differ with disease state (acute versus chronic), while dogs with acute diarrhoea, particularly AHD have marked alterations in their gut microbiome (Minamoto *et al*., 2014). In veterinary clinical practice, faecal microbiota

transplantation (FMT) is used to treat recurrent diarrhoea in dogs. The idea here is to repopulate or effectively change the microbiome to create an environment that exists in a healthy GI tract. This method has yielded a high rate of recovery in dog patients (Murphy *et al*., 2014; Youngster *et al*., 2014), IBD in humans and *C. difficile* infection (Kelly *et al*., 2015). Faecal biotherapy minimises costs for common GI recurrent problems associated with bacterial infections in dogs, and it is very easy to obtain and administer faeces in FMT. The technique involves administration of fresh faecal infusion from a healthy donor, laden with healthy populations of microflora to restore the disorderly microbial populations in the gut of a patient with enteropathy. Before the faecal transplant, fresh stools obtained from healthy dogs are screened for parasites and pathogenic bacteria, liquefied, and administered either as an enema or via rectal intubation or orally into the patient's GIT (Chaitman *et al*., 2016). Once administered in the ileum and colon, the patient is treated with loperamide to slow intestinal transit. Improvement in clinical signs is observed a few hours to days after transplant.

2.2.5.9 Immunotherapy

The use of immunosuppressive or immunomodulatory drugs is somewhat helpful in managing chronic cases of canine diarrhoea. Glucocorticoids are used most frequently with prednisone or prednisolone as drug of choice. The recommended dosage is 2 mg/kg Q24H to be given two to four weeks. Dexamethasone can also be used (0.25 mg/kg Q24H starting dose) owing to its fewer side-effects and/or reductions in refractory cases (Whitley and Day, 2011).

The immunosuppression from glucocorticoids could be insufficient in some immune-mediated cases of diarrhoea. Azathioprine, budesonide, cyclosporine, chlorambucil and mycophenolate may be added as an additional drug. The addition of any of these drugs allows increased immunosuppression and faster tapering of the glucocorticosteroid (Whitley and Day, 2011). Budesonide administered at 0.5–3.0 mg/animal is highly effective. It is a nonhalogenated corticosteroid with local action, high hepatic clearance, and low systemic activity. It is useful in cases that are very sensitive to prednisone or contraindications such as diabetes mellitus (Whitley and Day, 2011). Cyclosporine is the preferred second drug for managing canine and feline enteropathies. It induces rapid immunosuppression by reducing IL–2 productions from T-cells, thereby reducing the proliferation of T-cells and consequently B-cells. Its dosage is 5 mg/kg orally for Q12–

24H. Its adverse events associated with overdose are alopecia, inappetence, diarrhoea, vomiting, gingival hyperplasia, oral papillomatous lesions, idiosyncratic hepatopathy, as well as, exposure to opportunistic infectious diseases (Whitley and Day, 2011). In dogs with steroid-refractory IBD, treatment with cyclosporine achieved a response rate of (12/14) 85.7% (Allenspach *et al*., 2006). Azathioprine is a thiopurine which interferes with purine synthesis. It has a slower immunosuppressive activity compared to the other drugs. It is given 1–2 mg/kg *per os* Q24H to dogs. It has side-effects of inducing inappetence, vomiting, diarrhoea, pancreatitis; it is hepatotoxic and causes bone marrow degeneration. It is contraindicated in cats and pancreatitis in dogs (Whitley and Day, 2011). Mycophenolate is developed as an alternative for and has the same action as azathioprine, by blocking purine synthesis. Therefore, the two drugs are not administered concurrently. It acts faster and has a lower toxicity than azathioprine. Gastrointestinal adverse drug events may occur in some dogs (Whitley and Day, 2011). Chlorambucil is an excellent drug recommend for cats. It is administered at 2 mg PO Q48H (large cats) or 2 mg PO on alternate days (Monday/Wednesday/Friday for small cats) or 20 mg/m^2 every 14 days. Its side-effects may include inappetence, diarrhoea, vomiting and bone marrow suppression (Whitley and Day, 2011).

2.3 Vomiting in dogs

Persistent vomiting causes aspiration pneumonia, imbalances in electrolytes and metabolic acid-base, oesophagitis, and dehydration. Bradycardia may occur following vasovagal reflex stimulation (Rozanski and Rush, 2007; Tams, 2014). Forceful ejection of the upper GI and occasionally the duodenal contents via the mouth which occurs in a sequence of spontaneous spasmodic movements is termed vomiting (Rozanski and Rush, 2007; Armstrong, 2013b; Tams, 2014). After diarrhoea, vomiting constitutes the most treated GI disorder in small companion animals. Vomiting like diarrhoea is caused by several diseases affecting several organs in the body. In the US, vomiting accounted for 4.0% of the complaints presenting to veterinarians (Lund *et al*., 1999).

2.3.1 Causes of vomiting in dogs

Just like diarrhoea, vomiting has multiple causes which may be simple or complex conditions, making it very difficult to precisely diagnose and treat prudently. Vomiting can be prompted by both GI and extra-GI diseases (Rozanski and Rush, 2007; Elwood *et al*., 2010; Tams, 2014). A diagnostic registry service in the United States has listed

more than 400 likely aetiologies of vomiting in dogs, out of which many can be diagnosed successfully and managed judiciously (Tams, 2014). Some diagnostic tests have demonstrated their usefulness in vomiting dogs with diagnosis confirmed in 95.3% of 213 dog patients evaluated. Gastrointestinal, systemic, nongastrointestinal abdominal, neurological, and miscellaneous diseases were the main or among the main causes of vomiting in the dogs. Renal diseases were the commonest nongastrointestinal abdominal causes of vomiting in the dogs (Rosé and Neiger, 2013).

2.3.1.1 Gastrointestinal causes of vomiting

a) Obstructions due to foreign bodies, mesenteric torsion, neoplasia, volvulus, intussusception, and constipation.
b) Diseases caused by viruses (CPV, CDV, CCoV, rotavirus).
c) Diseases caused by bacteria (Salmonellosis, Campylobacteriosis, mycobacterial infection).
d) Parasites (*Ascarids*, *Coccidia*, *Giardia*, *Ollulanus tricuspis*, *Trichuris*, *Physaloptera*, salmon poisoning).
e) Gastroduodenal ulcerations, IBD, GI perforations, HGE.

2.3.1.2 Systemic nongastrointestinal causes of vomiting

a) Systemic disease e.g., acidosis and electrolyte disturbances, hepatic failure, renal failure, and sepsis.
b) Endocrinopathies, such as nonketotic hyperosmolar diabetes, diabetic ketoacidosis, hypoadrenocorticism.
c) Nervous disorders such as CNS trauma. encephalitis, meningitis, vestibular syndrome
d) Toxins and drugs e.g., NSAIDs, cardiac glycosides, chemotherapeutic agents, antibiotics.
e) Abdominal diseases e.g. peritonitis, pancreatitis, pyelonephritis, pyometra
f) Anaphylaxis.
g) Heatstroke, motion sickness, dietary indiscretion.

2.3.2 Pathophysiology of vomiting

Vomiting impulse ensues following the incitement of emetic centre situated at the medulla oblongata by either humoral or neural factors. Humoral factors disturb the emetic centre indirectly by activating *nucleus tractus solitarius* (NTS) and CRTZ

(chemoreceptor trigger zone), the most important relay area for afferent impulses arising in the GI tract, throat, and other viscera (Rozanski and Rush, 2007; Mercks, 2010; Armstrong, 2013b). The CRTZ is situated in the postrema territory, dorsally to the medulla oblongata and caudal to the fourth ventricle. Blood-brain barrier partially protects the CTRZ. Therefore, it is stimulated by some toxins, drugs, mediators, and hormones, etc., originating from either blood or cerebrospinal fluid. Neural stimulation occurs via pathways emanating from afferent vagosympathetic receptors located in many peripheral structures such as vestibular apparatus, cerebrocortical and limbic system (Rozanski and Rush, 2007; Armstrong, 2013b). Most vomiting in small animals, predominantly those occurring as a result of primary GI diseases, activate the neural pathways (Armstrong, 2013b). Chemoreceptors, osmoreceptors and stretch receptors, associated with the pathways are sited over all the body - GIT, visceral organs, pharynx, and peritoneum (Rozanski and Rush, 2007; Tripathi, 2008, Mercks, 2010). Duodenum hosts the largest number of these receptors. Factors such as abdominal distention, inflammatory processes, alterations in osmolality and irritations from chemicals easily stimulate the visceral receptors (Armstrong, 2013b).

2.3.3 Clinical features and consequences of vomiting

Vomiting is associated with nausea, reduced appetite, body fluid and electrolytes loss, resulting in dehydration, hypokalaemia, hypovolaemic shock, and a discordant metabolic acid-base and electrolyte balance, in addition to metabolic alkalosis or acidosis. The combined effects of these changes constitute a grave threat to the life of the patient (Boag *et al*., 2005). Some vomiting patients may develop aspiration pneumonia (Kogan *et al*., 2008). Protein-calorie malnutrition and emaciation are likely in severe vomiting that hinders dietary intake (Elwood *et al*., 2010).

2.3.4 Diagnostic approach to vomiting dogs

Vomiting like diarrhoea does not constitute a disease. It is a consequence of aetiological agents. Vomiting in dogs may be either acute or chronic. Acute vomiting that is mild and self-limiting need no intensive diagnosis but is managed symptomatically (Elwood, 2010). Due to multiple aetiological agents associated with vomiting in dogs, especially in chronic cases, accurate diagnosis and judicious management can be problematic for some veterinarians (Tam, 2014). Sometimes the diagnosis is not reached even with more extensive diagnostic evaluation, except the possible cause, such as dietary indiscretions

or errors is revealed by the history (Armstrong, 2013b). There is no one-test-fit-all appropriate for diagnosing vomiting in dogs or determining the course of treatment in all cases. A good history backed up with a thorough physical examination can provide substantial information on systemic causes or consequences of the problem (Merck, 2010). A good history and physical examination will differentiate dysphagia or regurgitation from vomiting, as well as give information on the duration and nature of vomitus. Palpating the abdominal may aid diagnosis or guide the clinician to suspect conditions such as tumours, intussusception, gastric dilatation volvulus, and foreign body (Rozanski and Rush, 2007).

Other essential diagnostic workups necessary for the initial evaluation of vomiting patients are CBC, biochemical profile, urinalysis, and faecal examination (Merck, 2010; Tams, 2014). Abdominal radiographs are essential for all patients with signs of systemic or metabolic complications, for example, abdominal cramps, dehydration, pyrexia, haematemesis, vomiting, or emaciation (Merck, 2010; Tams, 2014; Lee and Cohn, 2015). These diagnostic tests are the gold standard for vomiting dogs. They can aid in the diagnosis of both metabolic and electrolytes alterations as well as guide in understanding the changes associated with the primary causes (Rozanski and Rush, 2007; Lee and Cohn, 2015). Abnormalities detected by the tests can guide in initiating treatment or give direction for further diagnoses than identify the underlying causes that would require immediate intervention (Merck, 2010).

In-depth diagnosis is determined by the initial laboratory findings, how the patient is responding to treatment, and persisting clinical signs. Further diagnostic evaluation may include screening the patients against endoparasites (by faecal floatation, smear, wet mount, and *Giardia* antigen test), blood clotting profile, faecal occult blood, an assay of serum lead, ethylene glycol assay, and endoscopic examination (Tams, 2014). For dogs with protein-losing enteropathies, diagnostic tests such as an assay of α-antiprotease levels PCR, electron microscopic examination may be required to describe the diseases involved (Elwood *et al*., 2010; Armstrong, 2013b; Tams, 2014; Lee and Cohn, 2015). Parvovirus faecal antigen test is recommended for puppies or kittens regardless of their vaccination history (Armstrong, 2013b).

Furthermore, other useful diagnostics comprise resting or baseline cortisol or stimulation of ACTH to confirm hypoadrenocorticism where discordant Na/K ratio is observed.

Patients suspected of pancreatitis can benefit from pancreatic lipase immunoreactivity (PLI) assay. Assay of plasma bile acids is required in some important suspected liver diseases. Gastroesophageal reflux disease and hiatal herniation disorders may necessitate endoscopic examination and barium contrast radiography, respectively. If gastrinoma (Zollinger-Ellison Syndrome) is suspected, plasma gastrin levels assay will be appropriate (Tams, 2014). In addition, abdominal ultrasound is a useful tool for diagnosing chronic vomiting associated with GI lymphoma and gastric adenocarcinoma (Leib *et al*., 2010). Similarly, the usefulness of blood testing, faecal analysis, faecal cytology, urinalysis, ultrasound, and radiography in enabling or assisting diagnosis in dogs presenting with gastroenteritis has been described (Rosé and Neiger, 2013). This underscores the usefulness of these tests in the clinical assessment of dogs with gastroenteritis. Nonetheless, the tests should be economically and judiciously used as the need arises in order to minimise incurring unnecessary costs for the client.

2.3.5 Clinical management of vomiting dogs

Vomiting may be accompanied by nausea and anorexia and sometimes aspiration pneumonia (Kogan *et al*., 2008). Vomiting that is continuous such that it interferes with effective dietary intake predisposes to protein-calorie malnutrition (Elwood *et al*., 2010). Hence, supportive care targeted at treating or removing the aetiology, abdominal cramps, and control of vomiting, as well as correcting fluid, electrolytes and metabolic acid-base disturbances associated with frequent vomiting is warranted (Armstrong2013b). The supportive treatment alleviates suffering and avoids complications. Each patient's condition and the risk-benefit analysis will inform the choice for treating with drugs or to "wait and see" if there will be an improvement. In about 90.0% of dogs with vomiting, clinical signs will resolve within two days after onset (Hubbard *et al*., 2007).

Not all cases of vomiting warrant treatment. Dogs that ingest toxins can benefit from vomiting. Those with GI obstructions or presence of prokinetic effects need no antiemetic treatment. Antiemetics may mask pathognomic signs. Hence, the risk-benefit of with an antiemetic is assessed to prevent the likelihood of drug-induced adverse events (Hubbard *et al*., 2007). Though supportive therapy or treating the symptom often improves the patient's demeanour and comfort, it should not be taken as a replacement for making a correct diagnosis (Armstrong 2013b).

2.3.5.1 Fluid replacement therapy

Vomiting of GI contents usually results in a net loss of water and ions (e.g. Cl^-, K^+, Na^+, HCO_3^{2-}). Dehydration causes hypochloraemia, hypokalaemia, and hyponatraemia. Mildly dehydrated patients can be given isotonic fluids subcutaneously, between 10–20 mL/kg at each injection site. Balanced fluids (e.g. Normosol-R and PlasmaLyte) are given only intravenously, subcutaneous administration causes discomfort to the patient. Intravenous fluids are administered to animals that are moderately or severely dehydrated, usually 7.0% and above (Armstrong, 2013b). Depletion of total body potassium concentration following vomiting may cause GI hypomotility hence the necessity to supplement the loss. Similarly, metabolic acidosis is associated with an acid-base imbalance in canine GI disorders. Intravenous infusion of normal saline (0.9%) or lactated Ringer's solution will correct this imbalance (Armstrong, 2013b). Obstructions of the stomach or proximal duodenum by foreign objects are associated with either metabolic alkalosis or metabolic acidosis or normal acid-base status; hence laboratory evaluation is mandatory to specify the metabolic anomaly present without presumption (Boag *et al*., 2005).

2.3.5.2 Dietary management

Withholding food, usually 12 hours to 24 hours, in dogs with acute vomiting until the cessation of vomiting is beneficial in preventing aspiration pneumonia, extra loss of body fluid and to increase comfort (Armstrong, 2013b). Thereafter, a highly digestible single-protein diet or protein hydrolysate diet is fed in smaller quantities to the patient. *Ad libitum* feeding reduces stomach distention and secretion of gastric acid. Dogs benefit greatest when fed a highly digestible diet containing low fats. Diets with high fats to some extent aid gastric emptying in cats unlike in dogs. Diets which contain a single source of a protein deprived of carbohydrate, for example, cooked breast muscle of chicken. Once the clinical signs improve, the patient is reintroduced progressively to its regular food for a period of two to three days (Armstrong, 2013b).

2.3.5.3 Antiemetic therapy

Antiemetics are necessary for severe, discomforting, persistent vomiting that increases the likelihood of aspiration pneumonia, loss of body fluids, electrolytes, and acid-base balance. They are also warranted in cases that are not associated with obstructions of the GI or poison (Armstrong 2013b). The ideal antiemetic drug used should prevent both

peripheral and central stimulation of the CTRZ and should have a wider therapeutic index. Patients with persistent and/or severe vomiting are often dehydrated and have electrolytes and acid-base imbalance. Hence, an ideal antiemetic also should not have any side-effects on the heart or circulatory system to avoid disturbing the haemodynamic equilibrium in patients that are dehydrated. Again, the drugs should have no direct effects on GI motility (Elwood *et al*., 2010). There is no "one-fits-all" antiemetic drug of choice. The decision to use any drug should be guided by the observed clinical findings and condition of each individual patient. Antiemetics are usually administered concurrently with a prokinetic agent. Patients with refractory vomiting will benefit from combining antiemetics with different modes of action (Armstrong, 2013b). Several recommended antiemetics are used for controlling vomiting in the cat and dog.

2.3.5.3.1 Phenothiazines

Phenothiazines (0.01–0.05 mg/kg IM, SC or 1–3 mg/kg PO of acepromazine; 0.13mg/kg IM Q6–8H of prochlorperazine; and 0.5mg/kg IV, IM, SC Q6–8H of chlorpromazine; and antihistamines (2–4mg/kg PO Q8H of diphenhydramine) can give a desired result in some cases, however these drugs cause somnolence. Chlorpromazine and prochlorperazine are indicated only in normotensive and well-rehydrated patients. Phenothiazines are widely used in the management of seizures despite initial belief that they induce seizure threshold (Armstrong, 2013b). Acepromazine is beneficial in preventing vomiting in dogs (Valverde *et al*., 2004).

2.3.5.3.2 Domperidone

This antiemetic is a D_2 dopaminergic antagonist which increases the gastroesophageal sphincter tone. Its clinical usefulness in small animals remains elusive. It is administered to dogs at 0.05–0.1 mg/kg PO Q12–24H dose rate (Armstrong, 2013b).

2.3.5.3.3 Prokinetic drugs

These drugs are referred to as 5-HT4 serotonergic agonists and include

i. **Metoclopramide:** The dosage of administration is 0.2–0.5 mg/kg given IM, SC, PO, Q6–8H or 1–2 mg/kg infused IV slowly and continuously for Q24H. Metoclopramide (Reglan®) is reasonably a good central antiemetic drug for dogs than cats. It causes a reduction in GI reflux and inhibits CRTZ. It also causes an increment in GI motility as well as emptying inducing no acid secretion. It is

contraindicated in patients with intestinal obstruction, GI perforation, seizure disorder and diabetes (Rozanski and Rush, 2007; Tams, 2014).

ii. **Cisapride:** It is a GI prokinetic drug that is more potent than metoclopramide in managing gastric emptying disorders in small companion animals. Its stimulation of gastric motility occurs via 5-HT4 serotonergic receptors stimulation with a slight direct antiemetic effect. Cisapride is excellently efficacious in the management of colonic inertia and small intestinal ileus, gastroparesis, postoperative ileus, gastroesophageal reflux disease, idiopathic constipation, and where metoclopramide is not sufficiently effective (Tams, 2014).

iii. **Erythromycin** is not on the list of antiemetics but has a prokinetic effect that also stimulates the phase III migrating myoelectric complex activity in dogs. It is contraindicated in cats (Armstrong, 2013b).

2.3.5.3.4 Serotonin antagonists

Serotonin antagonists used in small animals are;

i. **Ondansetron** is initially administered orally at 0.5–1 mg/kg (Q12–24H) or intravenously at 0.5 mg/kg and subsequently infused at 0.5 mg/kg/hr for six hours.

ii. **Dolasetron:** Its recommended dosage is 0.6mg/kg IV or PO Q24H. The serotonin (5-HT3 receptor) antagonist antiemetic drugs produce a very good result. It remarkably decreases vomiting frequency in patients with persistent or severe vomiting as seen in CPV infection, pancreatitis and hepatic lipidosis in cats and dogs. They act either at the CRTZ or have some peripheral gastric prokinetic activity or both. They are very effective but expensive (Tams 2014).

iii. **Maropitant** citrate is a neurokinin-1 (NK-1) receptor antagonist. The drug is highly efficacious in controlling nausea and vomiting in dogs under field condition. For the control of vomiting, maropitant is dosed at 1mg/kg SC Q24H. It is administered at 8mg/kg orally Q24H for at most 48 hours to prevent motion sickness. It is contraindicated in GI obstruction, ingestion of poison, lactating and pregnant bitches, as well as dogs younger than 16 weeks old. This drug is considered a more excellent drug of choice than metoclopramide (Benchaoui *et al*., 2007; Vail, 2007). This drug has visceral analgesic effects besides its antiemetic activities (Niyom *et al*., 2013; Boscan *et al*., 2011). It is efficient in managing drug-induced nausea and vomiting, prevention of motion sickness, management of vomiting associated with renal diseases among others (Armstrong, 2013b; Tams, 2014).

2.3.5.4 Gastroprotective agents

Irritants and inflammation of the gastric mucosa facilitate peripheral pathways of vomiting. Gastroprotective or cytoprotective drugs (e.g. antacids and PPIs) and drugs acting locally on the gastric mucosa (e.g. sucralfate) give protection and relief from gastric irritations and inflammations. Histamine H_2–receptor antagonists used in the management of gastric ulceration and severe gastritis in cats and dogs are cimetidine, ranitidine, famotidine and nizatidine. Famotidine and nizatidine are more effective and superior to cimetidine and ranitidine for they have no inhibitory effects on the hepatic CYP-450 haemoprotein enzyme that is responsible for drug metabolism. They inhibit acetylcholinesterase activity thereby stimulating gastric emptying (Armstrong, 2013b). Of the gastric acid secretion inhibitors available, proton pump inhibitors (PPIs) are more potent. Omeprazole (0.7 mg/kg PO Q24H), sucralfate (0.25–1.0 g PO Q8–12H) and pantoprazole (0.7–1.0 mg/kg PO or IV PO Q24H) are PPIs commonly used in canines and felines to minimise effect of gastrointestinal compromise (Armstrong, 2013b).

2.3.5.5 Antibiotic therapy

Antibiotics are not essentially empirical therapy for managing cases of acute vomiting except there is fever or abnormal CBC suggestive of systemic infection. In such cases, antibiotics with wider activities, for example, amoxicillin combined with enrofloxacin, gives very good and broader coverage from bacteria translocating a breached gastric mucosal barrier. Probiotics or antibiotics, such as metronidazole and tylosin are useful for controlling acute diarrhoea accompanying vomiting (Armstrong, 2013b).

2.4 Canine parvovirus enteritis

This is caused by CPV a highly pathogenic and transmissible viral agent responsible for most gastroenteritis in dogs. The enteric disease and myocarditis are the two forms of disorder caused by CPV. The intestinal or enteric form is more serious in puppies that lack antibodies to the virus. Dogs with enteric form are lethargic, anorexic, pyrexic in addition to vomiting and/or diarrhoea as the major clinical signs. The cardiac form occurs commonly in neonates infected in-utero. It is characterised by respiratory or cardiovascular failures. This cardiac form is rarely reported nowadays due to vaccination of bitches. Passive transfer of antibodies in-utero and in colostrum protects puppies for a few weeks after whelping (Schatzberg *et al*., 2003). The disease does not discriminate between ages, breeds, or sexes, with puppies zero to six months old being predominantly

at a higher risk (Crawford and Sellon, 2010). Predisposing factors for parvovirus infection are related to lack of protective immunity, pre-existing bacterial infections, intestinal parasites, overcrowding, poor hygiene, and environmental stress (Hoskins, 1997; Lamm and Rezabek, 2008). Some dogs, such as Alsatian, Doberman and Rottweiler breed are more susceptible to parvovirus enteritis than others (Houston *et al*., 1996). In America and South Africa, the infection is more prevalent during summer period compared to winter (Shakespeare, 1999; Houston *et al*., 1996) and in the dry season than in wet seasons in Nigeria (Shima *et al*., 2015b; Gberindyer, 2016).

2.4.1 Evolution and molecular structure of canine parvovirus

Canine parvovirus belongs to the Parvoviridae family, which are tiny, non-encompassing and single-stranded DNA viruses that duplicate in very mitotic cells (Crawford and Sellon, 2010). The racoon parvovirus (RaPV), mink enteritis virus (MEV), and feline parvovirus (FPV) together with CPV evolved from a common ancestor and are now grouped into an exclusive specie Carnivore Protoparvovirus 1 (Cotmore *et al*., 2014; 2019). The virus evolved from feline parvovirus around 1977 and is substituted through successive evolutional changes by subtypes CPV-2a, CPV-2b and CPV-2c. Fundamentally, CPV genome includes 5200 nucleotides and comprised of three basic proteins (VP1, VP2, and VP3) and two non-structural proteins (NS1 and NS2) at the 3' and 5' end, separately (Agbandje *et al*., 1995). The VP2 constitute 90 % of the viral structural proteins; determines the host-range specificity, antigenicity, and interaction between the virus and its host. During the maturation process, cleavage of the VP2 protein by the host proteases give rise to VP3 (Decaro and Buonavoglia, 2012). The rate of detection of VP2 mRNA by *in-situ* hybridisation (ISH) signal was higher and most abundant gene compared to VP1 in dogs with CPV infection (Ford *et al*., 2017). Mutations occurring within the VP2 gene account for the CPV haplotypes (Truyen *et al*., 1995; Minakshi *et al*., 2016; Geetha, 2015). Plate 2.1 shows the evolution and an X-ray crystallography structure of CPV.

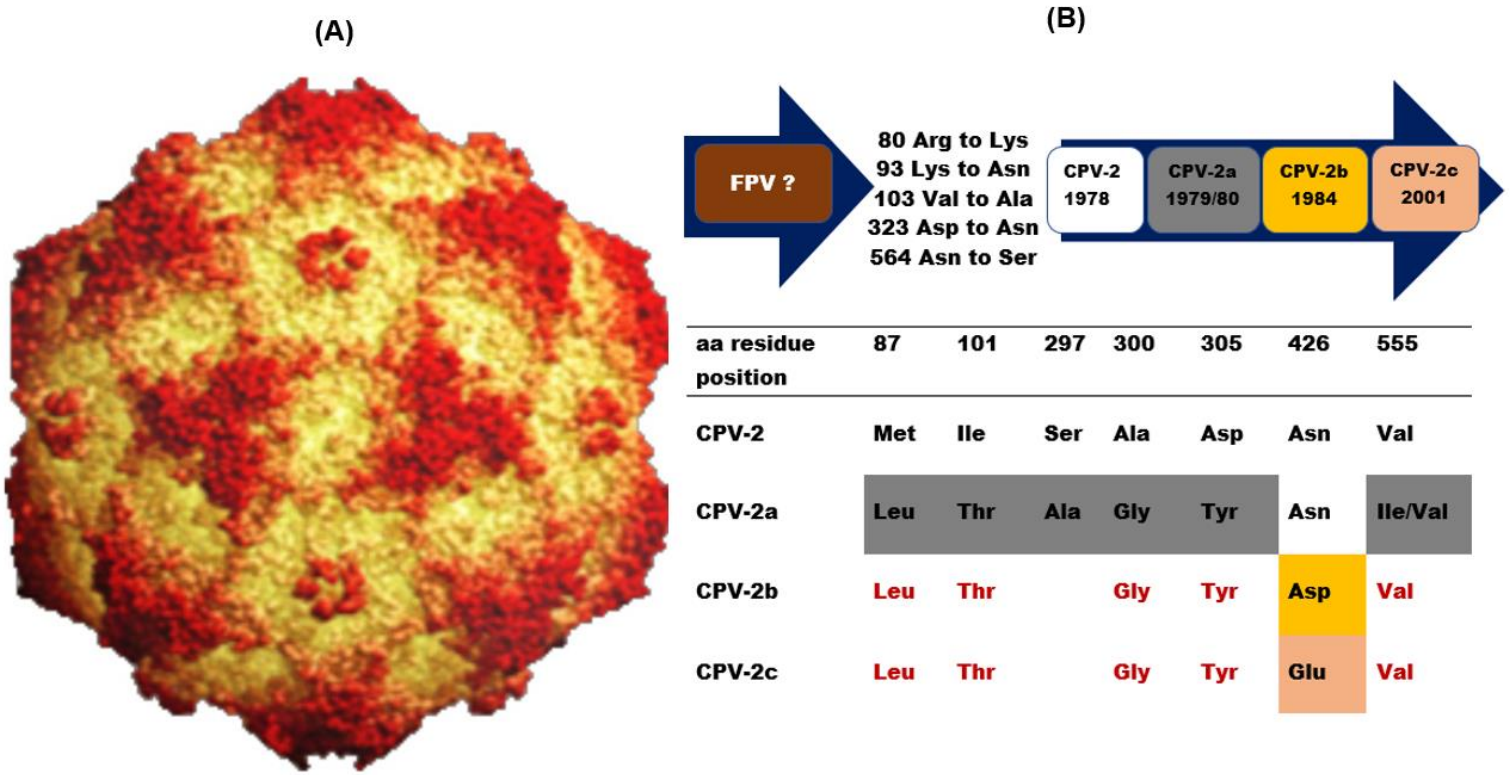

Plate 2.1: X-ray crystallographic molecular structure (A) and schematic illustration of evolution and VP2 protein sites influencing antigenicity among canine parvovirus subtypes (B)

Extracted from https://web.stanford.edu/group/virus/parvo/2000/cat-dog_parvovirus.html dated June 15, 2019. Ohneiser (2013)

2.4.2 Parvovirus replication

Parvovirus DNA replication and assemblage take place in the host cell's nucleus, making use of its cellular proteins. The host cell undergoes S phase for replication to be successful (Berns and Parrish, 2007). The whole replication process is called the Rolling-Hairpin mechanism. It involves the formation of a hairpin at the 3' end, which serves as a primer for replication (Cotmore and Tattersall, 1994). The non-structural (NS) NS1 is the initial gene product formed. The NS1 protein forms a covalent bond with the 5' end of the genome through nicking and helicase activities of NS1. The accumulation of NS1 instigates the cessation of host cell replication (Cotmore and Tattersall, 1987). The viral capsids are assembled in the nucleus where cytoplasmic translocation of the translated VP proteins takes place (Yuan and Parrish, 2001). The NS protein supports the integration of DNA into the preassembled capsids (Cotmore and Tattersall, 1994).

Following cell death and lysis, chromosome region maintenance 1 (Crm1) – a nuclear export factor interacts with NS2 triggering the release progeny and nuclear egress from the assembled capsids (Cotmore and Tattersall, 1987; Nuesch and Rommelaere, 2006). Furthermore, actin cytoskeleton modification occurred following the interaction of minute virus of mice (MVM) NS1 protein with casein kinase IIα, an enzyme that modifies gelsolin activity. The modified actin supports the transfer of MVM containing vesicles (Bär *et al*., 2008). The viral particles assembled are then engulfed by the coat protein complex II vesicles on the perinuclear area. The viral particles then travel via the endoplasmic reticulum (ER) and Golgi. During the egress, phosphorylation modifies the viral capsids increasing the infectivity of the virus. Transfer of MVM via the ER and Golgi regulates cytolysis, stimulating effectively the release of progeny (Bär *et al*., 2013). The MVM then leaves Golgi to the plasma membrane through LAMP-2 positive vesicles (Bär *et al*., 2013). The acidic milieu of the vesicles modifies the capsids on entry (Suikkanen *et al*., 2003), as well as viral maturation (Nuesch and Rommelaere, 2006; Bär *et al*., 2013). The process of parvovirus replication is presented in Figure 2.1.

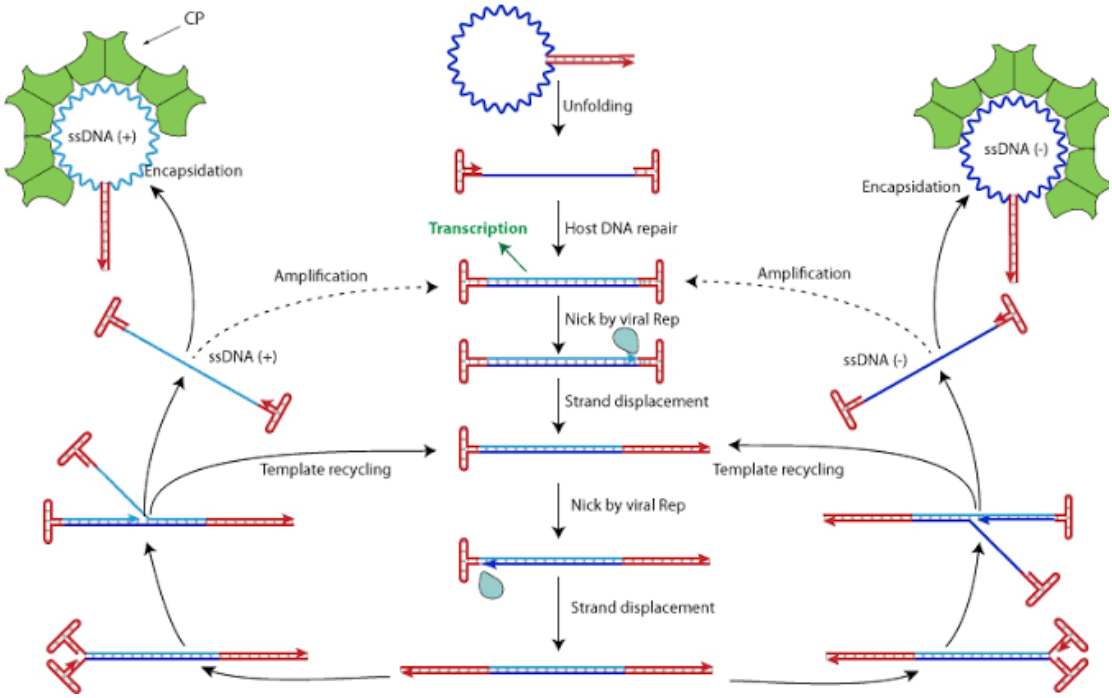

Figure 2.1: Rolling-Hairpin replication process by parvoviruses

Culled from http://viralzone.expasy.org/2656 dated June 15, 2019.

2.4.3 Transmission and pathogenesis of canine parvovirus

It can be transmitted via direct and indirect means. The virus spreads rapidly among dogs through faecal-oral routes via direct exposure to infectious faeces. It spread indirectly by fomites, environment contaminated with infective faeces, as well as, insect and rodent vectors (Crawford and Sellon, 2010; Kaur *et al*., 2015). Recovered dogs do not shield the virus in their faeces or transmit the disease. Recent knowledge showed that about 96.0% of puppies with CPE are exposed to viral infection and seroconversion process begins between day 2 and 42 following birth (Mila *et al*., 2014). Once the virus gets access to the host, its initial replication occurs in lymphoproliferative tissues from where it spreads to other highly mitotic cells of the intestinal crypts, myeloid and lymphoid tissues as well as the thymus (Carr-Smith *et al*., 1997; Dudley and Johnny, 2006). Viraemia is detected within five days following infection. The virus causes massive collapse and derangement of the intestinal crypts and epithelial villi integrity culminating in the loss of absorptive capacity (Black *et al*., 1979; Otto *et al*., 1997). A breach in the intestinal epithelial integrity and the presence of suppressed immunity gives room for bacteria to translocate culminating to bacteraemia or sepsis. CPV infects the precursors of myeloid and lymphoid cells leading to neutropaenia and lymphopaenia (Prittie, 2004; Dudley and Johnny, 2006; Crawford and Sellon, 2010). Faecal shedding of the viral antigens starts as from three days and cease 8–12 days post-infection. The peak of viral shedding corresponds to the time when the first clinical signs appear (Dudley and Johnny, 2006; Barr, 2009). Typically, clinical signs [such as severe diarrhoea, vomiting, dehydration, lethargy, hypoglycaemia, leukopaenia, myocarditis in neonates and high mortality] are apparent 5–7 days post infection (Streck *et al*., 2009).

Canine parvovirus affects the heart, kidneys, liver, lungs, and spleen meaning it is a viral infection with a highly systemic affinity (Appel *et al*., 1980; Hoskins, 1997). Infection at the time of myocardial cells proliferation causes myocarditis in neonates. Nonetheless, with widespread vaccination and MDA protection, the myocarditis form rarely occurs (Pollock and Coyne, 1993). Recent reports indicate that myocardial damage characterised by inflammation, necrosis and fibrosis can occur in young dogs under two years old (Ford *et al*., 2017). Because veterinarians believe vaccination has suppressed the widespread of CPV infection, the authors hypothesised that myocarditis, heart damage and myocardial fibrosis are common in young dogs but are often under-recognised in clinical practice. Some survivors of acute parvovirus infection may

develop heart failure secondary to lymphocytic myocarditis and fibrosis later in life (Meunier *et al*., 1984; Sykes, 2014; Jones, 2018).

2.4.4 Epidemiology of canine parvoviruses

The three biotypes of CPV occur worldwide with variations in regional distribution. Subtype–2c appears to be circulating at a relatively slow rate. The current 2a and 2b parvovirus isolates in circulation were first detected around 1980. The first detection of CPV–2c was in 2000 from Italian dogs and by 2007 it was detected in some regions around the globe including the USA. The biotypes have increased antigenic variations, virulence, and a very short incubation period (CPV–2a > CPV–2b > CPV–2c) different from the wildtype CPV. They are also reported to infect cats. The CPV–2a is predominantly found in France, Italy, New Zealand, and Taiwan (Chang *et al*., 1992; Martella *et al*., 2004; Ohneiser, 2013). The CPV–2b subtype is most common in USA, Switzerland, Japan, Germany, Brazil, and Austria (Parrish *et al*., 1988; Hirasawa *et al*., 1996; Pereira *et al*., 2000; Truyen, 2000). CPV–2a and CPV–2b subtypes are distributed in equal ratios in Spain (De Ybanez *et al*., 1995), India (Panda *et al*., 2009; Nandi *et al*., 2013) and UK (Greenwood *et al*., 1996) whereas, CPV–2c predominates the Vietnam, Europe, Spain, and USA isolates (Kapil *et al*., 2007; Decaro *et al*., 2006; Nakamura *et al*., 2004). In Africa, all the three subtypes are circulating in Morocco and Tunisia (Amrani *et al*., 2016; Touihri *et al*., 2009), while in South Africa, subtypes 2a and –2b are more common (Steinel, 1998; Dogonyaro *et al*., 2013). In Nigeria, all three haplotypes have been reported with the CPV–2a being the most widely detected strain in clinical samples (Apaa *et al*., 2016; Dogonyaro *et al*., 2013). The rate of detection of haplotypes 2b and 2c from clinic samples in Nigeria in the previous years was very low compared to haplotype 2a (Fagbohun and Omobowale, 2018).

2.4.5 Risk factors for canine parvovirus infection

Several factors predispose dogs to parvovirus disease, namely, absence and inadequate protective immunity, intercurrent GI parasitism, enteric infections, such as *Campylobacteriosis*, C*olibacillosis*, *Salmonellosis*, *Giardiosis* and Coronavirus, environmental stress, unhygienic environments, and overcrowding (Brunner and Swango 1985, Hoskins, 1997). The American Pitbull, Doberman pinscher, German shepherds, Labrador retriever and Rottweiler Terrier are breeds exceedingly susceptible (Houston *et al*., 1996). Inappropriate vaccination protocols, the period of the year and

geographical location are also key factors for CPV transmission in domesticated canine populations (Houston *et al*., 1996; Shakespeare, 1999; Shima *et al*., 2015b).

2.4.6 Diagnosis of canine parvovirus infection

Diagnosis is often based on a complete medical history, such as age, exposure to sick dogs, previous vaccinations or natural infection, acute onset of anorexia, vomiting, diarrhoea, and depression in puppies with questionable vaccination status – unvaccinated or improperly vaccinated (Pollock and Coyne, 1993). Definitive diagnosis is by detecting CPV antigens in the stool of the suspected dog, serology, virus isolation, PCR, necropsy, and histopathology (Costa *et al*., 2005). From 2–4 days post-infection, viral antigens are detectable in the stools by in-clinic immunoassay ELISAs. The kits are less sensitivity, and binding of antibodies by the bloody stools gives false-negative test (Barr, 2009). The PCR, *ISH* and immunohistochemistry are beneficial in the identification of parvovirus myocarditis (Ford *et al*., 2017; Jones, 2018).

2.4.7 Treatment of canine parvovirus enteritis

Supportive therapy is the most significant aspect of managing dogs with gastroenteritis. This involves administration of fluid intravenously to rehydration, correct electrolytes, and acid-base imbalance. Antibiotics are given to take care of secondary bacterial infections. Glucose and colloids are supplemented to correct hypoproteinaemia. In addition, antiemetics and an anthelmintic are given to control vomiting and endoparasites, respectively (Prittie, 2004; Manitone and Otto, 2005; Dudley and Johnny, 2006; Crawford and Sellon, 2010). Ringer's lactate solution contains a balanced electrolyte and is a fluid of choice in dogs with CPE. The success of the treatment is influenced by how early and aggressive symptomatic supportive therapy is initiated.

Vomiting is related to intestinal crypts obliteration, disturbed GI motility, and endotoxin-actuated incitement of the cytokine cascade activating local irritations and central stimulation of the emetic centre and CRTZ (Mantione and Otto, 2005). Antiemetics commonly used to control vomiting in CPV infections have been discussed earlier under control of vomiting in dogs in this study. The antiemetics commonly used are prokinetic and serotonin antagonists which include, metoclopramide, cisapride, erythromycin, ondansetron, dolasetron, and maropitant. Combining ampicillin (or other β–lactamase) or β–lactamase resistant penicillin [e.g. Augmentin (amoxicillin-clavulanic acid) combination] with an aminoglycoside [e.g. Gentamicin] gives effective

cover from Gram-positive anaerobes and Gram-negative aerobes (Macintire and Smith-Carr, 1989). The survival rate in CPE varies from 9% to 91% without or with treatment (Kalli *et al*., 2010). Many dogs may still die even with appropriate management due to CPV-related complications (De Laforcade *et al*., 2003; Kocatürk *et al*., 2010). Blood chemistry reflects dehydration and electrolyte disturbances, particularly hypokalaemia and hypoglycaemia. The progression of leukopaenia is prognostic. The presence of band cells in the blood is an indication for markable improvement in the prognosis (Barr, 2009). Older dogs have improved prognosis and shorter duration of hospitalisation than puppies. Prognosis is poor in young untreated dogs but puppies that responded well to treatment within the first three days post-admission usually good to excellent prognosis. Essentially, the immunity level and degree of the illness determines the prognosis for an individual animal (Otto and Drobatz, 1997).

2.4.8 Canine parvovirus prevention, vaccines, and immunity

Phylogenetically, all the viruses share a common ancestral origin (Horiuchi *et al*., 1998). Vaccination is considered as an effective means of preventing CPV infection and spread (Prittie, 2004). The current CPV vaccines produce persistently high antibody titres that protect dogs for at least two years. Interference of MDA with the vaccines reduces their potencies resulting in vaccination failure (Prittie, 2004). The available vaccines provide cross-immunity to all the known CPV subtypes (Parrish *et al*., 1985). However, prophylactic intervention using vaccines containing feline panleukopaenia virus is ineffective (Sakulwira *et al*., 2003). The present-day CPV vaccines still contain the wildtype CPV (Nandi and Kumar, 2010). Some current vaccines incorporate the 2a and 2b, but none incorporate the 2c subtype. However, if used appropriately, the vaccines cross-protect from other variants with immunity lasting longer (Spibey *et al*., 2008). Despite cross-immunity between the various CPV subtypes vaccines, there are anecdotal and confirmed cases of CPE in fully vaccinated dogs presented with gastroenteritis (Shima *et al*., 2015b; Woolford *et al*., 2017; Apaa *et al*., 2016). This has left many dog owners and veterinarians with a myth and suspicion about effectiveness and cross-protection offered by CPV vaccines. In vaccinated canines, CPV–2c-related infections are less common compared to infection with CPV–2a and CPV–2b strains (Hong *et al*., 2007). Faecal shedding of vaccine-virus after vaccination is uncommon. Faecal samples collected within 10 days after vaccine administration in dogs that developed parvovirus-like clinical signs did not reveal vaccine-virus strains (Miranda and Thompson, 2016).

A dog that survived natural infection is protected for life (Dudley and Johnny, 2006; Prittie, 2004), with the recovered animals maintaining high antibody titres for up to 16 months or more (Pollock and Carmichael, 1982). Plasma antibody titres and resistance to infection are correlated (Dudley and Johnny, 2006; Prittie, 2004). Thus, vaccine timing takes into consideration the need to provide early protection while minimising maternal antibodies interference. A window of increased susceptibility to the virus infection is however unavoidable (Buonavoglia *et al*., 1992). This occurs mostly when MDA thwarts the development of an adequate immune response to parvovirus vaccines such that it is rendered useless. When the level of MDA is low, their interference may be overcome by vaccines which potentiate high antibody titres (Dudley and Johnny, 2006). A bitch can only transfer between 50% to 60% of its acquired antibodies to the neonates through colostral and milk ingestion, and about 10% via transplacental transfer (O'Brien, 1994; Decaro *et al*., 2004). This MDA can protect the puppies for only a few weeks after birth. Therefore, CPV is rarely seen in neonates (Pollock and Carmichael, 1982). The maternally-derived antibody titre is influenced by the concentration in the bitch at the time of whelping, the volume of ingested colostrum by a puppy and litter size. It takes 10 days for the concentration of parvovirus maternal antibodies to reduce to 50% (half-life) in puppies. As the maternally derived antibody declines puppies become more vulnerable to diseases (Pollock and Carmichael, 1982; O'Brien, 1994).

Due to the challenges faced with CPV and pathogenic diseases control globally, Day *et al*. (2016) proposed a guideline and recommendation for vaccination to assist in regional vaccination policy (Table 2.1) and for incorporation of serological antibody testing (Figure 2.2) in small animal practice to ensure puppies and kittens get the needed protection against core diseases.

Vaccines recommended for all dogs all over the world, to be administered at specified intervals, in order to give them long-lasting immunity from communicable diseases with global interest and significance are termed "core vaccines" (Day *et al*., 2016). These include canine distemper, adenovirus, and parvovirus vaccines. Some vaccines that are not recommended or are optional are termed "non-core" vaccines. An example includes vaccination against CCoV is not compulsory for all dogs around the globe. They are restricted to geographical usage and how the lifestyle of a dog exposes it to the disease/s and risk-benefit ratios appraisal. Some vaccines with little scientific justification for

their use are not recommended. The initial core vaccination is given in a series. The puppy primary vaccination series started at six and eight weeks, is repeated at two to four-weekly intervals until at least four months of age, following an annual booster in 6 months or at 12 months (Day *et al*., 2016). Vaccinating dogs at six weeks of age can prevent them from being infected by CPV-2c (Glover *et al*., 2012). The age or vaccination start time and vaccination interval chosen will determine the number of puppy primary vaccination series a dog would receive.

Parvovirus is hardy and stable even in adverse ecological conditions; surviving on fomites for a longer period (Hoskins, 1997; Prittie, 2004). The virus is inactivated within an hour following exposure to sodium hypochlorite but is resistant to many other available disinfectants and detergents (Hoskins, 1997). Good hygienic practices in the kennels and by practitioners will complement vaccination in curtailing CPV spread.

Despite the good intervention programmes and availability of efficacious and effective vaccines, CPV infection has not been suppressed in the canine population in Nigeria. Exploration of vaccination practices or vaccine usage patterns, sequencing of field and vaccine-strains could explain why parvovirus infection occur in some vaccinated dogs.

2.5 Polypharmacy and irrational drug prescriptions in clinical practice

Accurate diagnosis of any disease and its management are an integral part of patient care. Polypharmacy is the inappropriate usage of multiple drugs concurrently by an individual (Hunter and Isaza, 2017). In human medicine, it is the prescription of at least 4-6 drugs to a patient to be used concurrently (Payne and Avery, 2011; Payne, 2016).

Polypharmacy and irrational drug prescription may be inevitable in the clinical management of many critical and challenging cases such as gastroenteritis to alleviate associated clinical signs (Veehof *et al*., 2000). Appropriate supportive and symptomatic management can be rewarding as there is no one-fit-all treatment for gastroenteritis. Irrational uses of medications are common in clinical practices worldwide; with abuse of antimicrobials taking a lead (German *et al*., 2010; Gberindyer *et al*., 2017; Ekakoro and Okafor, 2019). Inappropriate use of drugs is a potential hazard to the overall well-being of the patients and is not cost-effective (Bjerrum *et al*., 2008; Kirsten *et al*., 2010). Unlike irrational drug prescription, rational drug prescription reduces drug failures, adverse drug events, treatment cost, and drug resistance (Pramil *et al*., 2012).

Table 2.1. Proposed vaccination schedules for dogs and cats

Age at first presentation	Vaccination schedule guide					
		Weeks				
6 wks.		6	9	12	16	then 26 or 52
	OR	6	10	14	18	then 26 or 52
7 wks.		7	10	13	16	then 26 or 52
	OR	7	11	15	19	then 26 or 52
8 wks.		8	11	14	17	then 26 or 52
	OR	8	12	16	-	then 26 or 52
9 wks.		9	12	15	18	then 26 or 52
	OR	9	13	17	-	then 26 or 52

Index. The vaccination guide can be modified based on the regional vaccination policy and endemicity of the diseases. Culled from Day *et al*. (2016).

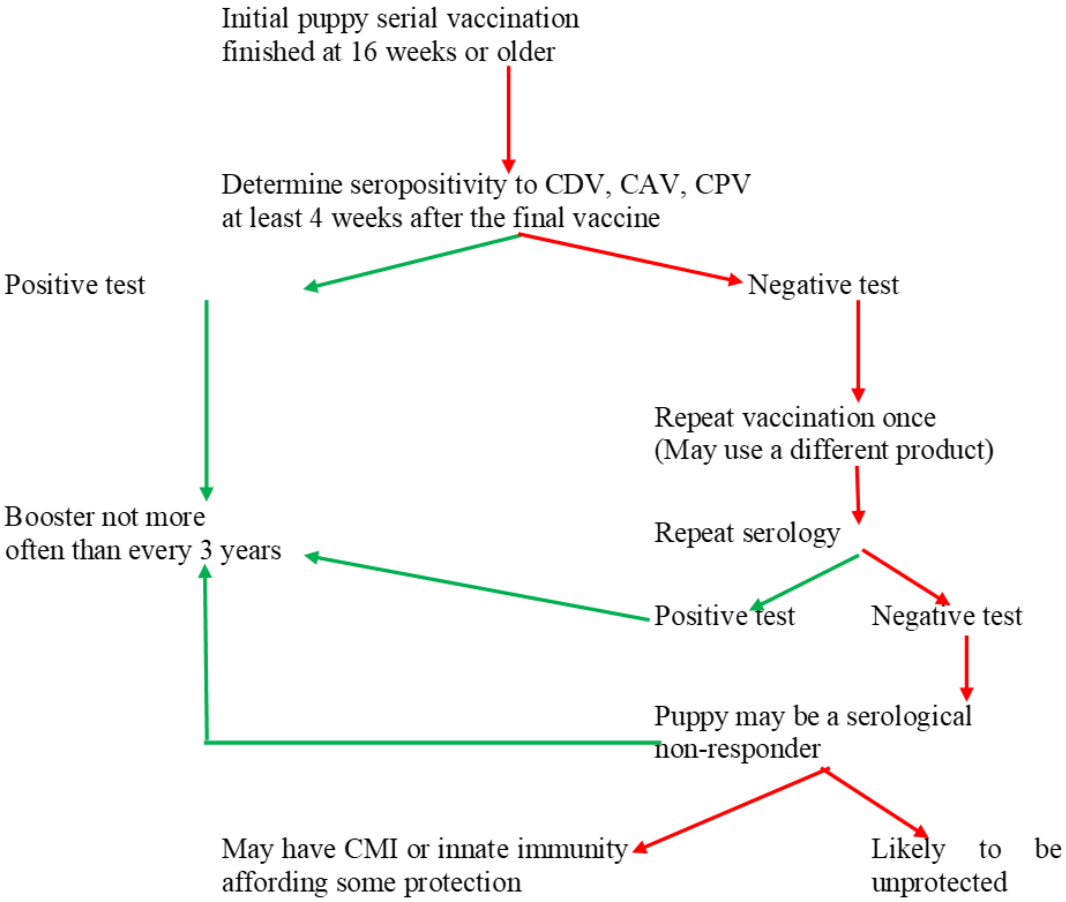

Figure 2.2: Flowchart for serological testing of puppies.

Culled from Day *et al*. (2016)

2.6 Aftermaths of gastrointestinal disorders following treatment

Gastrointestinal infections are associated with short- and long-term effects following treatment. The long-term effects are those occurring even after successful treatment. The aftermaths are triggered either by the disease itself or chemotherapeutic agents administered. For instance, survivors of acute parvovirus infection develop structural changes in their myocardial tissues even after successful treatment (Lenghaus and Studdert, 1984; Sime *et al*., 2015). The clinical implication of such structural changes in the myocardial tissue has not been elucidated. Survivors of the acute myocardial damage may die latest several weeks to months (Robinson *et al*., 1979). Severe destruction of the GI mucosa barrier heightens the chances of immunological diseases in later-life in dogs (Mohr *et al*., 2003). Exposing children too early to antibiotics aggravates the odds of allergies (Kummeling *et al*., 2007; Metsala *et al*., 2015). Humans with acute gastroenteritis are more likely to develop irritable bowel syndrome post-infection (Thabane *et al*., 2007; Thabane and Marshall, 2009; Schwille-Kiuntke *et al*., 2011; Klem *et al*., 2017).

Severe enteritis and early-life exposure to antibiotics predispose dogs to long-lasting GI diseases in their future life (Kilian *et al*., 2018). In osmotic diarrhoea, the damaged villi may remain shortened for life, predisposing a dog to chronic diarrhoea (Kilian *et al*., 2018). Survivors of acute canine gastroenteritis are prone to food-responsive diarrhoea caused by immunological responses – food allergies and/or non-immunological responses – food intolerances (Gaschen and Merchant, 2011; Verlinden *et al*., 2006). Breakdown in oral tolerance to the penetration of macromolecules may result in food hypersensitivity culminating in chronic diarrhoea (Chehade *et al*., 2005). Dog patients that survived GI infections may also develop auto-immunity. Approximately 10% of humans with acute self-limiting infectious gastroenteritis develops irritable bowel syndrome on account of autoantibodies produced in response to intestinal tissue antigen (Purdy *et al*., 2015).

Acute diarrhoea singly or in combination with antibiotics causes marked alteration of the intestinal microbiome of the patients, which can be short- or long-lasting (Jernberg *et al*., 2010; Guard *et al*., 2015). Their effects may culminate to a disproportion between intact normal intestinal microflora and the host's defence at the mucosal barrier (Elson *et al*., 2005; Guard *et al*., 2015). The antibiotics exposure at tender age following

alterations in the normal microbiome exacerbate the likelihood of developing allergies, asthma, obesity, IBD and atopy in later-life (Johnson *et al*., 2005; Ungaro *et al*., 2014; Metsala *et al*., 2015; Saari *et al*., 2015). The first six months is a critical period in life during which altered microbiota-induced immunological events promote allergic sensitisation in individuals (Russell *et al*., 2012; Arrieta *et al*., 2015). Humans that recovered from protozoa, parasites or bacteria enteritis often develop post-infectious irritable bowel syndrome persisting for several years (Zanini *et al*., 2012; Youn *et al*., 2012; Klem *et al*., 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Preamble

Based on the study objectives, this research is grouped into six parts: Objective 1 was to retrospectively quantify the prevalence of gastroenteritis in dogs presented for consultation in selected veterinary practices in Nigeria. Objective 2 was to retrospectively assess the drug prescription patterns for canine gastroenteritis patients using treatment records of dogs attending some veterinary clinics in Nigeria. Objective 3 was to conduct a prospective study that focused on clinicopathologic responses, predictors of duration of management and prognosis in canine gastroenteritis using clinical cases. The fourth objective was to carried out clinical assessment of cardiac functions in dogs diagnosed with an enteric form of CPV enteritis. The fifth objective was to evaluate the vaccination protocols of vaccinated dogs that were diagnosed with CPV disease. The sixth objective was to molecularly characterisation CPV isolates from vaccines licensed for vaccinating dogs in Nigeria and diarrhoeic samples. To achieve the objectives, different standard methods or approaches were used.

3.2 Ethical considerations

The procedures used in this research have received ethical clearance (Appendix 2) from the University of Ibadan, Animal Care and Use for Research Ethics Committee, with approval number UI-ACUREC/App/03/2017/007. In addition, consent of dog owners was obtained before their dogs were recruited into the study. Approval was also received from the Heads of the various clinics chosen as data collection centres.

3.3 Study locations

This research was undertaken in 7 veterinary clinics and 3 Veterinary Teaching Hospitals in the Federal Capital Territory Abuja, Ogun (Abeokuta), Oyo (Ibadan), Plateau (Jos), Benue (Makurdi), Anambra (Onitsha) and Delta (Warri) states (Figure 3.1). The selection of the study locations/veterinary practices was purposively based on consent granted and volume of small animal practice compared to other cities in Nigeria.

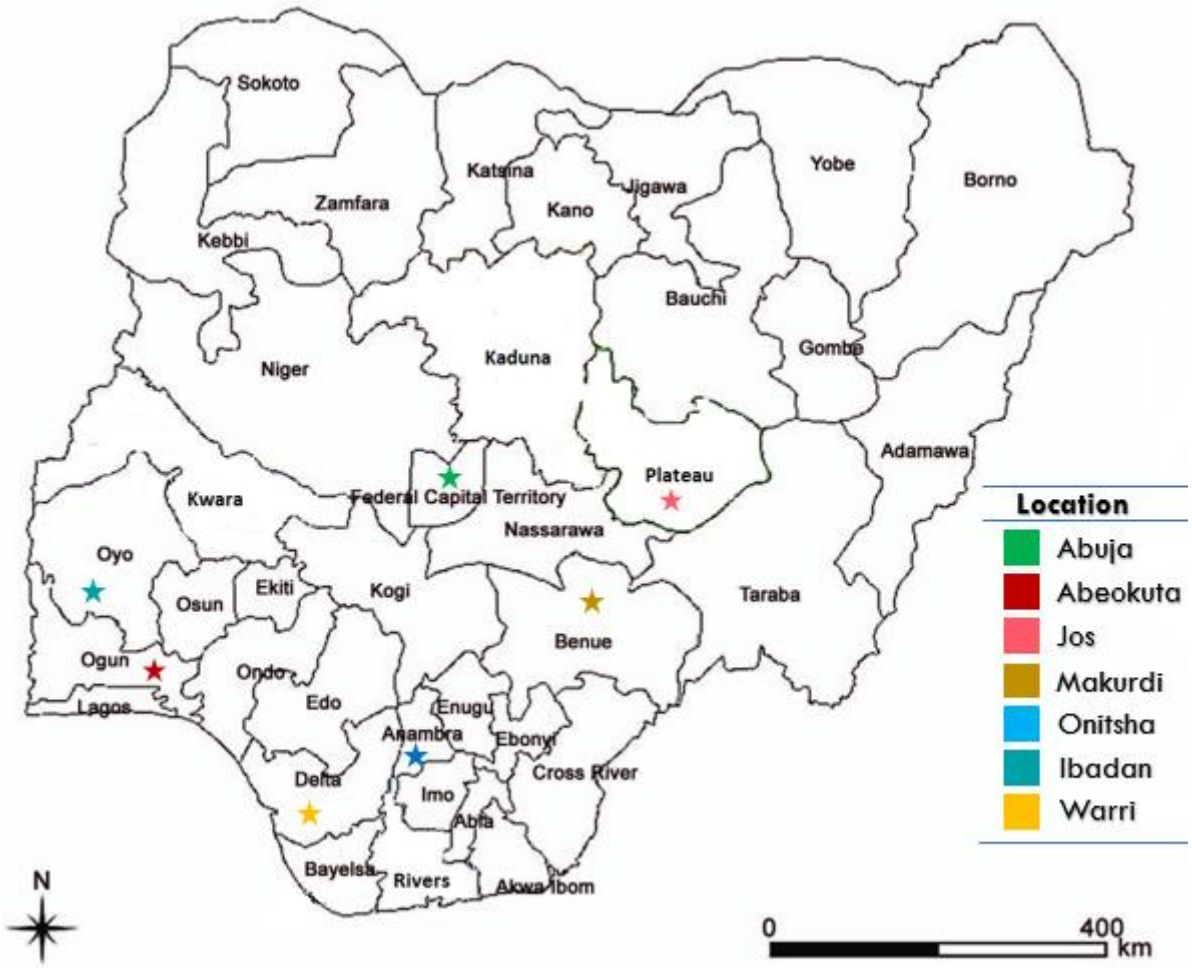

Figure 3.1: Map of Nigeria showing the study areas

Objective 1

3.4 Prevalence of gastroenteritis in dogs attending some veterinary practices in Nigeria

3.4.1 Study locations

Case records were retrieved, reviewed and data extracted from a total of ten veterinary practices from Abeokuta = 1; Ibadan = 2; Jos = 1; Makurdi = 2, Warri = 2, Abuja = 1, and Onitsha = 1 in Nigeria. The veterinary practices consisted of four private veterinary clinics, two state-owned veterinary clinics, and three Veterinary Teaching Hospitals. Selection of study locations was purposive and based on self-judgement or researcher's experience.

3.4.2 Study design

A one-year retrospective study was conducted on client-owned-dogs attending some selected veterinary practices in Nigeria from January to December 2016. The case records of patients presented to the selected veterinary practices were carefully reviewed with a keen interest in GI disorders, such as vomiting and/or diarrhoea.

3.4.3 Patient data

The parameters recorded for the enrolled patients include:

1. Signalment (age, sex, breed, body weight, and vaccination history).
2. Clinical signs of vomiting and/or diarrhoea and diagnosis.

3.4.4 Sample size

Computation of sample size was achieved with the G-Power® statistical programme (version 3.1.1, Germany); at a cut-off of sample effect size (w) = 0.3; α error probability = 0.05; degree of freedom (df) = 5; sample power (1 – ß error probability) = 0.95; critical Chi-squared ($\chi 2$) = 11.07, and a noncentrality parameter (λ) = 19.80. About 220 cases were reviewed per participating veterinary practice. Therefore, 3882 dogs were sampled.

3.4.5 Collation and analysis of data

Data obtained were organised in Microsoft Excel® 2016 and IBM SPSS Statistics version 20 (IBM Corporation, Chicago, USA). Comparison of the prevalence used bivariate statistics with Bonferroni adjusted p-value for multiple effects testing. The tests were performed as two-tails with the significant level set at $\alpha_{0.05}$. Descriptive statistics were used to report patients' characteristics and gastroenteritis prevalence.

Objective 2

3.5 Drug prescription patterns for dogs diagnosed with gastroenteritis in some veterinary practices in Nigeria

3.5.1 Study design, sample size and data collection

Treatment data of 537 [whose treatment records were available] out of 919 dogs diagnosed with gastroenteritis in the retrospective study in section 3.4 of this Chapter were used for this analysis.

Details of therapies administered were recorded for each dog patient; and assigned to one of the following classes: antihaemorrhagics, antibacterials, antiparasitics, antiemetics, steroids; antisialagogues, analgesics, and gastric protectants.

3.5.2 Data analysis

The degree of polypharmacy and drug prescription patterns for 537 dogs with canine gastroenteritis was measured using descriptive and bivariate statistics (German *et al*., 2010; Shea *et al*., 2011; Tamuno and Fadare, 2012). Briefly, the prescribing indicators measured were:

1. A fraction (%) of patients managed by using a specific drug class. This was determined as the sum of patients recommended/prescribed a class of drug divided by the aggregate of gastroenteritis patients.
2. The level of polypharmacy was estimated as the average number of medications recommended in each treatment regimen. This was determined as the sum of different medications prescribed divided by the total number of encounters.
3. The prescription rates of each drug category and subsequent individual drugs in each class. This was obtained by dividing the number of patient encounters in which the medication class was prescribed by the sum of encounters reviewed/surveyed, expressed as a percentage.
4. The percentage of encounters in which individual drug in a class was given to measure the prescription rate of each drug in its class.

Objectives 3

3.6 Clinicopathologic responses, predictors of the duration of management and prognosis in canine gastroenteritis

3.6.1 Study design

This was a prospective, observational analysis of dogs presented with gastroenteritis from four veterinary clinics comprising three Veterinary Teaching Hospitals and one private veterinary clinic in Nigeria. Selection criteria for inclusion into the study were: a dog must show signs of gastroenteritis and received no treatment from any other veterinary hospital or clinic, must have been screened for CPV, Coronavirus, Giardia, intestinal parasites, and any other suspected potential causes of gastroenteritis. Qualitative, haematology and biochemical data of the dogs were gathered once on the initial day of presentation. Costs relating to diagnosis and laboratory analysis were covered by this study. Treatment of the patients was done by the clinicians in the studied clinics following their own established protocols or as directed by the need of the individual patients. Qualitative data recorded for each patient at first presentation were breed, age, sex, deworming history, vaccination history, body condition score, husbandry practice, presence or absence of fever, anaemia, anorexia, depression, lethargy, dehydration, colic, faecal consistency, bloody diarrhoea, watery diarrhoea, duration of diarrhoea, frequency of diarrhoea and vomiting, presence or absence of concomitant diarrhoea with vomiting, type of disease, and presence or absence of intercurrent infections.

3.6.2 Sample size

A total of 157 dogs presenting diarrhoea and/or vomiting were sampled and screened for the various suspected diseases of interest. Thereafter, 104 dogs were selected randomly for haematological and biochemical analyses.

3.6.3 Clinical examination

The sampled dogs were physically and clinically examined by tactile percussion and abdominal palpation to detect the presence of colic, intussusception, or torsion etc. Dogs with progressive haemorrhagic gastroenteritis express a feeling of pain on abdominal palpation. The vital parameters were equally recorded. Mucous membranes and superficial lymph nodes were also checked for inflammation.

3.6.4 Sample collection

Faecal samples were used for coprological screen of the patients against CPV, coronavirus, Giardia, and other helminths. Heparinised vacutainer tubes were used to collect blood samples once on the day of the first presentation before any treatment started. The blood sampled was subjected to complete blood count and biochemical profiling. Blood was collected through cephalic venipuncture, taking into consideration the circulatory volume per body weight of each individual patient (i.e. approximately 0.6 mL/kg). About 2–4 mL of venous blood was collected and centrifuged. A portion of the whole blood was used for glucose testing, haematocrit, and complete blood count immediately after collection. Where it was impossible to run the biochemical tests immediately, the plasma was extracted, aliquoted and stored at –20 ℃ for later processing.

3.6.5 Laboratory techniques

Different laboratory techniques were used in diagnosing the possible causes of gastroenteritis in the dogs. There are multiple aetiologies known to be involved in companion animals. The presence of endoparasites and viruses were established through direct faecal smear, floatation, and sedimentation techniques, and by the application of in-clinic (SensPERT®, VetAll Laboratories, Korea) rapid antigen test kits for CPV, CCoV and *Giardia* (GIA).

3.6.5.1 Coprological examination for endoparasites

Three different techniques of endoparasites screening described by Urquhart *et al*. (1996) were used. Microscopic examination and morphological identification of parasites eggs and oocysts were achieved using characteristic keys described by Soulsby (1982).

3.6.5.1.1 Direct faecal smear

About 1–2mm^3 of faeces and equivalent drops of water were added to a glass slide, mixed, with coverslip placed over it and examined microscopically using x10 and x40 magnification. This method was used where it was impossible to have enough faecal samples for floatation technique.

3.6.5.1.2 Direct floatation method

Faeces weighing 2 grams were added to 10 mL of the floatation fluid of specific gravity between 1.18 to 1.2 (Saturated sodium chloride, 400 g of NaCl dissolved in 1000 mL of H_2O) and were thoroughly mixed. The supernatant was then used to fill a test tube with floatation liquid top-up to the brim. The test tube was then covered with a coverslip and spun at 1,000 rpm for two minutes, and examined the coverslip microscopically at ×40 objective lens.

3.5.5.1.3 Sedimentation method

Three grams (3 g) of faeces was homogenised with water. Debris was removed from the mixture by using a tea strainer placed over a beaker. Thereafter, the filtrate was kept for two minutes to settle, after which the supernatant was decanted. This step was repeated for one more time. About 2–3 drops of 5% methylene blue were then added to the sediments. Screening for parasites was carried out using a low power magnification (x40).

The presence of at least an egg or cyst irrespective of the techniques used was considered a positive test result. Oocytes and ova were morphologically identified as described by Soulsby (1982).

3.6.5.2 Screening for parvovirus, coronavirus, and Giardia

The patients were screened for three major reported causes of gastroenteritis in dogs (CPV–2, CCoV, and GIA) using rapid point-of-care immunochromatographic (IC) assay kits (SensPERT®, VetAll Laboratories, Kyunggi-Do, Korea). The test kits detect faecal antigens specific for CPV, CCoV, and *Giardia lamblia* (formerly *G. duodenalis*).

Principle: The SensPERT® CPV, CCoV and GIA in-clinic assay work on the principle of immunochromatography. Monoclonal antibodies from the kit bind to specific antigen epitopes and are absorbed by the nitrocellulose pad of the kit. The faecal antigens of the organisms bind to anti-parvovirus or anti-coronavirus or anti-Giardia conjugate of the pad, forming Ab-Ag complex. This complex forms Ab-Ag-Ab direct sandwich binding with the Ab of another monoclonal anti-parvovirus, anti-coronavirus, or anti-Giardia in the nitrocellulose pad. The appearance of the Control (C) and Test (T) bands in the result window determine the result. SensPERT assay kit contains a test panel, buffer, dropper, and swab. The test kit is stored between temperatures of 2℃ and 30 ℃.

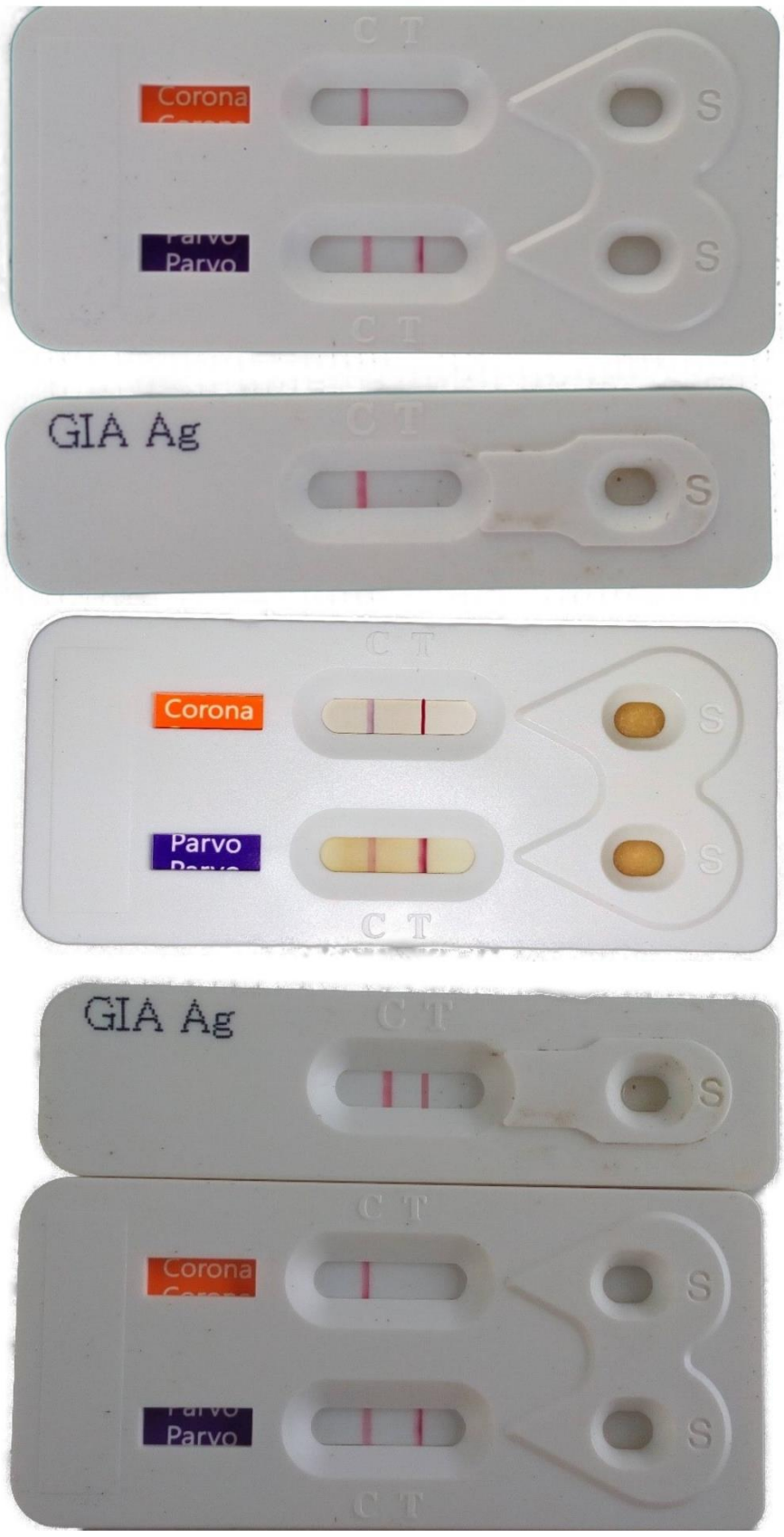

Plate 3.1: In-clinic assay kits used for CPV, CCoV, and Giardia screening

Test procedure: A reasonable amount of faeces was collected with the swab and mixed thoroughly with the buffer/diluent by swirling. The mixture was allowed for a few minutes to stand on the bench for heavy particles to sediment. Thereafter, the dropper was used to pipette the supernatant and dispensed about 100 µL (4 drops) into the sample well of the test panel labelled 'S'. The result was read within 5–10 minutes.

Result interpretation: The appearance of the test (T–) band in the result windowpane determines the presence or absence of the tested antigen. Control (C–) band will normally appear whether the tested faecal antigens (CPV, CCoV, GIA) are absence or presence. The appearance of C–band without T–band indicates the absence of the tested antigens (negative test). The appearance of both C– and T– bands signify the tested antigen is presence (positive test). The absence of C– or either T– and C–bands or appearance of T–band only indicate an invalid test and was repeated using a new kit and diluent. The bands appear within 5–10 minutes, after which it is considered as an invalid test, even if the test band appears (Plate 3.1).

3.5.5.3 The haemogram

3.5.5.3.1 Determination of packed cell volume

The microhaematocrit method was used to determine the PCV. This method makes use of microhaematocrit tube, microhaematocrit centrifuge, and a reader. Blood was taken from one end of the tube and the other open-end was closed using plasticine, and centrifugated for 3000 s at 12, 000 rpm. After centrifuging, the tube was removed with the length of the column of packed cells was read using the microhaematocrit reader. The length of the packed red cells converted to a percentage. The reference PCV value range for healthy Nigerian indigenous dog breed is 24–48% (Atata *et al*., 2018), and 35–57% for general dog breeds (Fielder, 2015).

3.6.5.3.2 Haemoglobin concentration

The cyanomethaemoglobin method adopted in measuring the Hb concentrations of the test blood samples. A buffered solution of potassium ferrocyanide and potassium cyanide to yield cyanomethaemoglobin was used to dilute the blood. The colorimetric method was used to measure the absorbance of the resultant solution using 540 nm wavelengths. The absorbance read in the colourimeter with a standard calibration graph and chart were used to determine the blood Hb concentration. The reference range for

Hb concentration for Nigerian indigenous dog breed is 8.6–17 g/dL (Atata *et al*., 2018); 11.9–18.9 g/dL for general dog breeds (Fielder, 2015).

3.6.5.3.3 The erythrogram

i. Total erythrocyte count

Total erythrocyte count (TEC) of blood sampled from the patients was performed manually by the visual method using the improved Neubauer counting chamber according to Jain (1986). Each blood sample was diluted by washing 20 µL of blood taken into a positive displacement pipette, into a 4mL of the diluent (Grower's solution) arriving at a final dilution ratio of 1:201 and mixed thoroughly. Through a steady suction, the pipette was filled with the diluted fluid to 101 Mark above the bulb, rotating gently while filling; then loaded into the haemocytometer counting chamber and allowed the cells to settle out of suspension. Thereafter, the number lying on five of the 0.04 mm^2 areas were counted using light microscopy using the ×40 objective. The cells in the total central 1 mm^2 areas of the counting chamber were counted if the number of cells counted on five of the 0.04 mm^2 areas was less than 500. The margin rule was applied to include or exclude cells lying on peripheral lines. The TEC was then determined from the sum of all cells in the five small squares multiplied by 10,000 to give the TEC per cubic millimetre using the formula:

$$\text{Total erythrocyte counts per litre (Y)} = \frac{\text{N}}{\text{A}\times\text{D}\times\text{DF}}$$

'N' connotes the number of cells; 'D' the chamber depth (0.1mm), 'A' area of chamber counted, while DF is the dilution factor (200). The conversion factor, 10^6 is used to convert cell per microliter to cells per litre (1 µL = 1 × 10^6 L). The Y denotes the red cell count, expressed as the number (X) raised to the power of 12 per litre (SI units) or to the power of 6 per microliter (US units). The reference red cell count range for healthy Nigerian indigenous dog breed is 3.1 – 6.8 × 10^{12}/L (Atata *et al*., 2018), and 4.9 – 7.9 × 10^6/µL for general dog breeds (Fielder, 2015).

ii. Erythrocyte indices

The mean corpuscular haemoglobin concentration (MCHC), corpuscular haemoglobin (MCH), and corpuscular volume (MCV) were computed from RBC, PCV and Hgb estimates obtained for each patient using the formula:

$$\text{Htc} = \text{MCV (fl)} \times \frac{[\text{RBC}]}{1000}$$

$$\text{MCH} = \frac{\text{Hgb}}{\text{RBC}} \times 10 \text{ (pg)}$$

$$\text{MCHC} = \frac{\text{Hgb}}{\text{PCV}} \times 100 \text{ (g/dL)}$$

$$\text{MCV (fl)} = \frac{\text{PCV}}{\text{RBC}} \times 10 \text{ (fL)}$$

The reference range for erythrocytes indices for Nigerian indigenous breed are MCHC = 29.4 – 35.8; MCV = 63.3 – 77 (Atata *et al*., 2018); MCH = 21–26.2; MCHC = 32–36.3; MCV = 66–77 for the general dog breeds (Fielder, 2015).

3.6.5.3.4 The leukogram

i. Total leukocyte counts

The TLC was determined using the improved Neubauer counting chamber. Blood samples were diluted by washing 50 µL of blood taken into a "shellback" WBC pipette into 950 µL of diluent to arrive at a final dilution ratio 1:20 and then mixed. The mixture was then loaded unto the improved Neubauer haemocytometer counting chamber with four large corners 1.0 mm^2 areas, and those in the central 1.0 mm^2 area to count the white cells. The margin rule was applied to include or exclude cells lying on the peripheral lines. The cells were microscopically counted using x10 objective magnification. The final total leucocyte counts were derived from the formula:

$$\text{White cell counts per litre } (Y) = \frac{\text{N}}{A \times D \times DF}$$

'DF' denotes dilution factor (1:20), 'N' cells counted, 'A' chamber area counted (4 mm^2), and D chamber depth (0.1 mm). The conversion factor, 10^6 is used to convert cell per microliter to cells per litre (1 µL = 1 $\times 10^6$ L). Y, the white cell count is expressed as the number (X) raised to the power of 9 per litre (SI units) or to the power of 3 per microliter (US units). The reference range for WBC count in normal Nigerian indigenous breed is 8 – 17 x 10^9/L (Atata *et al*., 2018); 5.0–14.1 × 10^3/µL for the general dog breeds (Fielder, 2015).

ii. Differential leukocyte counts

A smear of fresh blood samples from each patient was prepared using a slide-to-slide method. The was smear prepared, air-dried, fixed for 2 min with alcohol, then Giemsa stained for 8–10 min. The stained slide was rinsed with buffered water and air-dried. The films were inspected microscopically using the oil immersion objective (×100). The different cells were identified morphologically. A total of 200 cells were counted and

recorded as neutrophil, eosinophil, lymphocyte, and monocyte. The result was expressed as a percentage of individual cells. The reference intervals for differential cell counts in healthy Nigerian indigenous dog breed are Basophils 0–0 x 10^9/L; Eosinophils 0 – 1.67 x 10^9/L; Lymphocytes 0.23 – 4.87 x 10^9/L; Monocytes 0 – 1.37 x 10^9/L and Neutrophils 3.52 – 14.45 x 10^9/L (Atata *et al*., 2018); Basophils (0–1%), Eosinophils (0–9%), Lymphocytes (8–21%), Monocytes (2–10%), and Neutrophils (58–85%) for the general dog breeds (Fielder, 2015).

iii. Platelet count

Platelet count was performed by a visual method using the improved Neubauer counting chamber following standard method described by Jain (1986). A 1 in 20 dilutions of the blood sample was constituted in 1% ammonium oxalate and mixed for several minutes before filling the counting chamber, then set in a petri-dish with a piece of moisturised filter paper for 20 min for the cells to settle. The cells were counted in five of the 0.04mm^2 area using light microscope. The final platelet count was then calculated using the formula:

$$\text{Platelet count per litre } (Y) = \frac{\text{N}}{A \times D \times DF}$$

Where Y is the platelet count is expressed as the number (X) raised to the power of 3 per litre. 'A' represents the area of the chamber counted, 'N' the number of cells, 'D' counting chamber depth (0.1 mm), and DF the dilution factor (DF = 20). The conversion factor, 10^6 is used to convert cell per microliter to cells per litre (1 µL = 1 × 10^6 L). Y, the platelet count is expressed as the number (X) raised to the power of 6 per litre (SI units) or to the power of 3 per microliter (US units). The reference platelet count range for healthy dogs is 211–621 × 10^3/µL (Fielder, 2015).

3.6.5.3.5 Blood glucose level assay

A commercial glucose kit (Randox Laboratories Ltd, UK) was employed in testing blood glucose level in plasma of the patients based on glucose oxidase procedure described by the kit manufacturer. In this method, glucose oxidisation releases hydrogen peroxide which then reacts with phenol and 4–amino phenazone with peroxidase serving as a catalyst. This forms a red-violet quinonimine dye which serves as an indicator.

Procedure: Three cuvettes tagged as Reagent blank, Standard, and Sample were set up for the assay. Ten microliters (10 µL) each of the glucose standard and blood were

pipetted and added to cuvettes labelled as Standard and Sample, respectively with nothing added to the cuvette labelled blank. Next, Reagent 1 was pipetted (1000 µL) into the three cuvettes, mixed by shaking briefly 2 to 3 times. Incubation was done at 37 ℃ for 10 minutes before reading the result. The absorbance [A_{sample} and $A_{standard}$] were read at 500 nm in an hour and compared against that of the blank. The plasma glucose concentration of the test samples was calculated by applying the formula below:

$$\text{Glucose conc. (mg/dL)} = \frac{A_{Sample}}{A_{standard}} \times \text{conc. of standard (mg/dL)}$$

The normal glucose ranges from 2.8 – 8 mmol/L (or 50.45 - 144.14 mg/dL) in healthy Nigerian indigenous breed (Atata *et al*., 2018); with fasting blood sugar concentration range of 75 – 115 mg/dL and normal range of 76 – 119 mg/dL for the general dog breeds (Fielder, 2015).

3.6.5.4 Assay of plasma chemistry

Commercial kits produced by Randox Laboratories Ltd, UK were employed in assaying the plasma chemistry of the dogs under the investigation. Spectrophotometer (SM23A, Surgical field medical, England) was used to measure the absorbance. The plasma chemistry was assayed in accordance with methods by Coles (1986).

3.6.5.4.1 Total proteins, albumin, and globulin fractions assay

The manual Biuret method was used to determine the total proteins, albumin, and globulin fractions of the patients' plasma. These were carried out by following the procedure of the manufacturer of the kit.

i. Total proteins

Serum proteins were assayed by the Biuret method. In this method, when in an alkaline medium, cupric ion reacts with peptide bonds from proteins to form a coloured complex.

Technique: Three cuvettes labelled as Sample, Standard and Reagent blank were arranged on a rack. Thereafter, distilled water measuring 0.02 mL was pipetted into the blank cuvette, while CAL. standard (contains sodium azide and protein) measuring 0.02 mL and 0.02 mL of plasma was similarly pipetted into the respective cuvettes. Then 1.0 mL of R1 was pipetted into the three cuvettes and incubated on the bench for half an hour. A 550 nm wavelength was used to read absorbance. The absorbance A_{sample} and $A_{standard}$ were compared with that of the blank.

Total protein was determined manually using the formula:

$$\text{Total Protein Conc.} = \frac{A_{sample}}{A_{standard}} \times Standard\ Conc.\ (\text{g/dL}).$$

ii. Albumin

The manual method using bromocresol Green was employed in determining the concentration plasma albumin of the patients. The amount of albumin in the sample that binds to 3,3', 5,5'-Tetrabromo-m-cresol-sulphonphthalein (Bromo-Cresol-Green, BCG) is directly proportional to the absorbance. The complex formed (albumin-BCG) absorbs light maximally at 578 nm.

Procedure: Three cuvettes labelled as Reagent blank, Standard and Sample were arranged on a rack. Afterwards, distilled water (0.02 mL) was put in the Reagent blank, before 0.01 mL of CAL standard (protein) and 0.01 mL of were plasma added into their respective cuvettes. Subsequently, 3.0 mL of BCG Reagent (R1) was pipetted into the three cuvettes and gently swirled to mix then incubated for 5 min. A 630 nm wavelength was used to read absorbance. The absorbance A_{sample} and $A_{standard}$ were compared with that of the blank. Albumin concentration was calculated applying the formula:

$$\text{Albumin conc.} = \frac{A_{sample}}{A_{standard}} \times Standard\ Conc.\ (\text{g/dL})$$

Globulin concentration was obtained by deducting albumin concentration from total proteins. The reference total protein range in healthy Nigerian indigenous breed is 2.3 – 7.5 g/dL, globulin is 1.2 – 2.9 g/dL, and albumin is 1.0–3.1 g/dL (Atata *et al*., 2018); while total protein (5.4–7.5 g/dL), globulin (2.7–4.4 g/dL), albumin (2.3–3.1 g/dL), and albumin/globulin ratio (0.8–1.7) for the general dog breeds (Fielder, 2015).

3.6.5.4.2 Urea assay

The Urease–Berthelot technique was utilised in determining serum urea level in the samples. Hydrolysis of plasma urea to ammonia occurs when used urease as a catalyst.

Procedure: Three cuvettes labelled as Reagent blank, Standard and Sample were arranged on a rack. Thereafter, an equal volume (10 µL) of sterile water, Standard (CAL) and plasma was pipetted into each respective cuvette. Afterwards, 100 µL urease-buffer solution (R1) was pipetted into all the cuvettes. The content of each cuvette was slightly swirled, mixed, and incubated for 10 min at 37℃. Then, phenol-nitroprusside solution

and hypochlorite Reagent were pipetted 2.5 mL each into the three cuvettes, mixed immediately and incubated for 15 min at 37℃. The standard ($A_{standard}$) and samples (A_{sample}) were read at 546 nm and compared with blank. The manual calculation method was applied using the following formula:

$$\text{Urea Concentration} = \frac{A_{sample}}{A_{standard}} \times \text{Standard Conc. (g/L).}$$

The reference plasma urea concentration for healthy Nigerian indigenous breed ranges from 10 – 20 mg/dL (Atata *et al*., 2018); 8–28 mg/dL for the general dog breeds (Fielder, 2015).

3.6.5.4.3 Creatinine assay

The manual method described by the kit manufacturer was used for the quantification of the creatinine concentration from the clinical samples. In this method creatinine in the samples will react picric acid in an alkaline solution producing a coloured compound. The concentration of creatinine in the sample is directly proportionate to the amount of complex formed.

Procedure: Three cuvettes were labelled as Reagent blank (S_0), standard (S_1) and sample (S). With the aid of a micropipette, the Reagent blank cuvette (S_0) was dispensed with distilled water (50 µL), while100 µL each of the standard concentration and plasma sample were pipetted into cuvettes labelled S_1 and S, respectively. Thereafter, the working Reagent was pipetted (1000 µL) into the three cuvettes (S_0, S_1, S). The mixture was incubated for 30 seconds before reading the absorbance (A_1) of both the standard and plasma sample. The reading was repeated after 2 minutes i.e. absorbance (A_2). All the analyses were spectrophotometrically read at 492 nm wavelength.

The plasma creatinine level (mg/dL) was then determined as follows:

$$A_2 - A_1 = \Delta A_{sample} \text{ or } \Delta A_{standard}$$

$$\text{Plasma creatinine conc.} = \frac{\Delta \text{Asample}}{\Delta \text{Astandard}} \times \text{Standard conc. (mg/dL)}$$

The minimum detectable concentration of creatinine for this kit is 0.158 mg/dL.

The reference creatinine concentration range in healthy Nigerian indigenous breed is 0.4 – 1.8 mg/dL, while BUN/creatinine ratio is 4.1 – 10.2 (Atata *et al*., 2018), 0.5–1.7 mg/dL for the general dog breeds (Fielder, 2015).

3.6.5.4.4 Assay of Alanine aminotransferase

This was measured using the colourimetric method described by the manufacturer of the kit. The principle here is that ALT in the clinical sample reacts with 2,4 dinitrophenylhydrazine (DNPH) to form pyruvate hydrazine. The concentration of pyruvate hydrazine is proportionate to the amount ALT the sample contained.

Procedure: Two cuvettes were labelled as Reagent blank (S_0) and Sample (S_1), and then 100 µL of plasma was pipetted into S_1, while distilled water (100 µL) was put into S_0. Thereafter, 500 µL buffer substrate (R1) was pipetted into each of the cuvettes. The solutions were then gently mixed, while incubation was carried out at 37℃ for half an hour. After removing it from the bath, 500 µL of DNPH (R2) was added and kept on the bench for 20 min to incubate. After that, 5000 µL of NaOH solution was pipetted into both tubes (S_0 and S_1), mixed vigorously, and incubated for a further five minutes. Absorbance reading was done at 546 nm wavelength. The absorbance of the sample (A_{sample}) was compared to Reagent blank. The readings obtained and the plasma ALT activity was calculated from the calibration table provided with the kit.

The reference range of plasma ALT in Nigerian indigenous breed is 5 –100 U/L (Atata *et al*., 2018); 10–109 U/L for the general dog breeds (Fielder, 2015).

3.6.5.4.5 Aspartate aminotransferase level assay

The colourimetric method was used in determining the plasma AST concentration of the patients, as described by the kit manufacturer. The principle of this procedure is that reaction between 2,4 dinitrophenylhydrazine (DNPH) and AST from clinical sample forms oxaloacetate hydrazine. The concentration of pyruvate hydrazine and concentration of AST in the sample are directly proportional.

Procedure: Two cuvettes were labelled as Reagent blank (S_0) and Sample (S_1), then 100 µL of plasma pipetted into S_1, while 100 µL of distilled water to S_0. Thereafter, 500 µL buffer substrate (R1) was pipetted into each of the cuvettes. The solutions were gently mixed and incubation carried out at 37℃ in a water bath for 30 min. Following its removal, 500 µL of DNPH (R2) was added and incubated for a further 20 minutes.

Subsequently, 5000 µL solution of NaOH was pipetted into both tubes (S_0 and S_1), mixed vigorously, and incubated for further 5 min on the bench. Absorbance was read at 546 nm wavelength. The absorbance of the sample (A_{sample}) was compared to Reagent blank. The readings obtained and the plasma AST activity was extrapolated from the absorbance table supplied with the kit.

The reference range of AST in healthy Nigerian indigenous breed is 7 – 20 U/L (Atata *et al*., 2018); 13 – 15 U/L for the general dog breeds (Fielder, 2015).

3.6.5.4.6 Alkaline phosphatase level assay

This enzyme was assayed in the clinical samples using Randox ALP kit, adopting the manufacturer's procedure. The minimum detectable ALP concentration by the kit is 49.9 U/L. In this method, alkaline phosphatase catalyses hydrolysis of P–Nitrophenol phosphate (P–NPP) yielding two compounds: a colourless complex P–Nitrophenol phosphate and a coloured complex P–Nitrophenol. The P–Nitrophenol absorbs maximally at 405 nm. ALP catalyst activity corresponds to absorbance rate of 405 nm.

Procedure: To adjust the temperature, the spectrophotometer was switched on for 15–20 minutes. Thereafter, the wavelength was adjusted to 405 nm. One cuvette was set on the rack then 100 µL and 500 µL of plasma and the Reagent, respectively were pipetted into it and mixed thoroughly. Thereafter, the absorbance was read at a one-minute interval for three consecutive minutes. The concentration of ALP in the sample was obtained by applying the formula:

$$U/L = 2760 \times \Delta A \text{ } 405 \text{ nm/min}$$

Where 'ΔA' represents change in absorbance.

The reference range of ALP concentration in the plasma of healthy indigenous Nigerian indigenous breed is 7–113 U/L (Atata *et al*., 2018); 1–114 u/L for the general dog breeds (Fielder, 2015).

3.6.5.5 Assay of plasma electrolytes

In this study, calcium, potassium, sodium, chloride, sodium/potassium ratio, and calcium potassium ratio of the diarrhoeic patients were assayed.

3.6.5.5.1 Calcium level assay

Calcium concentrations of the plasma samples were estimated spectrophotometrically using a commercial kit (TECO Diagnostics, USA). The minimum detectable calcium concentration level for this kit is 1.16 mg/dL. The workability of the kit is that calcium ions in the samples react with O-Cresol phthalein complexone in an alkaline solution produces a violet complex which absorbs between 550–590 nm wavelengths.

Procedure: Three cuvettes were labelled as sample, standard and Reagent blank. Twenty-five microliters (25 µL) of distilled water were pipetted into S_0 cuvette, 25 µL of the standard into the S_1 cuvette and 25 µL of the sample into the sample cuvette. Thereafter, 0.5 mL of the chromogen (consisting of Hydrochloric acid, 8–Hydroxyquinoline and O-Cresol phthalein complexone) and 0.5 mL of 2-amino-2-methyl-propan-1-ol (buffer solution) were added into the three cuvettes, mixed thoroughly then incubated for 5–50 min. Afterwards, the absorbance A_{sample}, $A_{standard}$ and of the Reagent blank were read at 570 nm wavelength. The underlisted formula was utilised in computing the absolute calcium concentrations:

$$\text{Calcium concentration} = \frac{\text{A sample}}{\text{A standard}} \times \text{Standard conc. (mg/dL)}$$

The reference range of plasma calcium concentration for healthy Nigerian indigenous breed is 7.4 – 11.6 mg/dL (Atata *et al*., 2018); 9.1–11.7 mg/dL for the general dog breeds (Fielder, 2015).

3.6.5.5.2 Potassium level assay

Plasma potassium level in the patients was assayed by a commercial potassium test kit (TECO Diagnostics, USA), using the colourimetric method described by the manufacturer. The kit works based on the principle that potassium produces colloidal solution when reacts with sodium tetraphenyl boron. The potassium concentration in the sample is directly proportional to the turbidity formed ranging from 2–7 mEq/L.

Procedure: Four cuvettes, control (C), sample (S), standard (S_1) and Reagent blank (S_0) were arranged on a rack. Thereafter, 1000 µL of potassium Reagent was pipetted into each of the cuvettes. Afterwards, 10 µL of the test samples was to all the cuvettes, gently mixed, incubated at room temperature for three minutes. Thereafter, spectrophotometer initially adjusted to 500 nm wavelength was reset to zero using S_0 cuvette before the final absorbance of the rest of the cuvettes was measured. Samples with concentration

over 7 mEq/L were double diluted with normal saline in a ratio of 1:1 and reassessed. The result obtained was then multiplied by a factor of 2.

The plasma potassium concentration was then calculated using the formula:

$$Potassium\ conc.\ (mEq/L) = \frac{A_{Sample}}{A_{Standard}} \times Conc.\ of\ Standard\ (\text{mEq/L})$$

This kit has a sensitivity of 0.006 mEq/L of potassium. The reference range of plasma potassium level in healthy Nigerian indigenous breed is 3.4–5.7 mEq/L (Atata *et al*., 2018); 3.9–5.1 mEq/L for the general dog breeds (Fielder, 2015).

3.6.5.5.3 Sodium concentration assay

Plasma sodium concentration in the patients was assayed with a commercial sodium test kit (TECO Diagnostics, USA) using the procedure described by the manufacturer. In this method, the precipitation of sodium results in the formation of a triple salt – sodium magnesium uranyl acetate. Thereafter, ferrocyanide reacts with surplus uranium to produce a chromophore product. The concentration of sodium in the sample is inversely proportional to the chromophore absorbance. The minimum detectable limit of sodium by the kit is 0.5 mEq/L.

Procedure: Four cuvettes – Reagent blank (S_0), standard (S_1) control (C) and sample (S) were arranged on a rack. About 1000 µL of filtrate Reagent was dispensed in each of the cuvettes. Subsequently, 50 µL plasma samples was pipetted into S_1, C and S cuvettes, while in S_0 distilled water was added before mixing by vortexing for 3 min. Centrifugation of the content was for 10 min at 1500 rpm. The harvested supernatant fluid was assayed as follows:

A new set of labelled test tubes corresponding to the filtrate tubes were set on a rack and 1000 µL of Acid Reagent added to all of them. The supernatant fluid (50 µL) was pipetted into the cuvettes, mixed, followed by addition the of 50 µL of Colour Reagent and mixing. The spectrophotometer was adjusted to 550 nm wavelength before zeroed with distilled water, and the absorbance of the cuvettes measured. In this procedure, there is a reduction in absorbance rather than the typical absorbance increase observed in previous methods. The blank has higher absorbance compared to the test samples. The plasma sodium concentration of the patients was then calculated using the formula:

$$\text{Sodium Conc. (mEq)} = \frac{A_{Blank} - A_{Sample}}{A_{Blank} - A_{Standard}} \times \text{Conc. of Standard}$$

Where A = Absorbance

The reference range of plasma sodium concentration for healthy Nigerian indigenous breed is 142 –157 mEq/L (Atata *et al*., 2018); 142–152 mEq/L for the general dog breeds (Fielder, 2015).

3.6.5.5.4 Chloride concentration assay

Chloride assay was measured as per the protocol of the chloride test kit manufacturer (TECO Diagnostics, USA). In this method, a reaction between chloride ions from the serum sample and mercuric ions releases thiocyanate ions. The thiocyanate then forms a coloured complex product with ferric ions, absorbing light at 480 nm. The concentration of chloride in the sample determines the colour intensity formed.

Procedure: Three test tubes - blank (S_0), calibrator (S_1) and sample (S) were set on a rack. Chloride Reagent (1.5 mL) was pipetted into each of the tubes. After that, 10 µL each of the chloride calibrator and sample were pipetted into their respective tubes and then mixed. Incubation was carried out on the bench for 5 min. Spectrophotometer wavelength was adjusted to 480 nm after which it was reset to zero using the Reagent blank. A wavelength of 480–520 nm was used in measuring the absorbance (A). Plasma chloride concentration was computed applying the formula:

$$\text{Chloride concentration} = \frac{A_{Sample}}{A_{calibrator}} \times \text{Conc. of the calibrator (mEq/L).}$$

The reference range of plasma chloride concentration in apparently healthy Nigerian indigenous breed is 100 –132 mEq/L (Atata *et al*., 2018); 110–124 mEq/L for the general dog breeds (Fielder, 2015).

3.6.5.5.5 Determination of sodium/potassium ratio

This was determined in the plasma of the enteritis dogs by dividing the sodium concentration by the potassium concentration obtained for each patient.

$$\text{Na: K ratio} = \frac{\text{Sodium conc. (mEq/L)}}{\text{Potassium conc. (mEq/L)}}$$

The reference range for sodium/potassium ratio in healthy dogs is 25–40 (Sodikoff, 1995).

3.6.6 Data handling and analysis

This has been achieved in three aspects. The first part used exploratory data analysis to obtain descriptive statistics for the 157 dogs studied. For the second and third aspects of the analysis, the patients were grouped according to the treatment response as survivors (n = 70) and non-survivor dogs (n = 34), previous vaccinations history (vaccinated or unvaccinated), previous deworming history (dewormed in the last three months or not dewormed). Other qualitative variables were the lifestyle of the dog (restricted or stray), and body condition score (Good/fair or emaciated), type of disease (viral or non-viral), presence or absence of co-infection, breed of dog, age, sex, presence or absence of fever, anaemia, anorexia, depression, lethargy, dehydration, colic, faecal consistency, bloody diarrhoea, watery diarrhoea, duration of diarrhoea, frequency of diarrhoea, frequency of vomiting, concomitant vomiting with diarrhoea at presentation.

For the second analysis, descriptive statistics for the studied variables were evaluated and expressed as the mean and percentage of patients with clinicopathologic responses. The mean haematologic and biochemical variables of dogs that survived and those that died were compared using the t-test. A two-tailed distribution rule for heteroscedastic data with an assumption of unequal variances was adopted. A test of association between the prevalence of gastroenteritis and the studied variables of interest were assessed using Chi-square test.

For the third analysis, Pearson's correlation and logistic regression for binary outcome were used to determine the strength of the associations and the predictive value of the haematological, biochemical, and qualitative variables influencing treatment outcomes (death versus recovery) and the DOM, below or above the median duration). The DOM as used here was computed as the difference in days between presentation and when the dog was certified fit. The haematological and biochemical parameters of interest were coded as being either within or outside the reference limits, while the qualitative variables were coded as either present or absent. Pearson's correlation was performed and variables exhibiting significant relationships with clinical outcomes and the DOM were subjected to logistic regression model for binary outcomes. The final regression model was evaluated for goodness-of-fit by applying established rules (Hosmer and Lemeshow, 2000). The data analyses were conducted in Microsoft Excel 2016 and SPSS statistical programme (ver.20) at the $\alpha_{0.05}$ significance level.

Objective 4

3.7 Assessment of electrocardiographic and biochemical responses in dogs with canine parvovirus enteritis

3.7.1 Study design

This aspect of the research was conducted on dogs with gastroenteritis presented to the Infectious Diseases Unit, University of Ibadan Veterinary Teaching Hospital. Only dogs presented with gastroenteritis and were not treated prior to admission and that tested positive to parvovirus faecal antigen test (SensPERT®, VetAll laboratory, Korea) and by polymerase chain reaction assay partook in this analysis.

3.7.2 Sample size

Forty (40) parvo-positive dogs were purposively sampled for this study. Dog patients assessed were only those confirmed positive for CPV by in-practice test kit and by PCR.

3.7.3 Screening of the dogs against CPV infection

At presentation, dogs that met the inclusion criteria mentioned above were examined physically and clinically by the researcher. Stools were collected from the patients per rectum using swab sticks and were assayed for CPV–2 using on-the-spot immunochromatographic ELISA kit (SensPERT®, VetAll Laboratories, Korea), as described earlier on in section 3.6.5.2 of this Chapter. The CPV positive dogs (71.34%) in the in-clinic test were further confirmed by polymerase chain reaction assay.

3.7.4 Laboratory analysis

Blood specimens were collected without anticoagulant to harvest serum. After collection, the blood was left on the bench for about half an hour to clot before centrifuging at 1500 rpm for 300 s. The sera were then subjected to biochemical assay for calcium, chloride, potassium, and sodium concentrations using spectrophotometric methods according to standard techniques by Coles (1986). All the biochemical analyses were assayed with commercially available test kits (Randox Laboratories Ltd., Ardmore, United Kingdom) using procedures described earlier on in section 3.6.5.5 of this Chapter.

3.7.5 Electrocardiogram evaluation

The ECG of the confirmed parvovirus dogs was recorded using a 6/7 Channel ECG machine (EDAN VE–1010, Shanghai, China). The patients were controlled physically,

put on right lateral recumbence with forelimbs held at right angle to the long body axis, holding the hindlimbs in a semi-flexed position as ECG tracing was recorded. A standard technique with surface electrodes previously described was adopted (Tilley, 1995). The electrodes were attached to the skin using filed down crocodile clips at the level of the olecranon on the caudal aspect of the forelimb, over the patellar ligaments on the cranial aspect of the hind limbs and the chest. The methylated spirit was used to aseptically prepared the points where the alligator clips were attached to the skin. Ample amount of ultrasound gel was applied between the skin and the crocodile clips to improve electrical contact with the body of the patients. Recordings were made at a paper speed of 25mm/s and vertical ECG calibration of 10 mm/mV. Lead II was used to interpret electrocardiogram variables and compared with the ECG reference values for the dogs. Variables evaluated were cardiac arrhythmias, heart rate, QRS, PR, and P–wave intervals, R– amplitude, T-amplitude, QT segment and Bazett's correction of the QT interval.

3.7.6 Data analysis

Data handling and analysis were conducted in SPSS v20 and evaluated at $\alpha_{0.05}$ significance. Group data, such as dog characteristics and arrhythmia variables were computed as mean values and compared with reference ECG values for healthy dogs.

Objective 5

3.8 Vaccine administration protocols of vaccinated dogs diagnosed with canine parvovirus enteritis in Nigeria

3.8.1 Data source

This was a prospective study of 94 confirmed cases of vaccination failure out of the 157 dogs that were screened under section 3.6 of this Chapter. Data of dogs positive in the in-clinic test were further confirmed by means of PCR assay. The information recorded for each animal was name of the clinic, case occurrence date, patient identity, breed, age, sex, vaccination status, vaccination date and vaccine brand/type.

3.8.2 Data management and analysis

Age in years and months were converted to weeks only. Sex was grouped as male or female. Only cases with evidence of vaccination were included in this analysis. Unvaccinated or cases with unknown vaccination were excluded from this analysis. Based on the vaccination history, vaccinated dogs were regrouped according to age at first and last vaccination. Where more than one vaccine dose was administered, the vaccination interval was calculated and recorded in weeks. The number of doses administered prior to disease was recorded. Data management was achieved using Microsoft Excel 2016 before analysed with SPSS version 20. Data analysis involved estimating the age at which the dog was presented with parvovirus, the number of doses received and the mean vaccination interval.

Objective 6

3.9 Prevalence and molecular analysis of canine parvoviruses from vaccines and clinical samples in Nigeria

3.9.1 Materials

Equipment and reagents used here were:

A. In-practice CPV antigen screening test kit (SensPERT®, VetAll Laboratories, Korea)
B. Quick-DNA™ Miniprep Plus extraction kit (ZYMORESEARCH™, USA)
C. PCR Master Mix (2X) (BioLab, New England)
    i. 4 mM $MgCl_2$,
    ii. 0.4 mM of each dNTP (dATP, dCTP, dGTP and dTTP),
    iii. reaction buffer,
    iv. 0.05 U/µL Taq DNA polymerase,
D. Nuclease-free water
E. Vortex
F. Adjustable pipettes（10, 20, 200, 100 µl）
G. Microcentrifuge (adjustable, up to 13 000 rpm)
H. Sterile, RNase-free pipette tips with an aerosol barrier
I. Ethanol (96–100%)
J. Positive control
K. Thermocycler
L. Microcentrifuge tubes（0.2, 1.5 ml）
M. Molecular Imager® Gel Doc™ XR+ System with Image Lab™ Software (Bio-Rad, Hercules, CA).
N. Samples:
    i. Faecal
    ii. CPV vaccines
    iii. DNA extracts
O. Primers:
    i. VP2-Forward
    ii. VP2-Reverse

3.9.2 Study design

Random diarrhoeic samples from dogs from ten selected veterinary practices in seven cities covering four out of the six geopolitical zones in Nigeria namely; Northcentral: Abuja, Jos, Makurdi; Southwest: Abeokuta, Ibadan; Southeast: Onitsha; and South-south: Warri, with perceived high rate of caseload in small animal practice/dog population were analysed. Signalment/demographic statistics were collected with a predesigned form (Appendix 3). Collected faecal samples were subjected to coprological examination. The faecal samples obtained were first screening for CPV, using rapid point-of-care immunochromatography (IC) assay, followed by PCR and PCR product sequencing. Canine parvovirus vaccines licenced for vaccinating dogs in Nigeria were also sampled for sequencing and characterization of the vaccine-strains contained for comparison with the disease strains.

3.9.3 Sampling

3.9.3.1 Animal sampling

A total of 112 faecal samples out of 157 patients with acute gastroenteritis screened for CPV in the in-clinic testing were randomly selected. Samples for molecular assay were collected using either sterile pipettes, 2 mL syringes or cotton swabs as appropriate and were immersed or dispensed immediately into Eppendorf tubes containing virus transport medium (VTM). The samples were then transported and stored at –20°C until molecular assay at Dr O. A. Fagbohun Molecular Laboratory, University of Ibadan. The sample distribution was as follows: 72 in Ibadan , 25 in Warri, 22 in Abeokuta, 20 in Jos, 11 in Makurdi, 4 in Abuja, and 3 in Onitsha.

3.9.3.2 Vaccine sampling and blinding

Six brands of parvovirus vaccines licenced for canine vaccination were obtained from various veterinary and pharmaceutical shops in Nigeria. The vaccines sampled were randomly labelled from NGA-Vacc1 to NGA-Vacc6 blinding them from the analysers. Vaccines sampled were those expiring in at least six months at the time of experiment. A single lot of each vaccine brand was used for this assay.

3.9.4 Extraction of vaccine and faecal DNA

Vaccine and faecal DNA were extracted by means of Quick-DNA™ Miniprep Plus (ZYMORESEARCH™, USA), in accordance with the procedure of the manufacturer. The lyophilised parvovirus vaccines were each diluted with 200 µL of Nuclease-free

water. Briefly, 1,060 µL of Storage Buffer was pipetted into individual 20 mg tube of Proteinase K and stored at -20℃. Thereafter, 200 µL of the liquefied vaccine or faecal samples were pipetted into each 2 mL microcentrifuge tube; then added 200 µL of Biofluid and 20 µL of Proteinase K in microcentrifuge tubes containing faecal samples. The admixtures were then vortexed thoroughly after which the tubes were incubated for 10 minutes at 55℃ to digest. Then added 420 µL of Genomic Binding Buffer to all the microcentrifuge tubes containing 420 µL of the digested samples and vortexed thoroughly. The admixture was afterwards transferred to Zymo-Spin™ IIC-XL Column in Collection Tubes. The tubes were subsequently centrifugated at 12, 000 × g for 60 s. The Collection Tubes with the flow-throughs were discarded. Afterwards, the Zymo-Spin™ IIC-XL Columns were transferred to a fresh set of Collection tubes, added 400 µL of DNA Pre-Wash Buffer, and centrifugated for 60 s. The flow-throughs with the Collection tubes were discarded, followed by adding 700 µL of gDNA Wash Buffer to all the Spin Columns and centrifugated for 60 s. The flow-throughs were discarded before further 200 µL of gDNA Wash Buffer was pipetted into the Columns and centrifugated for extra minute. Then Collection tubes with the flow-throughs were discarded. DNA eluted by transferring the Spin Columns to clean microcentrifuge tubes and added 50 µL of DNA Elution Buffer to the Spin Column membranes. This mixture was subsequently allowed to incubate for 300 s, before centrifuging for a minute to elute DNA. The eluted DNAs were labelled appropriately and stored at -20℃ until PCR assay.

3.9.5 Parvovirus PCR assay

The PCR was performed with One Taq Quick-load Master Mix (2X) (BioLabs), predesigned VP2 gene-specific primers (Mira *et al*., 2018), Nuclease-free water and DNA extract in a 50–µL reaction mix (Table 3.1). The primers were manufactured by Iqaba Biotechnology, SA (Table 3.2). Primer stock of 100 µM solutions was prepared from lyophilised primers by diluting with an appropriate volume of nuclease-free water (Appendix 8). A 10 µM primer working solutions were then prepared from the primer stocks by diluting one part of the stock solution in nine parts of nuclease-free water, aliquoted in sterile Eppendorf tubes and stored at -20℃. The thermal conditions used for TaqPol activation and amplification of the DNA are depicted in Table 3.3. PCR product size of 700 bp amplicons were obtained.

3.9.6 Analysis of PCR products

A 2% agarose gel electrophoresis with the addition of 0.005% ethidium bromide protocol was used in analysing the amplicons as detailed below:

1. A 100 ml of 2% w/v agarose gel (Agarose, Sigma, St. Louis, Mo, USA) was prepared.
2. Ethidium bromide (5 µL of 5 mg/mL) was supplemented, swirled gently to mix and poured in the gel tank platform before the gel cools and solidifies completely.
3. A 100 kb DNA ladder (BioLabs) was used.
4. Then 3 µl of orange/blue dye (BioLabs) followed by 5 µl of PCR product were placed on a clean parafilm and mixed.
5. The sample-dye mixture and the marker were then loaded into the wells.
6. The gel electrophoresis was conducted at a voltage = 85 V, amperage = 400 mAmps for 1 hour in 1X TBE buffer.
7. The stained gel was imaged in Molecular Imager. An amplicon of 700 bp, based on the set of primers used, was considered positive for CPV DNA.

3.9.7 Parvovirus DNA sequence and phylogenetic analyses

Partial CPV VP2 gene sequencing was successfully performed on 11/16 randomly selected positive samples from the seven cities and 5/6 of the vaccines. The DNA sequencing was done at the University of Cornell, Infectious Diseases and Molecular Diagnostic Laboratory, USA. DNA sequence assemblage and editing were achieved using BioEdit v7.2 (Hall, 1999). BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi) tool was used to look for identical sequences in the public domain. Thereafter, multiple alignment of the clinical, vaccine, and reference sequences acquired from GenBank was conducted in CLC Main Workbench sequence viewer 8.1 (QIAGEN, Aarhus A/S). The Neighbour-Join and BioNJ algorithms in MEGA X evolutionary analytic software was adopted in the evolutionary genetic analysis (Kumar *et al*., 2018). Tamura-Nei model and Maximum Likelihood method were employed to infer the phylogenetic relationships of the biotypes (Tamura and Nei, 1993).

3.9.8 Statistical analysis

Descriptive statistic was used in estimating the prevalence of CPV. The categorical data were analysed using SPSS statistical software v20 (IBM SPSS Inc., Chicago, IL, USA).

Table 3.1: Composition of the 50-µL reaction mix used for the PCR assay

Reagents	**The volume required per sample (µl)**	**Final concentration**
One Taq Quick-load Master Mix (2X) with std buffer	25	1X
10 µM CPV-VP2-850F	1	0.5 µM
10 µM CPV-VP2-1550R	1	0.5 µM
Nuclease-free water	18	
Template DNA	5	
Total	**50**	

Table 3.2: Primers sequences used for DNA amplification

Primer name	Sequences	Size	Reference
CPV VP2			
CPV-850 Forward	5′-GAGCATT GGGCTTACCA-3′	700	Mira *et al*., 2018
CPV-1550 Reverse	5′-GCAAGATGCATCAGGATC-3′	bp	

Table 3.3: Thermocycling conditions used for the PCR assay

Step	**Temperature**	**Duration**	**Cycles**
Initial denaturation	90℃	2 min	1
Denaturation	95℃	20 s	
Annealing	43℃	60 s	35
Extension	72℃	90 s	
Final extension	72℃	7 min	1
Holding	4℃	∞	1

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Retrospective study of canine gastroenteritis prevalence in Nigeria

Gastroenteritis is a common presenting condition in canine practice however there is more anecdotal evidence than published data available on it in Nigeria. The findings reported here revealed a high prevalence of gastroenteritis of 41.2% (919/2231) based on the total number of sick dogs presented and a prevalence of 23.7% (919/3882) based on a combined total of sick and apparently healthy dogs presented at consultation during the period under investigation. This indicates endemicity of canine parvovirus and other conditions that predispose dogs to gastroenteritis in Nigeria. A total of 1312/2231 (58.8%) of the patients were diagnosed with other clinical conditions (Table 4.1). It can be inferred from these data that one out of every three sick dogs presented during the period under review had gastroenteritis. This finding of a very high prevalence of canine gastroenteritis is comparable to existing reports on canine GI disorders. In Egypt, a prevalence of 56.5% was described (Rakha *et al*., 2015), 52.0% in Turkey (Yilmaz *et al*., 2002), and 36.1% in India (Dutta *et al*., 2013). In South Africa, 11.5% of sick dogs presented for veterinary attention were diagnosed with gastroenteritis (Shakespeare, 1999). Prevalence reports from different studies could be influenced by differences in geographical locations, management, husbandry practices, the lifestyle of the dog such as roaming and scavenging freely, study period, and study designs. Diarrhoea only (40.8%) and concomitant vomiting with diarrhoea (34.5%) were the most common presenting complaints compared to vomiting (24.7%). This lends support from previous reports that also recorded a higher incidence of diarrhoea than vomiting in dogs (Tarafder and Samad, 2010; Atsbaha *et al*., 2014; Rakha *et al*., 2015). Canine diarrhoea is more precisely related to the lifestyle of dog than a specific infectious agent (Stavisky *et al*., 2011). In this study, it is related to endemicity of CPV and other infectious disease in Nigeria.

A total of 515 out of the 919 patients had their data on breed documented. Gastroenteritis was associated with dog breed (Table 4.2 and Figure 4.1). Large breed dogs (Alsatian, Boerboel and Rottweiler) with 270 cases were at elevated risk ($\alpha = 0.014$) of developing gastroenteritis compared to the giant, medium, and small breed dogs. Within the giant breed dogs, Caucasian (85.5%) was significantly ($\alpha=0.017$) more susceptible to gastroenteritis than Mastiffs (14.5%). This is because large dogs have voracious appetite and are fond of picking hence are more predispose to infections. These findings lend credence to existing reports in which the incidence of gastroenteritis was significantly influenced by dog breed (Sævik *et al*., 2012). Tarafder and Samad (2010) also found that GI disturbances were more prevalent in Alsatian compared with other dog breeds.

Breed predisposition to gastroenteritis could be related to genetic influence, husbandry practice, as well as the lifestyle exposure risks (Stavisky *et al*., 2011; Sævik *et al*., 2012). Invariably, overrepresentation due to preference of exotic breeds (Alsatian, Boerboel, Rottweiler, Caucasian, and Mastiff, etc.) over Nigerian Indigenous breed or certain purebreds, for breeding and security purposes could help explain the higher prevalence of gastroenteritis recorded in exotic breeds compared with crosses, Nigerian Indigenous breed, Lhasa Apso, Terriers, and other breeds in this study. Dog owners are more likely to give medical attention to exotic or purebred or pedigree than local or mixed breed dogs owing to their economic values (Shima *et al*., 2015a). Ultimately, the role of immunity to diseases and genetic dissimilarities in breeds cannot be overemphasised.

In consonant with existing reports, the association between observed clinical signs of gastroenteritis (diarrhoea, vomiting and concomitant diarrhoea with vomiting) was not significant at a 95% C.I. in this study. However, since $\alpha=0.05$ in observed clinical signs of gastroenteritis, it is noteworthy that these values are strongly suggestive of a significant relationship between gastroenteritis and the breed of dog. Future studies should explore this relationship for determining whether the clinical signs of gastroenteritis are more prevalent in some dog breeds than in others, as this data suggest.

Alsatians had the highest incidence of gastroenteritis (diarrhoea only – 11.7%, vomiting only – 6.8% and concomitant vomiting with diarrhoea – 8.5%) compared with other breeds under investigation. Within the same dog breed, the incidence of concomitant vomiting with diarrhoea (6.6%) was higher than diarrhoea only (4.9%) and vomiting (3.1%) in Rottweiler. These breed differences might be genetically influenced. Viewed

differently, this is a function of the portion of the GIT most affected: stomach as against small intestine or large intestines. The positive influence of gastroenteritis on breed corroborates the reports of Sævik *et al*. (2012). In a different scenario, no clear overall difference in the frequency of vomiting or diarrhoea between different dog breeds was described by Hubbard *et al*. (2007), who attributed it to the very low numbers of dogs belonging to several breeds registered in their study.

Table 4.3 depicts the effect of sex, age and canine Distemper, Hepatitis, Leptospirosis and Para-influenza (DHLPP) polyvalent vaccination status on the incidence of gastroenteritis in the dogs studied. Out of the 919 patients diagnosed with gastroenteritis, only 513 had data available on sex. Diarrhoea (41.9%) was the major clinical sign observed in the dogs. Vomiting and/or diarrhoea was highest in the male (52.8%) compared to female dogs (47.2%). The effect of sex on gastroenteritis was not statistically significant (α=0.825).

There are diverging reports regarding the influence of sex of the dog on gastroenteritis. Rakha *et al*. (2015) reported a substantially higher prevalence in male dogs. In contrast, some researchers (Hubbard *et al*., 2007) observed that sex was not significantly associated with gastroenteritis even though more male dogs were treated for diarrhoea compared to their female peers, supporting the findings in this study. Similarly, a case-control study by Stavisky *et al*. (2011) revealed that diarrhoea was highest in males when compared with female dogs. The findings reported here and two other separate studies corroborated that sex of the dogs is not a significant predisposing factor of gastroenteritis (Wells and Hepper, 1999; Proschowsky *et al*., 2003), despite males (52.8%) having the highest prevalence compared to female dogs (47.2%). The explanation for this remains elusive. Nonetheless, this may be related to over-representation owing to the preference of male dogs over their female peers for security and breeding purposes in Nigeria. Several authors have reported that sniffing and licking of the anogenital (Bradshaw and Lea, 1992; Maarschalkerweerd *et al*., 1997), and roaming are behaviours predominant in male dogs (Westgarth *et al*., 2008) that exposed them to infections such as CPV (Shima *et al*., 2015a, 2015b), mischievous or malicious poisonings (Shima *et al*., 2015c).

Out of the 919 dog patients diagnosed with gastroenteritis, only 442 had data available on age. The incidence of gastroenteritis was highest in puppies 1 to 6 months old (63.1%)

compared to older dogs. Again, diarrhoea (40.1%) was the highest presenting clinical sign observed among the age groupings (Table 4.3).

Age of the dogs was not a significant (α=0.181) factor of gastroenteritis. However, the prevalence of gastroenteritis skewed with age, with a higher prevalence of gastroenteritis (63.1%) occurring in younger dogs. The studied dogs had a median age of 4 months implying that dogs below one year are more susceptible to gastroenteritis. This finding is consistent with the reports that gastroenteritis occurs more frequently in puppies and declines with as they grow older (Castro *et al*., 2007a; Sævik *et al*., 2012). This is attributed to the abundance of rapidly dividing cells such as enterocytes in very young animals like puppies, introduction to solid diets, and difference in vaccination status (vaccinated versus unvaccinated). Age did not substantially influence the prevalence of gastroenteritis in this investigation (α=0.165), reinforcing the findings by Hubbard *et al*. (2007) but diverging from the report of Rakha *et al*. (2015). Furthermore, higher number of cases of gastroenteritis in puppies within the age bracket of one to three months old reported here agrees with the reports of Rakha *et al*. (2015), as well as, reports of several other studies that focused on specific infectious diseases related to gastroenteritis (Hamnes *et al*., 2007; Kempf *et al*., 2010). This age predisposition to gastroenteritis could be related to stress, undeveloped immunity, and shorter lifespan of maternally acquired antibodies which usually wane about 12 weeks of age making puppies vulnerable to infectious GI diseases such as intestinal parasites (Jarret and Ramsey, 2001; Tennant, 2001; Berset-Istratescu *et al*., 2014).

With regards to DHLPP vaccination, only 699 dogs had available data on their vaccination status documented. Gastroenteritis was more common in patients with uncertain vaccination history (74.8%) than in those that were vaccinated (14.2%) and unvaccinated (11.0%) patients. Canine DHLPP polyvalent vaccination history was significantly (α = 0.047) associated with the prevalence of canine gastroenteritis in the studied dogs (Table 4.3). It is not surprising that unvaccinated dogs and those with uncertain or doubtful vaccination history had higher prevalence of gastroenteritis compared to vaccinated ones. They were unprotected from infectious diseases; gastroenteritis was indisputably due to vaccine-preventable infections, such as parvovirus. Vaccination failure or failure to vaccinate properly may be explanations for the cases of gastroenteritis recorded in the vaccinated dogs. In this study there was a

predominance of gastroenteritis caused by CPV over other aetiologies. Lower cases of gastroenteritis reported in vaccinated dogs here support the view that vaccination lowers the chances of infectious diseases incidence in animals (Waner, 2004; Gill *et al*., 2004; Day *et al*., 2016).

Cases of gastroenteritis was reported all year round but the prevalence was significantly (R^2 = 0.809; α=0.03) associated with the period of the year (Figure 4.2). Its peak was recorded in January (13.2%) and a trough between August (5.8%) and September (5.3%), corroborating reports from some parts of Nigeria (Shima *et al*. 2015b; Gberindyer, 2016), but contrasting the report of Mohammed *et al*. (2005) that canine gastroenteritis is more prevalent between May and June in Vom, Nigeria. This is because Jos and Vom have cooler climate mimicking that of temperate regions of the world. In South Africa, the prevalence of gastroenteritis is highest in summer than in the winter months (Shakespeare, 1999). In Canada, a higher number of cases occur between July and September (Houston *et al*., 1996). Differences in seasonality and prevalence reports of different studies may be connected to dissimilarities in the climate of different regions, husbandry practices, as well as, dissimilarities in exposure to diverse causative agents. The lowest number of cases was recorded between August and September. This could be associated with the heavy downpour generally experienced across Nigeria during this period. Rainfall patterns and changes in climate have modulating effects on the propagation and the seasonal distribution of infectious agents (Ricardo-Izurieta and Clem, 2008; Shima *et al*., 2015a).

There are diverging reports regarding seasonality of GI problems in dogs. In Egypt, the season of the year had no significant effect on its prevalence; however, the peak incidence is generally recorded during the summer (Rakha *et al*., 2015). In Norway, canine gastroenteritis showed seasonal variations with summer as the period with the highest prevalence (Sævik *et al*., 2012).

There is no clear-cut association (α=0.05) of gastroenteritis with either wet or dry season in Nigeria (Figure 4.3). However, with an α-value of 0.05 and higher prevalence registered during the dry season (54.1%), the chance of a significant association between season and prevalence cannot be dismissed (Dahiru, 2008; Thisted, 2010). Environmental stress due to harsh weather, experienced in Nigeria usually during the dry season, predisposes animals to infections, hence the higher prevalence of

gastroenteritis recorded in the dry season (54.1%) compared to wet season (45.9%). This finding lends credence to existing reports in Nigeria (Shima *et al*., 2015b; Gberindyer, 2016).

The diverging reports on seasonality of GI disorders could be as a result of dissimilarities in the climatic conditions from various regions, husbandry system, dissimilarities in exposure to the diverse aetiological agents as well as variations in seasons.

Changes in climate controls seasonality and occurrence of many infectious diseases. Due to changes in climate and the environment, pathogenic diseases can abruptly alter or drift from their usual trends of occurrence over time (Ricardo-Izurieta and Clem, 2008; Shima *et al*., 2015a).

Table 4.4 shows differential diagnoses for gastroenteritis cases studied. The most cited causes by the clinicians were helminthoses, CPV, dietary indiscretions, poisoning, bacterial infections, and foreign bodies. These are the well-documented causes of gastroenteritis in dogs (Leib, 2005; Gough, 2007; Shima *et al*., 2015a, 2015c). Out of the diagnoses reached in this study, only about 5.0% were made based on laboratory diagnosis, 95.0% were presumptively treated based on circumstantial evidence, such as the age of the dog, vaccination and deworming history, and previous experience of the clinicians. This shows the underutilisation of laboratory diagnoses in the management of canine gastroenteritis in Nigeria. This may be a way of cutting costs for the clients or attributed to lack of laboratories in many veterinary clinics in Nigeria. This observation corroborates the report of Hubbard *et al*. (2007), that 5.0% to 10.0% of dogs presented with gastroenteritis, the aetiology was never determined. Inappropriate diagnosis in most cases results in misdiagnosis and injudicious use of drugs (Marks, 2015).

The dogs with gastroenteritis had a mean body temperature of 39.1℃ ± 0.9 SD (95% CI: 39.0℃ to 39.2℃). This is within the normal reference range of 37.9℃ to 39.9℃ for apparently healthy dogs (Fielder, 2015). This corroborates the report of Gberindyer (2016). The explanation for this could be that mask changes in physiologic parameters made abnormal temperatures to go undetected.

This retrospective study was limited by poor record-keeping such as incomplete recording of signalment, diagnosis and therapies administered. Hence, the data used here are not homogenous enough.

Table 4.1: Prevalence of canine gastroenteritis in Nigeria.

Category	Sub-group	No. of dogs	Prevalence (%)	
			Sick dogs	Total
Sick dogs	Gastroenteritis	919	41.2	23.7
	Other sicknesses	1312	58.8	33.8
	Sub-total	2231	-	57.5
APH	-	1651	-	42.5
Total	-	3882	100.0	100.0

Index: APH, apparently healthy dogs

Table 4.2: Breed prevalence of canine gastroenteritis in Nigeria

Category	Gastroenteritis prevalence, n (%)				α–value
	Diarrhoea	Vomiting	CDV	Total (%)	
Giant breed†					
Caucasian	34	8	17	59(85.5)	0.017
Mastiff	4	4	2	10 (14.5)	
Sub-total	38(60.9)	12(11.6)	19(27.5)	69(100)	
Large breed†					
Alsatian	60	35	44	139(51.5)	0.384
Boerboel	22	12	22	56(20.7)	
Rottweiler	25	16	34	75(27.8)	
Sub-total	107(39.6)	63(23.3)	100(37.1)	270(100)	
Medium breed†					
Crosses	34	15	23	72(49.3)	0.466
Indigenous	20	18	19	57 (39.0)	
Others	5	5	7	17 (11.7)	
Sub-total	59(40.4)	38(26.0)	49(33.6)	146(100)	
Small breed†					
Lhasa Apso	2	7	3	12(40.0)	0.360
Terriers	4	7	7	18(60.0)	
Sub-total	6(20.0)	14(46.7)	10(33.3)	30(100)	
Overall total	210(40.8)	127(24.7)	178(34.5)	515(100)	

Index: CDV, concomitant diarrhoea with vomiting. Others (Cane Corso = 6; Doberman = 4; Great Dane = 2; Pit bull = 4; St. Bernard = 1). Terriers (Spitz = 2; Yorkshire = 3; Pomeranian = 1; Eskimo = 1; Chow-chow = 3). Breed categories† (giant, large, medium, and small breeds) showed a statistically significantly difference in susceptibility to gastroenteritis (α=0.014).

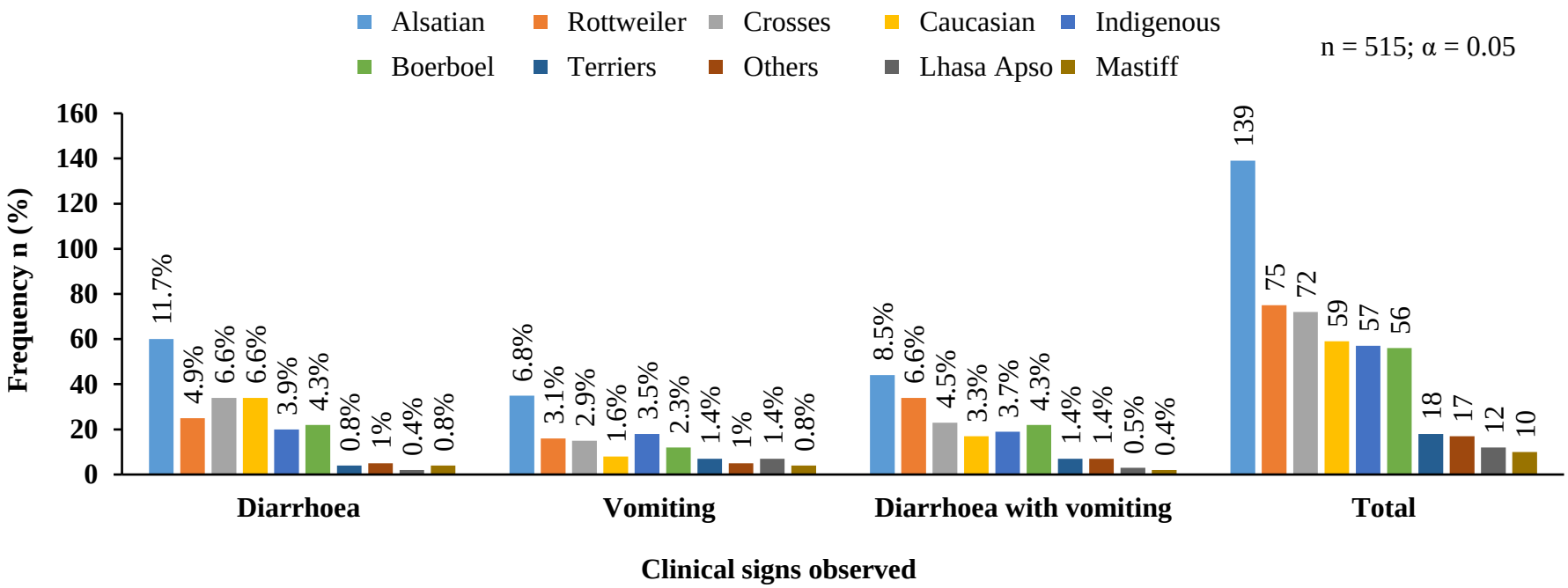

Figure 4.1: Breed susceptibility to the incidence of gastroenteritis

Index: Others = least represented breeds (Cane Corso = 6; Doberman = 4; Great Dane = 2; Pit bull = 4; St. Bernard = 1). Terriers (Spitz = 2; Yorkshire = 3; Pomeranian = 1; Eskimo = 1; Chow = 3)

Table 4.3: Influence of dog characteristics on the prevalence of gastroenteritis

Breed category	Gastroenteritis signs, n (%)				α–value
	Diarrhoea	Vomiting	CDV	Total	
Sex					
Male	117	65	89	271(52.8)	
Female	98	60	84	242(47.2)	0.825
Total	215(41.9)	125(24.4)	173(33.7)	513(100)	
Age (months) *					
0–6	114	57	108	279(63.1)	
7–12	24	12	18	54(12.2)	0.165
≥13	39	35	35	109(24.7)	
Total	177(40.1)	104(23.5)	161(36.4)	442(100)	
DHLPP vaccination history					
Vaccinated	35	24	40	99(14.2)	
Unvaccinated	35	14	28	77(11.0)	0.047
Uncertain	268	106	149	523(74.8)	
Total	338(48.4)	144(20.6)	217(31.0)	699(100)	

Index: CVD, concomitant vomiting with diarrhoea; n, the frequency of occurrence; *the mean age of the dogs was 11.79±19.05 SD (95% confidence interval (CI): 10.01 to 13.57), the median of 4 months, and a range of 1 to 156 months. DHLPP = canine distemper, hepatitis, leptospirosis, parvovirus polyvalent vaccine.

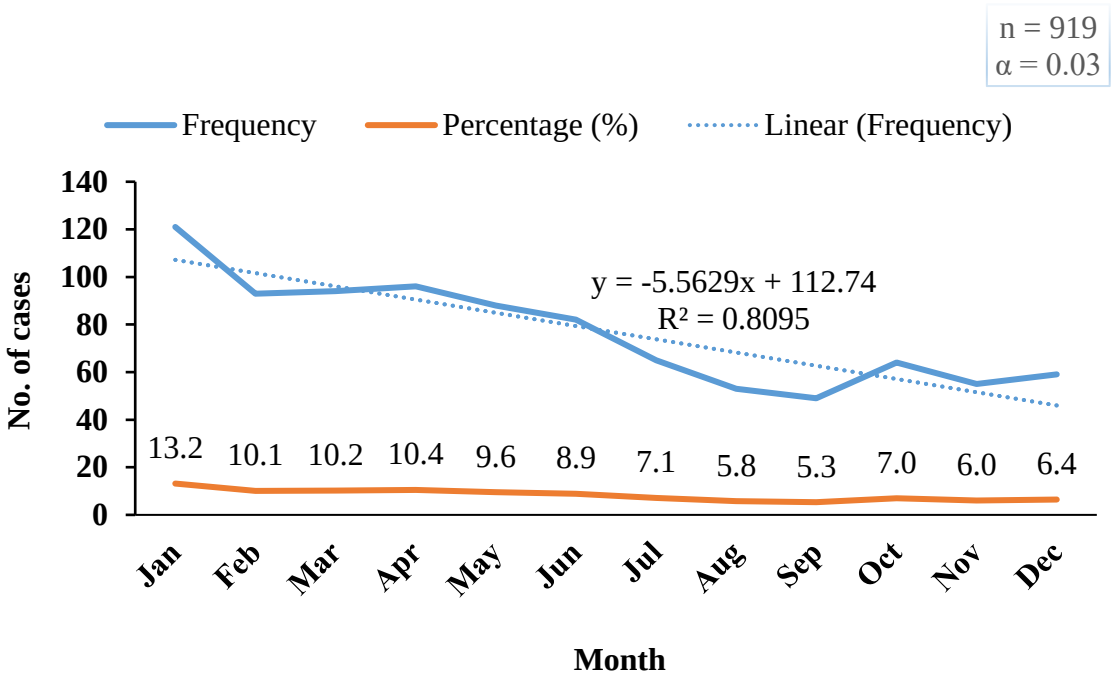

Figure 4.2: Monthly incidence of canine gastroenteritis in Nigeria. recorded from January to December 2016

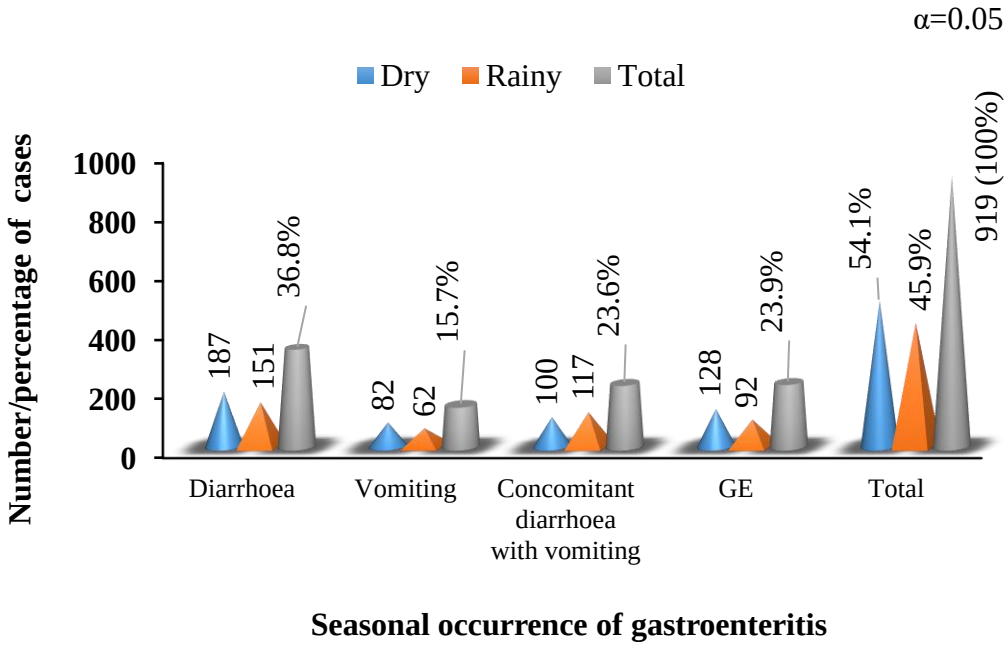

Figure 4.3: Seasonal prevalence of canine gastroenteritis in Nigeria recorded from January to December 2016

Index: GE, no diagnoses were reached and no data on clinical signs other than gastroenteritis

Table 4.4: Common causes of canine gastroenteritis diagnosed in Nigeria

Diagnosis	Clinical presentation							
	All findings		Diarrhoea		Vomiting		CDV	
	n	%	n	%	n	%	n	%
Helminthosis	232	40.4	157	27.4	41	7.1	34	5.9
Canine parvovirus	203	35.4	55	9.6	20	3.5	128	22.3
Poisoning	46	8.0	28	4.9	11	1.9	7	1.2
Dietary indiscretions	17	3.0	11	1.9	4	0.7	2	0.4
Bacterial infections	15	2.6	6	1.1	9	1.6	-	-
Haemorrhagic enteritis	17	3.0	6	1.1	-	-	11	1.9
Foreign bodies	12	2.1	1	0.2	10	1.7	1	0.2
Leptospirosis	11	1.9	4	0.7	2	0.4	5	0.9
Drug toxicity	4	0.7	1	0.2	2	0.4	1	0.2
Canine distemper	3	0.5	1	0.2	1	0.2	1	0.2
Liver diseases	2	0.3	1	0.2	-	-	1	0.2
Aflatoxicosis	2	0.4	-	-	-	-	2	0.4
Oestral changes	2	0.4	-	-	2	0.4	-	-
Intussusception	2	0.4	-	-	2	0.4	-	-
Motion sickness	2	0.4	-	-	2	0.4	-	-
Constipation	1	0.2	-	-	1	0.2	-	-
Gastric ulcers	1	0.2	-	-	1	0.2	-	-
Pyometra	1	0.2	-	-	1	0.2	-	-
Renal disease	1	0.2	-	-	-	-	1	0.2
Total	**574**	**100**	**271**	**47.2**	**109**	**19.0**	**194**	**33.8**

4.2 Retrospective study of drug prescription patterns for presenting dogs with gastroenteritis in Nigeria

Data evaluating therapies used in managing canine GI diseases in veterinary practices are largely limited in Nigeria and Africa at large. A total of 537 dog patients with treatment data available were assessed for drug prescription patterns. In this report, 45.8% of patients diagnosed with gastroenteritis were prescribed an average of 5.4 different drugs (ranging from 1 to 8 different drugs), excluding minerals, vitamins, fluid, and electrolytes, at a single consultation; 54.2%, 43.2% and 2.6% were prescribed between 1-3 drugs, 4-6 drugs, and 7-10 drugs, respectively (Figure 4.4). This study showed that polypharmacy practice and injudicious prescriptions were common in the clinical management of canine gastroenteritis in Nigeria. Polypharmacy as used here refers to the prescription of more drugs above the medical need and overall safety or wellbeing of an animal patient. These findings corroborate report on the management of CPE in which 67.1% of the dog patients were prescribed at least four drugs (Gberindyer *et al*., 2017). The prescribing habit was significantly ($\alpha<0.001$) associated with the clinical signs of gastroenteritis observed. Patients presented with concomitant vomiting with diarrhoea (21.1%) were more prone to polypharmacy compared to patients presenting with vomiting (7.8%) or diarrhoea (14.3%) only, probably, the clinicians considered such cases as more serious or perceived multiple aetiological agents involvement. Vomiting and diarrhoea are critical means of fluid and electrolytes loss in patients with gastroenteritis. Notwithstanding, setting limits on the number of drugs prescribed to a patient to take at a time will reduce the drug-to-drug interaction and effects on safety and therapeutic success, yet, no such limits are available in veterinary medicine. According to Veehof *et al*. (2000), some patient's illness may warrant the prescription of multiple drugs, especially seriously ill patients to alleviate associated clinical signs. Nevertheless, this can have adverse effect on compromised liver and kidneys, as well as on the general welfare of the animal. Despite its merits and demerits, polypharmacy should be considered an emerging issue requiring a rational and structured approach to addressing it in canine practice.

In Figure 4.5, antibacterials, antiparasitics, and antiemetics were extensively used in 75.4%, 56.1%, and 30.0% respectively of the 537 patients with treatment data available. This may be to putatively control secondary bacterial infection, helminths and vomiting common in these cases.

Dexamethasone (16.2%) was the steroid of choice used commonly in the patients sampled (Figure 4.5) and was prescribed at a rate of 5.2% (Figure 4.6). These findings concur with the findings of German *et al*. (2010), in which steroids, particularly dexamethasone and prednisolone, were prescribed in 19.0% of the cases. These drugs and other immunosuppressive /immunomodulatory drugs are used in managing chronic diarrhoeas. They exert their activities by reducing IL-2 production from T-cells, thereby reducing the proliferation of T-cells and consequently B-cells (Whitley and Day, 2011). This probably informed their use in the studied dogs.

Antimicrobials were the most extensively prescribed drugs in dogs presented with GI disorders. About 75.4% out of 537 dogs studied were prescribed at least one antibacterial agent (Figure 4.5), at a prescription rate of 48.3% out of 1669 prescriptions made compared to other drug categories (Figure 4.6). This finding is comparable to a report from the United Kingdom in which 71.0% of dogs with diarrhoea were prescribed at least an antibacterial by the first-opinion practitioners (German *et al*., 2010), a 42.3% prescription rate of antibiotics in the management of CPE in Nigeria (Gberindyer *et al*., 2017), and report of irrational use of antimicrobial in the United States of America (Ekakoro and Okafor, 2019).

Metronidazole (23.8%), gentamicin (22.0 %), oxytetracycline (17.6%), and amoxicillin (17.2%) were the extensively prescribed antibacterials out of the 806 prescriptions made (Figure 4.7). Canine parvovirus enteritis remains a very common and highly prevalent viral disease in Nigeria (Shima *et al*., 2015b). In a survey of drugs used in the management of parvovirus enteritis in Nigeria, amoxicillin, gentamicin, metronidazole and oxytetracycline ranked in the topmost prescribed antibacterials (Gberindyer *et al*., 2017). These drugs are cheaper and effective against *E. coli*, *Campylobacter spp*, *Salmonella spp*, and other aerobic and Gram-negative bacteria are significant causes of enteropathies in man and animals (Baxia, 2010). Apart from its activity against anaerobic bacteria, metronidazole remains a drug of choice in cases of giardiasis, protozoan diseases associated with many cases of diarrhoea and occasional vomiting in dogs (Vesy and Peterson, 1999). Perhaps these could have informed the choice and their extensive prescription in the management of canine gastroenteritis by the clinicians.

Antibacterials with the lowest prescription rates (Figure 4.7) were amoxicillin-clavulanate, doxycycline and sulphadimidine with/without trimethoprim.

Fluoroquinolones (ciprofloxacin – 2.5 % and enrofloxacin – 1.4%) were occasionally used. This finding corroborates previous reports (German *et al*., 2010; Gberindyer *et al*., 2017). This low fluoroquinolones prescription rate may be equated to their ability to cause damage to growing cartilage, as well as their contraindication in growing animals (Prittie, 2004; Plumb, 2008). Fluoroquinolones also cause damage to the 8th cranial nerve and vestibulocochlear nerve affecting balancing and vision, and are nephrotoxic. Majority of the patients were young growing dogs hence were considered unsuitable for fluoroquinolones prescription. Fluoroquinolones are recommended in cases that have failed to respond well to other categories of antibacterials, as to minimise antimicrobial resistance (BVA, 2015).

The top five GI and hepatobiliary antibiotics of choice recommended for small animals are enrofloxacin, metronidazole, tylosin, amoxicillin-clavulanic acid and neomycin (Webb, 2018). As good as antibiotics are in the management of gastroenteritis in animals, they are associated with many adverse drug events as well as antibiotic resistance (Iraguen *et al.*, 2010; Bigby, 2011; Voie and Lavergne, 2012; Gberindyer *et al.*, 2018); therefore, their use should be judicious, purposefully, and ideally used as directed by antimicrobial susceptibility test results.

An average of 1.5 antibacterial was prescribed to patients with gastroenteritis during a single consultation. However, the clinicians were significantly ($\alpha<0.001$) unlikely to prescribe a combination of four different antibacterial agents to patients with canine gastroenteritis at a time. Approximately 27.2% of the patients with canine gastroenteritis were prescribed above two different antibacterials in a treatment regimen (Figure 4.8). What informed the decision to use more than two antibacterials remains unexplained. These therapies may deviate from the specified recommendations for prudent use of medicines (The European Union, 2015; BSAVA, 2020). The extensive use of antibacterials was observed previously in general and specialised veterinary practices (Rantala *et al*., 2004) and the practice has continued to this day. In this investigation, only 10.1% of the gastroenteritis cases were suspected to be caused by bacterial infections, yet, antibacterial agents were the therapy of choice in 75.4% dogs. The antibacterial drugs mostly were empirically prescribed with no confirmation of the causative infectious agents involved. This could be an attempt to eliminate putative bacteria suspected as the cause of the gastroenteritis or to prevent secondary invaders.

This also suggests that therapies in clinical practices deviate from recommendations of administering antibacterials only after culture and sensitivity test. Antibacterials use in canine gastroenteritis is limited to animals manifesting systemic clinical signs of illness such as alterations in body temperature, or where a specific pathogen has been isolated, or where the GI mucosa is breached, characterised by the presence of haematochezia and/or melaena (Battersby and Harvey, 2006; Hall and German, 2010; Armstrong, 2013a; Marks, 2015). Antimicrobials are usually prescribed to dogs suffering from life-threatening gastroenteritis. However, previous studies showed that clinical response failed to improve in aseptic dogs with bloody diarrhoea that received antibiotics therapy (Unterer *et al*., 2011). Owing to the upsurge in the use of antibacterials, and the rising concerns, coupled with evidence of resistance to antimicrobials of bacteria isolates from pets (Normand *et al*., 2000; Carattoli *et al*., 2005; Lloyd, 2007; Papich, 2010; BSAVA, 2020), diet modification, anthelmintics and probiotics supplementation, and addition of an antidiarrheal in some cases, have been suggested as the first-line therapies for acute diarrhoea, besides prudent use of antibacterials (Armstrong, 2013a).

Figure 4.9 presents the types of antiparasitic drugs prescribed for patients diagnosed with gastroenteritis in Nigeria. Endoparasites burden predispose GI disturbances culminating in nausea, vomiting and diarrhoea in an affected dog. They add stress to the patient and enhance the intestinal cell turnover, thereby worsening the severity of other GI diseases (Prittie, 2004; Humm and Hughes, 2009). Hence, this might be the rationale for the extensive use of antiparasitics (23.8%) by clinicians (Figure 4.6). Ivermectin injectable (46.2%), pyrantel pamoate (15.1%), fixed-dose ratio formulation of pyrantel pamoate, praziquantel and fenbentel (13.6%), and levamisole injectable (8.8%) were the preferred drugs. These drugs were prescribed empirically to eliminate putative invasive endoparasites. The widespread use of ivermectin and levamisole injectable may be related to their efficacy against an array of parasites, their ready availability as well as the ease of administration (subcutaneously) in vomiting patients. More so, the widespread use of ivermectin may be informed by its dual purpose of eliminating both endoparasites and ectoparasites and being more economical. The least prescribed antiparasitic drugs were oral formulations.

Antiemetics were mostly prescribed in patients with signs of vomiting (Figure 4.10); this corroborates the report of German *et al*. (2010). Antiemetics are beneficial in

vomiting patients as they improve comfort, appetite and minimises loss of body fluid (Spencer and Tappin, 2014), yet were withheld in 70.0% of the patients with vomiting. This may be due to the reluctance to use antiemetics where clinical signs were perceived to be self-limiting or due to concerns over potential adverse effects (Elwood *et al*., 2010). Metoclopramide and promethazine were the major antiemetics frequently prescribed during the period under review. The prescription rate for metoclopramide was highest (94.0%) and about 16 times compared to that of promethazine (6.0%).

Antihaemorrhagic drugs and gastroprotectants were commonly prescribed when bloody diarrhoea or melaena was present. Dicynone and cimetidine were the antihaemorrhagics and gastroprotectants respectively of choice largely used in the management of canine gastroenteritis in Nigeria.

About 8.2% of the dogs with gastroenteritis were prescribed nonsteroidal anti-inflammatory analgesics (NSAIDs) probably due to the perceived visceral pains sometimes accompanies severe enteropathies. Injectable piroxicam, diclofenac injectable, acetaminophen and ibuprofen tablets were prescribed at the rates of 52.6%, 28.8%, 13.2% and 5.3%, respectively out of the 38 analgesics prescribed (Figure 4.11). NSAIDs are contraindicated in life-threatening gastroenteritis due to their negative effect of diminishing blood flow to the GI mucosa, leading to gastric ulcers and increased risk of acute renal failure in dehydrated patients (Tripathi, 2008; Leekha *et al*., 2011; Spencer and Tappin, 2014). Rather butorphanol and fentanyl are the preferred analgesics for managing pains associated with enteropathies in companion animals (Spencer and Tappin, 2014).

Antidiarrheals commonly used in the control of gastroenteritis in the patients were mist kaolin – 82.3% and loperamide – 17.7% (Figure 4.12). They are known as absorbents and motility modifiers, respectively, with some level of efficacy of the drugs in the control of diarrhoea reported in humans (Battersby and Harvey, 2006; Szajewska *et al*., 2006; Li *et al*., 2007). Loperamide can be useful in acute diarrhoea but is contraindicated in malnourished, dehydrated as well as systemically ill patients due to potential side-effects (Li *et al*., 2007). Mist kaolin and loperamide are extensively used in gastroenteritis, but their efficacy is yet to be evaluated in veterinary medicine. Mist kaolin coats GI mucosa and prevents the translocation of the enteric bacterial toxin. Opioid motility modifiers (Loperamide and Diphenoxylate) are only of benefit to

individuals with large bowel diarrhoea or colitis (Armstrong, 2013a). They are contraindicated in suspected infectious diarrhoea or diarrhoea due to toxins (Boothe, 2012; Armstrong, 2013a; Kelly, 2016) since they encourage the development of endotoxaemia when used alone (Philip, 2015), or prolong the undesirable presence of toxins in the GI tract (Boothe, 2012). These drugs were combined with antibiotics. This combination reduces the build-up and passage of infectious stools in the environment (Gberindyer, 2016).

Anticholinergic drugs are ineffective in treating acute diarrhoea (Reves *et al*., 1983). Nevertheless, atropine sulphate and hyoscine were used commonly in suspected cases of poisoning and to prevent salivation. The prescription rate of atropine – 81.0% was four times higher than that of hyoscine – 19.0% (Figure 4.13).

The therapies used in several cases seem to deviate from recommendations for prudent use of medicines. Polypharmacy and injudicious prescriptions require a rational and structured approach to addressing it in veterinary practice in Nigeria.

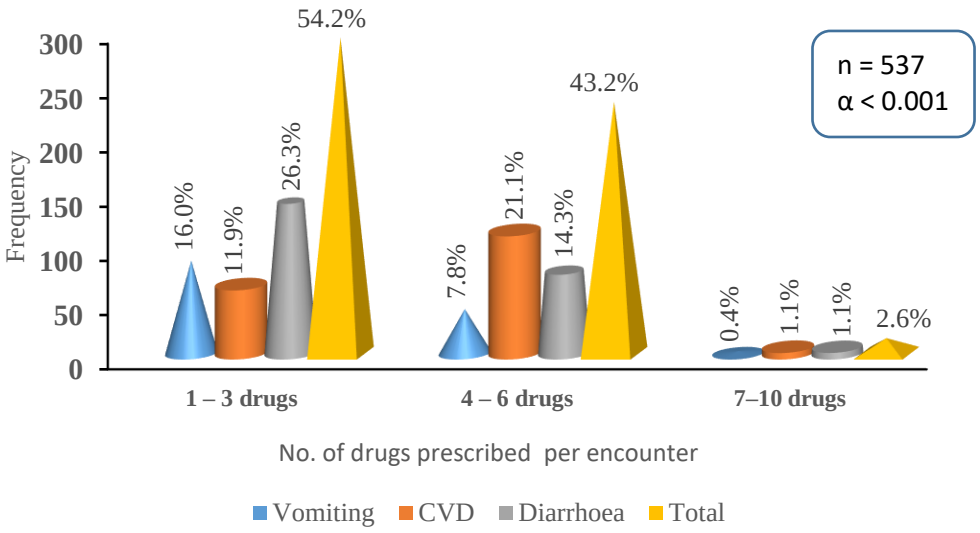

Figure 4.4: Polypharmacy practice for canine gastroenteritis patients

Index: CVD - concomitant vomiting with diarrhoea; an average of 5.4 drugs ranging from 1 to 8 different drugs was prescribed to a dog diagnosed with gastroenteritis in a single treatment regimen.

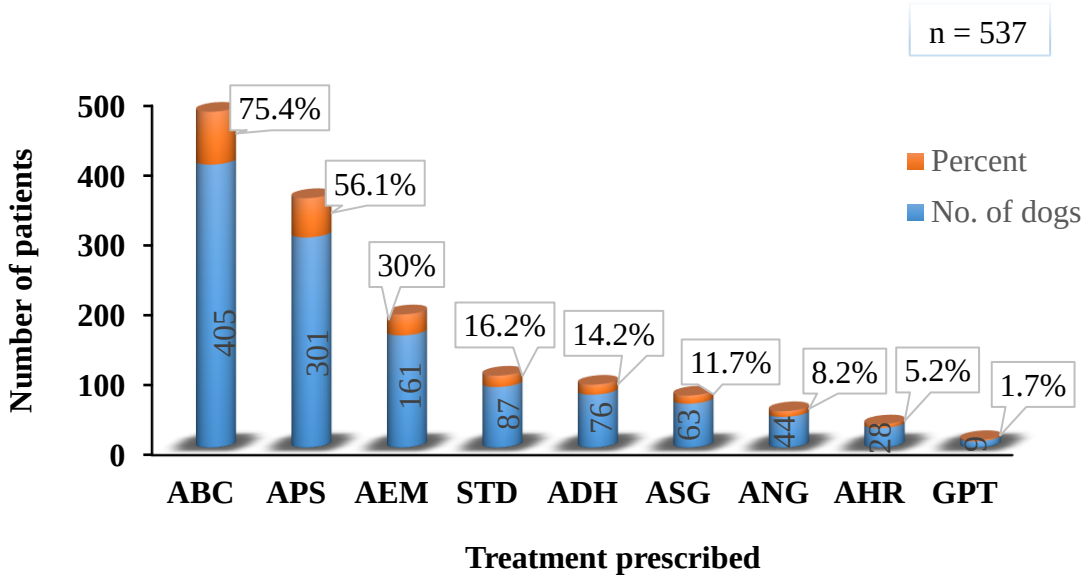

Figure 4.5: Number of gastroenteritis patients prescribed a class of drug

Index: ABC, antibacterials; APS, antiparasitics; AEM, Antiemetics; STD, steroid; ASG, Antisialogogues; ANG, Analgesics; AHR, antihaemorrhagics; GPT, gastric protectants

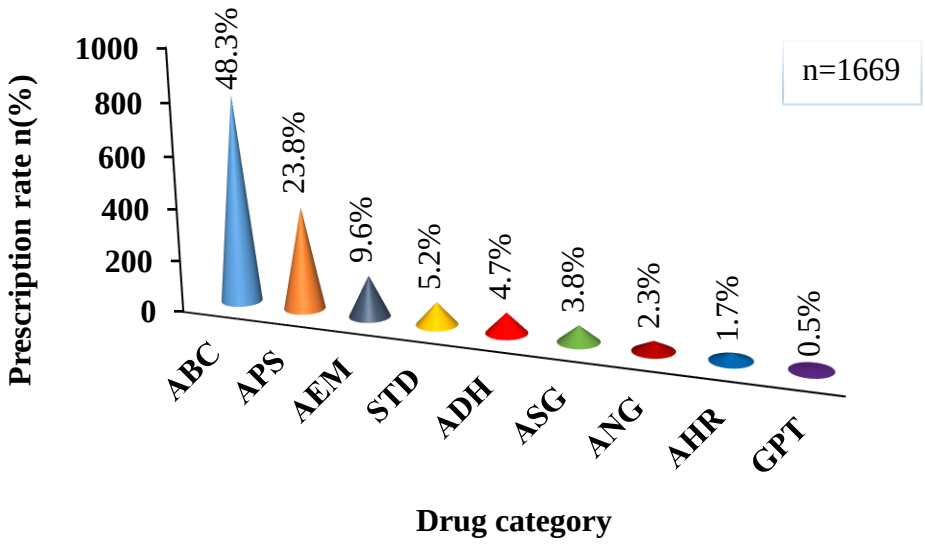

Figure 4.6: Drug prescription rate for 515 patients with canine gastroenteritis

Index: ABC, antibacterial; APS, antiparasitics; AEM, Antiemetics; STD, steroids; ASG, Antisialogogues ANG, Analgesics; AHR, antihaemorrhagics; GPT, gastric protectants

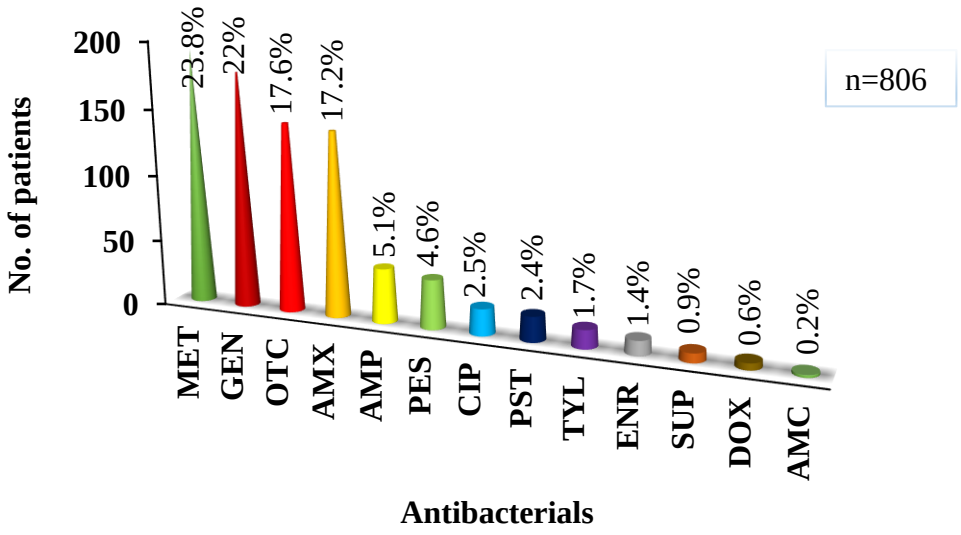

Figure 4.7: Antibacterial prescription trend for canine gastroenteritis patients

Index: MET, Metronidazole; GEN, Gentamicin; OTC, Oxytetracycline; AMX, Amoxicillin; AMP, Ampicillin-cloxacillin; PES, Penicillin-streptomycin, CIP, Ciprofloxacin; PST, Phthalylsulfathiazole (Thalazole); TYL, Tylosine; ENR, Enrofloxacin; SUP, Sulphadimidine/trimethoprim; DOX, Doxycycline; AMC, Amoxicillin-clavulanate.

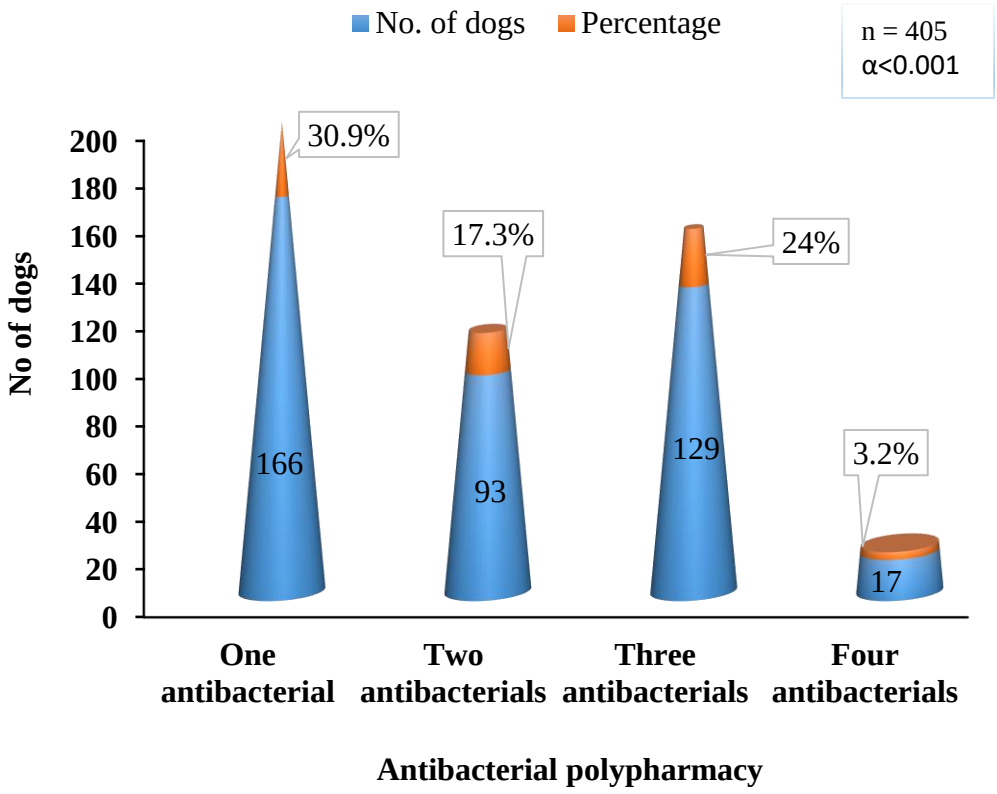

Figure 4.8: Number of antibacterials prescribed to a patient at a consultation

Index: An average of 1.5 antibacterial was prescribed to be taken at a time.

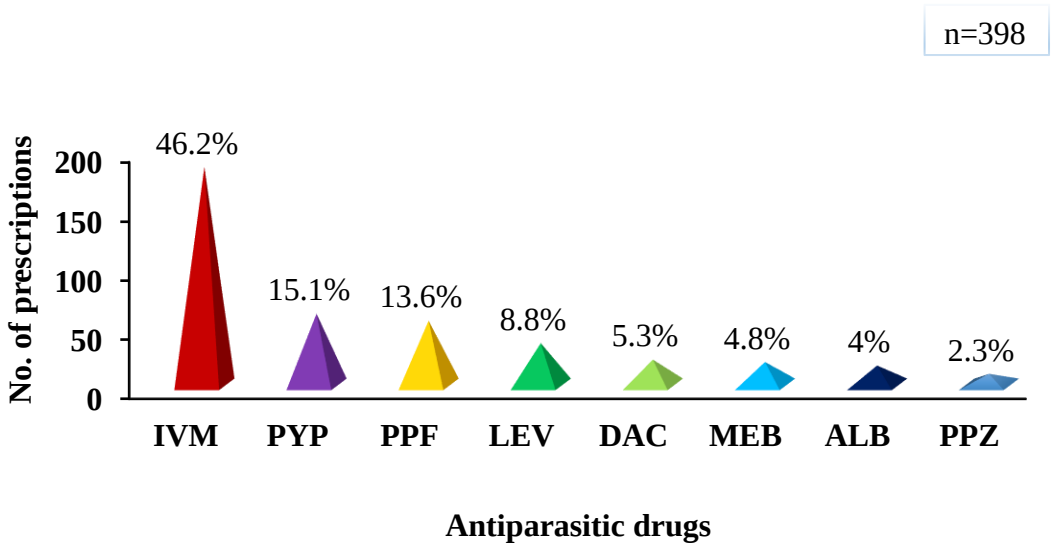

Figure 4.9: Prescription rate of antiparasitic drugs for gastroenteritis patients

Index: IVM, Ivermectin, PYP, Pyrantel pamoate; PPF, Fixed-dose ratio formulation of pyrantel pamoate, praziquantel and fenbendazole; LEV, Levamisole; DAC, Diminazene aceturate; MEB, Mebendazole; ALB, Albendazole; PPZ, Piperazine

n = 161

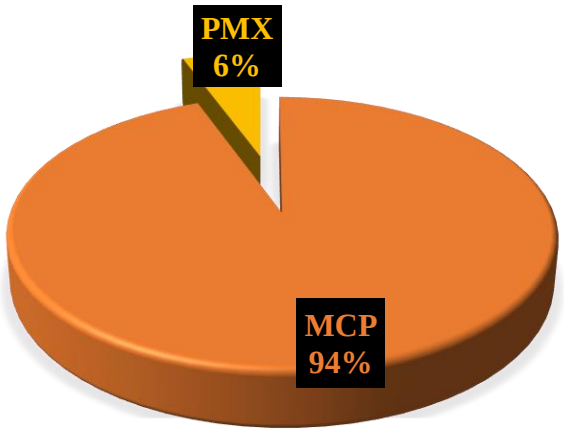

Figure 4.10: Prescription rates of antiemetics for canine gastroenteritis patients

Index: PMX, Promethazine, MCP, Metoclopramide

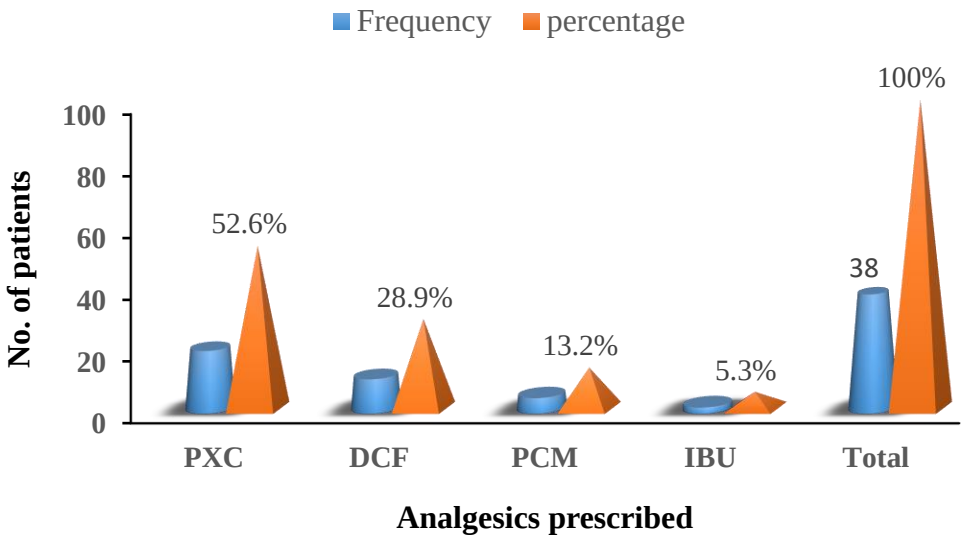

Figure 4.11: Prescription rate of analgesics for canine gastroenteritis patients

Index: PXC, Piroxicam; DCF, Diclofenac sodium; PCM, Acetaminophen; IBU, Ibuprofen

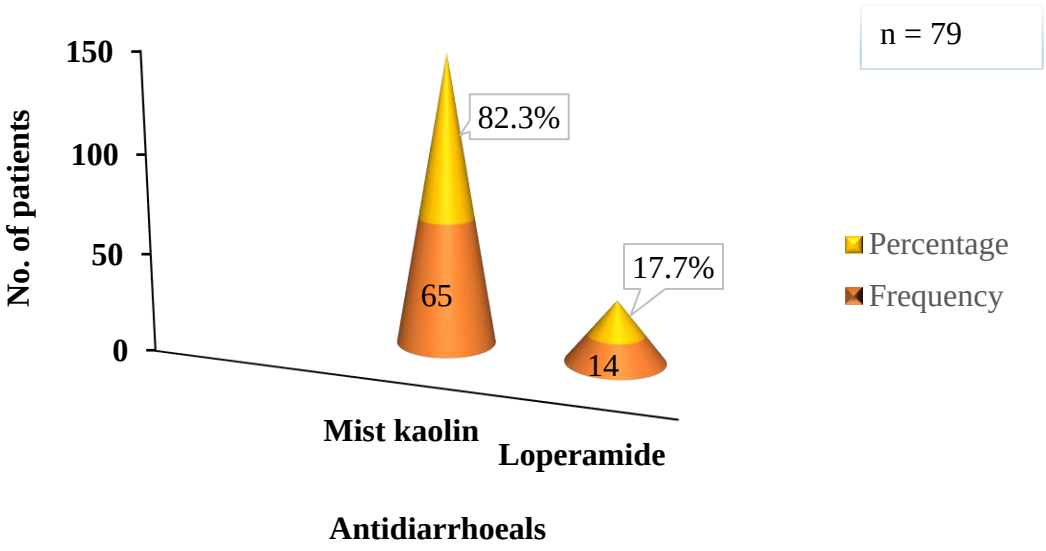

Figure 4.12: Prescription rate of antidiarrhoeals for canine gastroenteritis patients

Index: MKA, Mist kaolin; LOP, Loperamide

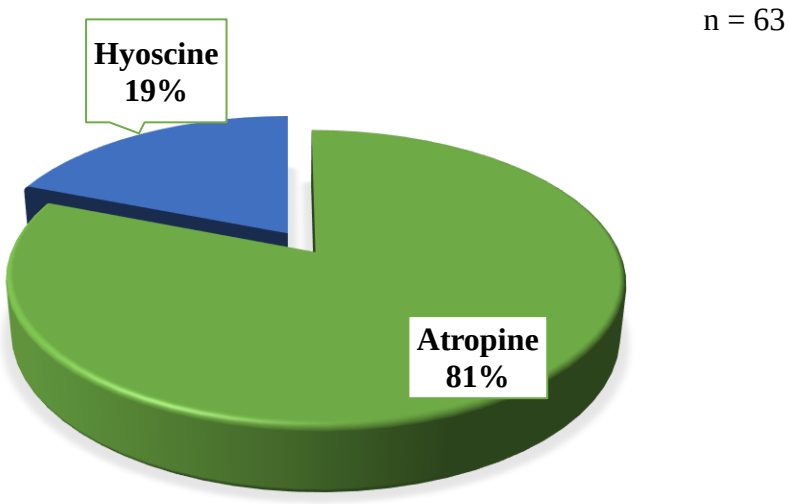

Figure 4.13: Prescription rate of antisialogogues for canine gastroenteritis patients

4.3 Clinical findings in dogs presenting with gastroenteritis in Nigeria

Results of the prospective clinical pathology study have shown that CPV (93.0%) and GI parasites, such as helminths and protozoa (12.1%) were associated with gastroenteritis in the cases studied. Canine coronavirus (2.6%), liver disease (0.6%) and undetermined causes (1.9%) were detected in smaller proportions. The GI parasites included a non-zoonotic helminth – *Isospora* and some helminths of zoonotic importance – *Ancylostoma*, *Toxocara* and *Giardia* (Table 4.5). The prevalence of 93.0% of CPV and 2.6% coronavirus differs from the report of Kempf *et al*. (2010) who found in equal proportions the prevalence of CPV (19.9%) and CCoV (17.3%) in European dogs; but comparable with the reported prevalence of 62.8% CPV and 12.8% CCoV in a study from Thailand (Sakulwira *et al*., 2003). Also, the finding of a very high prevalence of parvovirus infection reported here agrees with 61.0%, 75.0% and 96.4% a few years ago (Fagbohun and Omobowale, 2018; Adejumobi *et al*., 2017; Apaa *et al*., 2016) but much higher than 47.7% reported in Nigeria six years ago (Chollom *et al*., 2013); indicating that CPV is circulating in the Nigerian dog populations at an irrepressible rate. Therefore, CPV should be suspected in all cases of acute canine gastroenteritis and should be screened first for CPV before attempting more expensive and comprehensive diagnoses.

It is noteworthy that this is the first detection of coronavirus in Nigeria. Its low prevalence may be as a result of the unfavourable tropical conditions or geographical location that did not support its development, survival and spread in Nigeria. Coronavirus infection is more prevalent in temperate regions (Mochizuki *et al*., 2001; Castro *et al*., 2013). This reported prevalence of CCoV does not warrant vaccination as it is not a core disease of dogs in Nigeria. Further studies are desirable to sequence and characterise CCoV strains and to explore the scientific basis and benefit of vaccination before attempting its vaccination in Nigeria.

As opposed to the 12.1 % prevalence of GI parasites reported here, 62.8%, 67.1%, 71.8% and 73.3%, and 89.0% of dogs from Ghana, Tanzania, Nigeria and Ethiopia, respectively (Muhairwa *et al*., 2008; Abere *et al*., 2013; Johnson *et al*., 2015; Tion *et al*., 2016; Ola-Fadunsin, 2018) had endoparasites in their stools. The low prevalence reported here may be attributed to anthelminthic treatment which reduced the shedding of parasites ova or proliferation of parasites in the patients sampled. About 45.0% of the

patients sampled received anthelminthic treatment within the last three months prior to sickness. Also, the low prevalence may be influenced by study design, location, and detection method and husbandry practice. This study focused on clinical cases of gastroenteritis, unlike studies conducted by other researchers who focused on asymptomatic dogs.

The prevalence of *Ancylostoma* of 5.7% reported here is lower than 41.5% obtained in Nigerian previously (Tion *et al*., 2016), 48.0% from Malaysia (Mahdy *et al*., 2012), 58.9% from Thailand (Traub *et al*., 2008), 66.3% from China (Wang *et al*., 2006), and 13.2% from India (De *et al*., 2017), but slightly higher than 3.2% reported from Pakistan (Ali *et al*. 2013). Very low prevalence of *Ancylostoma* in this study is attributed to sampling of anthelminthic-treated dogs. Average Nigerian dog owners are into production for commercial purposes and imbibe the habit of deworming regularly their dogs.

Toxocara prevalence (0.6%) in the studied dogs agrees with the lower prevalence reported in time past (Adamu *et al*., 2012; Ogbaje *et al*., 2015; Adimanyi and Omudu, 2016). In contrast, other studies found higher *Toxocara* infection prevalence in some parts of Nigeria (Tion *et al*., 2016) and Ethiopia (Dagmawi *et al*., 2012).

Isospora was found to be prevalent in 4.5% of the dogs screened. This is lower than the prevalence of 11.7% and 3.1% of *Isospora* in local and exotic dogs, respectively reported by Adejinmi and Osayomi (2010) in Nigeria, and 16.3% in Pakistani dogs (Younas *et al*., 2014). In agreement with the prevalence reported herein, other investigators found in dogs *Isospora* prevalence below 10.0% (Little *et al*., 2009; Papazahariadou *et al*., 2007; Johnson *et al*., 2015).

Mixed-species *Ancylostoma* and *Isospora* infection was detected in 0.6% of the patients sampled. This agrees with reports from different authors regarding canine GI parasites mixed-species infection. However, mixed-species infection prevalence in the studied dogs is lesser than reported rates within the range of 21.3% to 89.0% (Mahmuda *et al*., 2012; Abere *et al*., 2013; Tion *et al*., 2016). This extremely low prevalence of mixed-species endoparasites infection in the dogs studied could be attributed to the sampling of anthelmintic-treated owned-dogs. Higher rates may occur in stray and unowned dogs (Johnson *et al*., 2015).

Giardia is a frequent finding in patients presenting with GI signs (Bouzid *et al*., 2015). The *Giardia* prevalence of 0.6% in this report is lower than the pooled global *Giardia* prevalence of 15.2% in canines (Bouzid *et al*., 2015) and 20.4% reported in Belgium (Claerebout *et al*., 2009). *Giardia* and *Toxocara* species are the regularly detected parasites in dogs presenting with gastroenteritis from United Kingdom (Batchelor *et al*., 2008). The global *Giardia* prevalence for dogs is in the range of 0.0% to 61.0% (Martinez-Carrasco *et al*., 2007; Himsworth *et al*., 2010; Mohamed *et al*., 2013). Reported prevalence rates which are in good agreement with 0.6% reported here are 0.0% in symptomatic and asymptomatic dogs from the US (Mohamed *et al*., 2013), 1% in asymptomatic dogs in Iran (Gharekhani *et al*., 2014), 2.0% from Cambodian dogs (Schar *et al*., 2014) and 3.0% from dogs in Romania (Amfim *et al*., 2011). Geographical position, the age and origin of the dogs, housing conditions, detection method as well as, anthelminthic treatment prior to the disease are probably the explanatory reasons for the very low *Giardia* prevalence in sampled patients (Ballweber *et al*., 2010). The only *Giardia* positive sample in this report was from a six-month-old local breed dog that has never received anthelminthic treatment, roam and scavenge freely. Dogs kept as pets and cared for have the lowest detection rate of *Giardia* (Bouzid *et al*., 2015). Symptomatic and young dogs have higher odds of detecting *Giardia* in their faeces compared to asymptomatic and older dogs (Bouzid *et al*., 2015). Diagnostic methods, such as ELISA, IFA and PCR have higher sensitivities and specificities than microscopic examination (Bouzid *et al*., 2015). Both microscopy and faecal antigen testing were used to screen the dogs sampled against *Giardia*. This point-of-care rapid faecal antigen ELISA test kit used herein has a sensitivity of 95.8% and specificity of 100.0%. About 90.5% of the dogs screened were restricted, cared for, and were dewormed (45.0%) within the past three months prior to this study. Therefore, the prevalence of *Giardia* of 0.6% reported here reasonably represents a true prevalence for the study population.

The detection of *Ancylostoma*, *Giardia* and *Toxocara* species have both clinical and zoonotic implications for the dogs, their owners, and veterinary caregivers. *Toxocara* species is associated with infection of the eyes, visceral organs, brain; covert *Toxocariosis* as well as other atopic symptoms in humans (Overgaauw and van Knapen, 2013). *Ancylostoma caninum* causes eosinophilic gastroenteritis in humans (Walker *et al*., 1995). Chronic *Giardiasis* causes debilitating gastroenteritis, as well as, increases the load of other diseases (Cotton *et al*., 2011). *Giardia* prevalence, excretion patterns

and the existence of zoonotic assemblages A and B are important for transmitting this parasite to man (Caccio *et al*., 2005). The assemblage of *Giardia* species detected was not investigated. This calls for further investigation into *Giardia* assemblage in dogs in Nigeria. Again, intestinal parasite burdens increase stress, the rate of intestinal epithelial cells destruction and worsen the severity of other intercurrent diseases (Prittie, 2004; Humm and Hughes, 2009). Clinicians, caregivers, and dog owners should be cautious about the zoonotic potential of these GI parasites when handling dogs to avoid contracting them.

Table 4.6 depicts the rate of gastroenteritis according to the demographic characteristics of the dogs studied. A total of 157 dogs weighing between 0.5 kg and 37 kg sampled comprised 12 breeds of dogs, predominantly exotic (91.8%) and few Nigerian native breeds (8.2%). Bias from the overrepresentation of purebreds than Crossbred, Nigerian native breed is due to commercialization and use of exotic dogs as security in homes and the ability of clients to afford veterinary care for exotic breeds or present them for veterinary consultation influences the skewed breed prevalence. Most dog owners and companies in Nigeria prefer exotic breeds to mix or Indigenous dogs (Shima *et al*., 2015a). This also lends credence to reports of Otolorin *et al.*, (2014) that 66.0% of dogs attending veterinary clinics in Nigeria are exotic dogs. Nevertheless, breed susceptibility of non-Indigenous dogs to infectious causes of gastroenteritis could be an explanatory variable. Genetic diversity is established as a vital determinant of susceptibility and resistance to diseases in animals (Leroy, 2011).

Regarding the age, gastroenteritis was recorded across dogs of all ages (Table 4.6). However, young dogs below 12 months were the most affected. A total of 115 (73.2%) dogs were below 7 months of age, 23 (14.7 %) were between the ages of 7–12 months, 19 (12.1%) were above 12 months. The dogs were mostly males (86: 54.8%). This finding supports the hypothesis that gastroenteritis is more common in early-life but decrease as the animals grow older (Wells and Hepper, 1999; Sævik *et al*., 2012). Furthermore, puppies have an incompetent immune system, and the maternally derived antibodies decline around the age of 12 weeks, increasing their vulnerability to infections, such as diarrhoea-related diseases (Jarret and Ramsey, 2001). Similarly, stress due to weaning, transportation, re-homing, and introduction of a new diet following weaning increase susceptibility to gastroenteritis in puppies (Tennant, 2001).

More male (54.8%) than female dogs (45.2%) were treated for gastroenteritis in this study (Table 4.6). The reason for this is difficult to justify, but this may be as a result of overrepresentation and preference of males over female dogs for security in homes and companies in Nigeria (Shima *et al*., 2015). About 52.0% of the patients had thin or poor body condition scores. This may be due to dehydrating gastroenteritis and a reduction in dietary intakes. Gastroenteritis irrespective of the aetiology causes dehydration and electrolytes imbalance.

Restricted or non-strayed dogs had the highest incidence of gastroenteritis (89.8%) than those that stray (Table 4.6). This could be biased by population distribution. DHLPP polyvalent vaccinations and therapeutic anthelminthic lowers the risk of infectious gastroenteritis in dogs (McCaw and Hoskins, 2006). However, this finding showed that gastroenteritis was much in vaccinated (60.5%) and anthelmintic (44.6%) treated dogs. This could be attributed to inappropriate vaccination or deworming protocols. Systemic anthelminthic prophylaxis meaningfully reduces the risk of helminthosis in dogs (Brunner and Swango, 1985). This study did not collect information on the type of anthelminthic treatment. Again, vaccinated dogs are less likely to develop viral enteritis in ideal situations (Kalli *et al*., 2010). Deworming benefit dogs by reducing intestinal parasites burdens (Brunner and Swango, 1985).

Table 4.7 shows the major presenting complaints and common physical findings recorded at presentation. The signs frequently observed in the dogs sampled were anorexia (84.7%), colic (6.4%), vomiting (72.0%), diarrhoea (100.0%), depression/lethargy (43.9%), dehydration (72.6%), fever (24.0%) and mucosal pallor (20.9%), corroborating the reports of Xavier de Castro *et al*. (2007b). The clinical signs could be attributed to inflammations affecting the digestive system, coupled with the release of interleukin–1 and cachectin (Hankes *et al*., 1992; Sharp *et al*., 2010). Gastroenteritis cases persisting for at least two days were presented for veterinary consultation. The dogs were presented within 2.69±1.7 SD days following observation of clinical signs. This tallies with the reports that most dog owners 'wait to see' if clinical signs persist before presenting their dogs for treatment (Hubbard *et al*., 2007; Armstrong, 2013a).

Plate 4.1 to Plate 4.3 is a pictorial presentation of the characteristic diarrhoea and vomiting observed in the patients upon presentation.

Table 4.5: Aetiologies and intercurrent infections identified in the patients

Aetiologies	Sub-category	Count (n)	*Percentage (%)
CPV	-	146	93.0
CCoV	-	4	2.6
GI parasites		19	12.1
	Ancylostoma	9	5.7
	Isospora	7	4.5
	Ancylostoma + *Isospora*	1	0.6
	Toxocara	1	0.6
	Giardia	1	0.6
Undetermined	-	3	1.9
Hepatic disease	-	1	0.6
Intercurrent diseases		48	30.6
	Acariasis	34	21.7
	Piroplasmosis	10	6.4
	Cutaneous myiasis	2	1.3
	Pneumonia	1	0.6
	Severe Otitis	1	0.6
Total		**157**	**100**

Index: *The percentage was obtained by dividing the frequency of each aetiology divided by the total animal examined irrespective of whether they occur concurrently or not.

Table 4.6: Characteristics of the patients presented with acute gastroenteritis

Variables	Category	Count (n)	Percentage (%)
Breed	Alsatian	37	23.6
	Caucasian	24	15.3
	Boerboel	25	15.9
	Rottweiler	24	15.3
	Crossbreeds	18	11.5
	Native	14	8.2
	Bullmastiff	5	3.2
	Lhasa Apso	4	2.5
	Pitbull	2	1.3
	Doberman	2	1.3
	Chihuahua	1	0.6
	Great Dane	1	0.6
Age (months)[†]	0-6	115	73.2
	7-12	23	14.7
	>12	19	12.1
Sex	Female	71	45.2
	Male	86	54.8
Body condition score	Emaciated	11	7.0
	Thin	70	44.6
	Moderate	54	34.4
	Stout	22	14.0
Lifestyle	Non-strayed	141	89.8
	Strays	13	8.3
	Mixed	3	1.9
Anthelmintic therapy in the past 3 months	Untreated	86	54.8
	Treated	71	44.6
Vaccination history	Vaccinated	95	60.5
	Unvaccinated	62	39.5
Total		**157**	**100**

Index: [†] (Mean ± SD 7.5 ± 10.2, Ranging from 6 weeks to 60 months); body weight (Mean ± SD 12.5 ± 8.8 kg, Ranging from 0.5 kg to 37 kg).

Table 4.7: Physical examination findings in the dogs presented with gastroenteritis

Variables	Category	Frequency (n)	Percentage (%)
Anorexia	-	133	84.7
Colic	-	10	6.4
Dehydration	-	114	72.6
Depression	-	69	43.9
Diarrhoea	Haemorrhagic	110	70.1
	Non-haemorrhagic	47	29.9
Lethargy	-	58	36.9
Fever	-	38	24.2
Mucosa colour	Pink	82	52.2
	Blanched	33	21
	Pale-pink	28	17.8
	Congested	13	8.3
	Jaundiced	1	0.6
Vomiting	-	113	72
Faecal consistency	Watery without solids	132	84.1
	Sloppy and mucoid	15	9.5
	Soft stool	10	6.4

Index: Rectal temperature (Mean ± SD 39.3 ± 0.9, Range = 36 to 41.7 ℃); *Heart rate (Mean = 136.7 ± 29.3 SD, Range = 74 to 188 beats/min); *Respiratory rate (Mean = 35.2 ± 16.2 SD, Range = 12 to 60 cycles/min). Average duration that elapsed before presentation was 2.7±1.7 SD days with a range of 1 to 14.

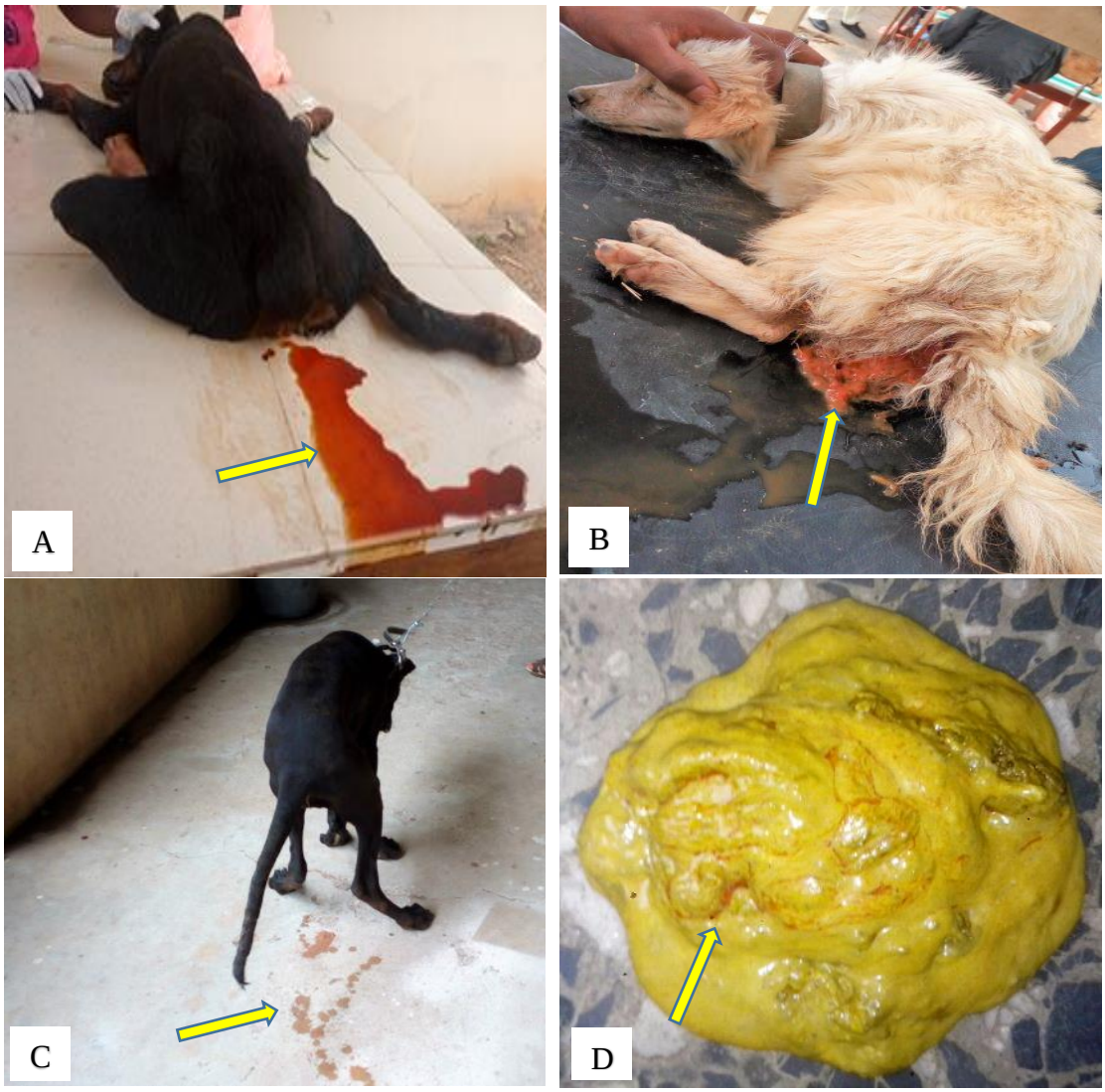

Plate 4.1: Patients presented with characteristic diarrhoea

Index: A-C = characteristic haemorrhagic diarrhoea; d = blood-tinged bulk faeces

Plate 4.2: Dogs presented with vomiting typical of parvovirus infection

Index: a–b = absence of blood in the vomitus, **c–d** = haematemesis

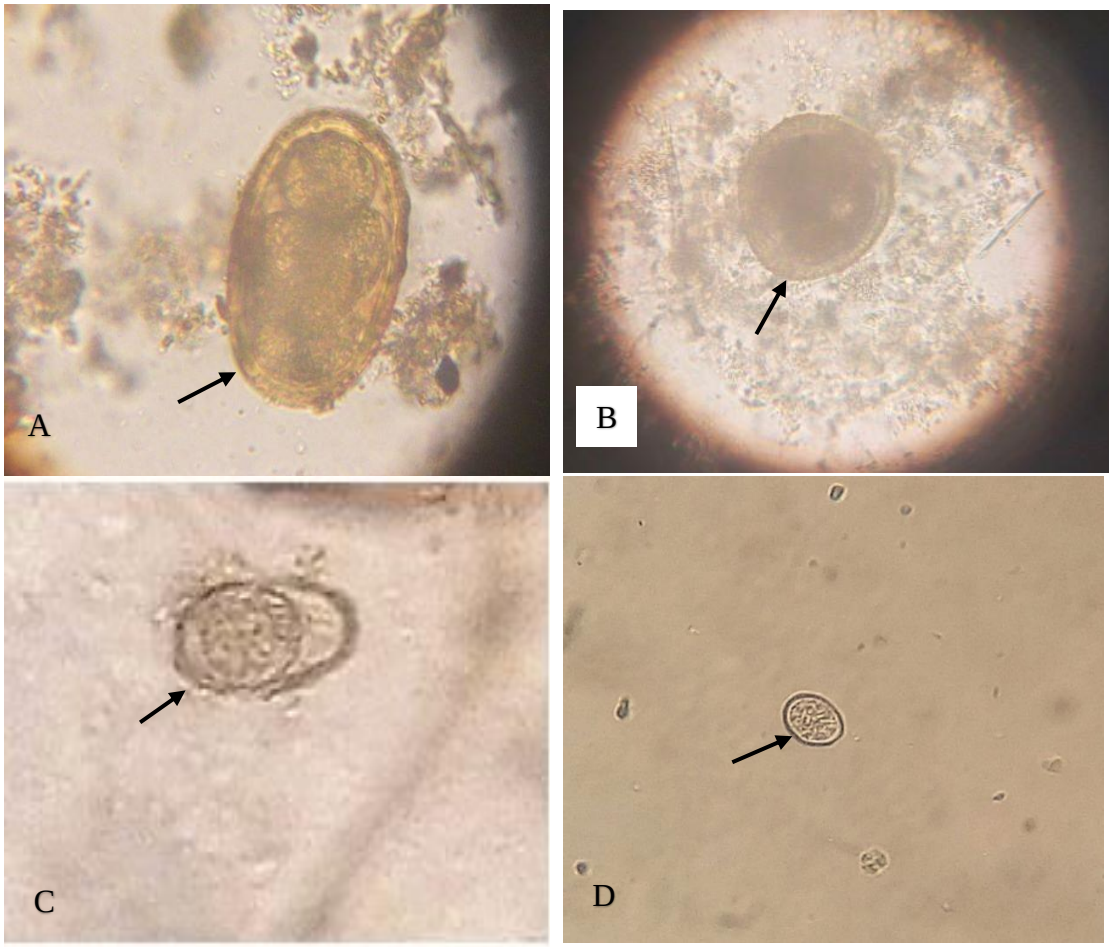

Plate 4.3: Helminths species recovered from faecal samples of some patients

Index: (a) *Ancylostoma caninum egg*; (b)*Toxocara canis* egg (c) *Isospora* species oocyst (d) unsporulated *Isospora* oocyst

4.4 Clinicopathologic responses in patients with canine gastroenteritis

Clinicopathologic studies are very limited on gastroenteritis in dogs, especially in Africa, while the available ones focused on specific diseases such as viral enteritis (e.g. CCoV and CPV infections). The present study also evaluated the clinical, haematologic, and biochemical profiles of dogs presenting with gastroenteritis, as well as assessed the determinants of clinical outcomes and the DOM in canine gastroenteritis. Treatment options used in managing the cases is shown in Appendix 7.

The haematological findings in this study were anaemia, marked thrombocytopaenia, band neutrophilia, marked lymphocytosis and mild hyperfibrinogenaemia. Mean Hb (11.6 ± 0.4), MCV (61.4 ± 0.4), MCH (20.1 ± 0.1), band neutrophils (3.2 ± 0.4), lymphocytes (27 ± 1.5) fell outside their normal reference limits, while MCHC (32.3 ± 0.2), WBC (7.8 ± 0.6), monocytes (3.4 ± 0.2), and eosinophils (2.4 ± 0.3) fell within their normal reference limits (Table 4.8). Anaemia was recorded in 47.1%, leukopaenia in 30.8%, leucocytosis in 9.6%, thrombocytopaenia in 47.1%, neutropaenia in 26.9%, lymphocytosis in 52.9%, pancytopaenia in 27.9%, hyperfibrinogenaemia in 19.2% and hypofibrinogenaemia in 15.4% of the patients with canine gastroenteritis (Table 4.9). The high prevalence of haematological changes observed here is comparable to existing findings in dogs with CPE (Kalli *et al*., 2010). About 93.0% of cases of gastroenteritis investigated were caused by CPV infection.

In Table 4.10, a comparative haematology index of the survivors with non-surviving patients obtained at first presentation revealed diminutive differences ($\alpha>0.05$). The mean haematocrit in non-survivors (34.3 ± 1.1%) was lower than that of the survivors (36.2 ± 1.7%). The MCV was below the normal reference values in both survivors (61.6 ± 0.5 fL) and non-survivors (60.8 ± 0.6 fL) despite being relatively higher in survivors. Similarly, the MCH of survivors (20.2 ± 0.1 g/dL) and non-survivors (19.8 ± 0.3 g/dL) were both below the normal reference values. Thrombocytopaenia was common to both survivors (195.8 ± 18.7 $\times 10^3$/µL platelets) and non-survivors (200.3 ± 24.4 $\times 10^3$/µL platelets). The mean segmented neutrophils were within normal reference values even though survivors have higher segmented neutrophils (66.9 ± 1.8%) compared to non-survivors (61.8 ± 3.1%). Survivors had band neutrophilia (3.3 ± 0.6%) compared to non-survivors (3 ± 0.5%). Lymphocytosis was marked in both survivors (26.2 ± 1.7%) and non-survivors (28.8 ± 2.9%) patients with slightly higher lymphocytes observed in non-

survivors. These findings are comparable to the previous reports (Kalli *et al*., 2010; Bastan *et al*., 2013). Anaemia was highest in survivors compared to non-survivors with the mean PCV being slightly above the minimum reference values. This is difficult to explain. Nonetheless, it could be that the survivors had non-regenerative anaemia and/or anaemia due to helminthosis that improved upon treatment. This unusual finding calls for further investigation. Band neutrophilia was marked in survivor patients. The most abundant white blood cells in canines are neutrophils which determine changes in the total leucocyte count. The neutrophils oversee antibody-dependent cellular cytotoxicity and the destruction of invading pathogenic organisms – algae, bacteria, fungi, parasites, yeast, and viruses (Latimer, 1995; Otto and Drobatz, 1997; Smith, 2000; Moore and Bender, 2000).

Biochemical changes observed in the dogs studied were azotaemia (mean urea = 37 ± 0.7 mg/dL), hyperglycaemia (138.4 ± 0.7 mg/dL), hyperglobulinaemia (4.7 ± 0.1 g/dL), elevated A/G ratio (2.1 ± 0.1), hypercreatinaemia (1.9 ± 0.04 mg/dL), elevated ALP (118.4 ± 1.1 u/L) and ALT (117.3 ± 1 u/L) with mean values above their upper reference limits. Besides, the mean albumin (2.41 ± 0.1) and AST (13.7 ± 0.1) concentrations were within reference limits (Table 4.11).

Azotaemia was recorded in 75.0% of the patients, hyperproteinaemia in 33.7%, hypoalbuminaemia in 37.5%, hyperalbuminaemia in 16.3%, increased A/G ratio in 55.8%, hypercreatinaemia in 45.2%, elevated ALP in 54.8%, elevated ALT in 72.1% and low levels of AST in 9.6% of the patients. Hyperglycaemia was recorded in 27.9% of the patients studied (Table 4.12).

No significant ($\alpha>0.05$) deviations occurred in the mean biochemical values between survivors and non-survivors. Both groups revealed elevated globulin, A:G ratios, blood glucose, BUN, creatinine, ALP and ALT levels with diminutive differences at the initial time of admission (Table 4.13).

Hyperglycaemia was probably not a consequence of diabetes but rather dehydration with subsequent stress and ketosis that interferes with glucose utilisation in dogs with gastroenteritis. This hyperglycaemia was transient and resolved upon rehydration. In children with gastroenteritis, it is a consistent finding (Seth and Aneja, 1995; Mallesh and Shepur, 2015). Elevated ALP, ALT, BUN, and creatinine concentrations can be

associated with dehydration, hypovolaemia resulting in deficient oxygenation of the liver, or absorbed toxins following GI barrier derangement (Berghoff and Steiner, 2011; Shah *et al*., 2013). Elevated ALP activity has also been associated with young age in canine viral infections (Macintire and Smith, 1997; Jacobs *et al*., 1980). The hyperglobulinaemia and hypoalbuminaemia observed in the patients could be attributed to reduced food intake, food indigestion and poor absorption of nutrients, and GI diseases such as CPV that causes loss of proteins, GI bleeding or decrease hepatic synthesis of albumin by the patients (Feldman and Nelson, 2004; Craven *et al*., 2011; Dossin and Lavour, 2011). Hyperproteinaemia was due to dehydration as a result of fluid loss during gastroenteritis (Odunayo, 2018).

Hypocobolaminaemia may be related to hypoalbuminaemia and poor prognosis associated with severe and persistent canine enteropathies (Allenspach *et al*., 2007). Hyperglobulinaemia recorded in some of the studied patients may be associated with hepatic acute-phase proteins production following its stimulation by leukocytes endogenous mediators released from damaged tissues and inflammatory processes (Broek, 1990). Similarly, infectious pathogens causing damage to intestinal integrity and SIRS that facilitates upsurge in vascular penetrability may be associated with PLE (Macartney *et al*., 1984; Mazzaferro *et al*., 2002). Azotaemia was probably due to dehydration that occurs following fluid loss from gastroenteritis. An elevated level of BUN concentration is a frequent occurrence in canine gastroenteritis (Jani *et al*., 1992; Bhat *et al*., 2013). This increase may reflect pre-renal uraemia owing to a reduction in glomeruli filtration rate and tissue catabolism in pyretic patients (Shinde *et al*., 2000).

The major discordant electrolyte changes recorded in the patients diagnosed with gastroenteritis at presentation were hyponatraemia (137.7 ± 0.6), hypokalaemia (4.1 ± 0.1), hypercalcaemia (11.3 ± 0.1), hypochloraemia (115.3 ± 0.7) and elevated sodium to potassium ratio (34.5 ± 0.5) as shown in (Table 4.14). Patients presented with hypokalaemia accounted for 26.0%, hyponatraemia in 61.5%, mild hypercalcaemia in 25.0%, elevated Na/K ratio in 7.7%, and hypochloraemia in 18.3 % (Table 4.15). However, the mean electrolytes values at initial admission fell within the normal reference ranges. This shows that results expressed in percentage are more informative and reliable than one expressed as mean values when assessing haematological, biochemical and electrolyte changes in a study population. The observed changes in the

mean values of electrolyte parameters between survivors and non-survivors were diminutive and not statistically significant ($\alpha>0.05$). Hyponatraemia was the only abnormality found in both groups. Other parameters were within the lower normal reference values (Table 4.16).

Hypokalaemia perhaps was due to loss of potassium ions alongside other electrolytes in diarrhoeic fluid and the inability of the colon to conserve much of this electrolyte in the diarrhoeic patients (Ettinger and Feldman, 2010; Oswald *et al*., 2015). Similarly, hypochloraemia was also associated with loss of chloride ions diarrhoeic fluid. The mild, transient hypercalcaemia recorded in the studied patients perhaps resulted from substantial but reversible dehydrating gastroenteritis or relative increase in intravascular protein concentrations, particularly albumin, following the loss of body fluid through vomiting and diarrhoea (Fan, 2007). This underscores the need to monitor total serum calcium (tCa) or ionised calcium (iCa) concentrations in canine patients presenting with gastroenteritis to institute early corrective measures to prevent complications. As opposed to the finding in this study, hypocalcaemia was reported in humans with acute gastroenteritis (Devrajani *et al*., 2009).

Hyperkalaemia may occur secondary to diabetes mellitus, hypoadrenocorticism, kidney failure or pseudo hyperkalaemia (Schaer, 1994). According to some reports, extreme leukocytosis and thrombocytosis such as seen during blood clotting process cause the discharge of substantial quantities of potassium ions into the bloodstream leading to hyperkalaemia (Chakrabarty, 2005). This could explain the reason for the hyperkalaemia recorded in one of the patients with haemorrhagic gastroenteritis studied. Alterations in the Na/K ratio were recorded in 10.7% of the patients with gastroenteritis studied. This may be associated with metabolic or systemic diseases altering electrolyte balance in the body. Hypoadrenocorticism, pyometra, pancreatic, renal diseases (Roth and Tyler, 1999), severe GI disease (Feldman and Nelson, 2004; Nielsen *et al*., 2008), besides endoparasites (DiBartola *et al*., 1985; Graves *et al*., 1994; Pak, 2000) are conditions frequently seen with low Na/K ratios. CPV and parasites were the most detected pathogens in the study dogs. At the initial time of presentation, the dogs demonstrated mainly decrease electrolyte concentrations. These findings were anticipated as diarrhoea with villous atrophy is usually accompanied by a net loss of water, Na^+, K^+, and Cl^- and nutrients inclusively (Argenzio, 1992).

Table 4.8: Mean haematology values of the patients at presentation

Parameter	Units	Mean ± SEM	Observed range	Ref. interval*
PCV	%	35.0 ± 0.9	33.2 – 36.8	35 – 57
Hb	g/dL	11.6 ± 0.4^{+}	10.9 – 12.4	11.9 – 18.9
RBCs	$\times 10^6/\mu L$	5.8 ± 0.2	5.4 – 6.1	4.9 – 7.9
MCV	fL	61.4 ± 0.4^{+}	60.7 – 62.1	66 – 77
MCH	Pg	20.1 ± 0.1^{+}	19.8 – 20.4	21 – 26.2
MCHC	g/dL	32.3 ± 0.2	32.0 – 32.7	32 – 36.3
Platelets	$\times 10^3/\mu L$	197.4 ± 14.8^{+}	168.4 – 226.3	211 – 621
WBCs	$\times 10^3/\mu L$	7.8 ± 0.6	6.6 – 9.1	5 – 14.1
Neutrophils	%	63.9 ± 1.6	60.8 – 66.9	58 – 85
Band neutrophils	%	3.2 ± 0.4^{+}	2.3 – 4.1	0 – 3
Lymphocytes	%	27 ± 1.5^{+}	24.1 – 29.9	8 – 21
Monocytes	%	3.4 ± 0.2	2.9 – 3.9	2 – 10
Eosinophils	%	2.4 ± 0.3	1.7 – 3.0	0 – 9
Basophils	%	0.0	0.0	0 – 1
Fibrinogen	mg/dL	288.2 ± 18.2	252.5 – 323.9	150 – 300

Index: $^{+}$Values outside normal reference range; *Reference range (Fielder, 2015)

Table 4.9: Number and percentage of patients with haematologic alterations

Changes	Units	n (%)	Observed range	Ref. range*
Anaemia (PCV)	%	49 (47.1)	26 – 28.9	35 – 57
Haemoconcentration	%	1 (0.9)	–	35 – 57
Low Hb	g/dL	41 (39.4)	8.8 – 10	11.9 – 18.9
Low RBC	$\times 10^6/\mu L$	25 (24.0)	3.8 – 4.5	4.95 – 7.87
High RBC	$\times 10^6/\mu L$	9 (8.6)	8.5 – 8.8	4.95 – 7.87
Low MCV	fL	60 (57.7)	60.1 – 61.2	66 – 77
Low MCH	pg	59 (56.7)	19.5 – 20	21 – 26.2
Low MCHC	g/dL	13 (12.5)	28.8 – 31.1	32 – 36.3
Leukopaenia	$\times 10^3/\mu L$	32 (30.8)	3.4 – 4	5 – 14.1
Leucocytosis	$\times 10^3/\mu L$	10 (9.6)	15.9 – 28.8	5 – 14.1
Thrombocytopaenia	µL	49 (47.1)	122.7 – 150.5	211 – 621
Thrombocytosis	µL	1 (0.9)	–	211 – 621
Neutropenia	%	28 (26.9)	40.4 – 47.8	58 – 85
Neutrophilia	%	8 (7.7)	86.4 – 91.4	58 – 85
Lymphopenia	%	7 (6.7)	4.5 – 6.1	8 – 21
Lymphocytosis	%	55 (52.9)	34.2 – 40.6	8 – 21
Monocytopaenia	%	15 (14.4)	0.2 – 0.7	2 – 10
Monocytosis	%	3 (2.8)	10 – 14	2 – 10
Eosinophilia	%	1 (0.9)	–	0 – 9
Pancytopenia*		29 (27.9)	PCV:26.4–31.1; WBC:4.2–5.5; PLT:109.9–143.9	35 – 57 5.0 – 14.1 211 – 621
Hypofibrinogenaemia	mg/dL	16 (15.4)	–	150 – 300
Hyperfibrinogenaemia	mg/dL	20 (19.2)	446.3 – 513.6	150 – 300

Index: n (%) = No. dogs with abnormality/no. dogs examined and percentage; *Reference range (Fielder, 2015); *Pancytopenia connotes PCV <37% or <30% (for dogs older or below 5 months, respectively), WBC $<6\times10^3$/L, and platelets $<200 \times 10^3$/L (Weiss *et al.*,1999).

Table 4.10: Comparative haematology profile of survivors with non-survivors

Parameter	Unit	Non-survivors (n = 34) Mean ± SEM	Survivors (n = 70) Mean ± SEM	α-value	Reference Interval*
PCV	%	36.2 ± 1.7[a]	34.3 ± 1.1[a+]	0.361	35 – 57
Hb	g/dL	11.6 ± 0.7[a]	11.7 ± 0.5[a+]	0.962	11.9 – 18.9
RBCs	$\times 10^6/\mu L$	5.8 ± 0.3[a]	5.8 ± 0.2[a+]	0.891	4.9 – 7.9
MCV	fL	60.8 ± 0.6[a+]	61.6 ± 0.5[a+]	0.284	66 – 77
MCH	Pg	19.8 ± 0.3[a+]	20.2 ± 0.1[a+]	0.172	21 – 26.2
MCHC	g/dL	32.1 ± 0.4[a]	32.4 ± 0.2[a]	0.378	32 – 36.3
Platelets	$\times 10^3/\mu L$	200.3 ± 24.4[a+]	195.8±18.7[a+]	0.887	211 – 621
WBCs	$\times 10^3/\mu L$	6.9 ± 1.1[a]	8.3 ± 0.8[a]	0.297	5 – 14.1
Neutrophils	%	61.8 ± 3.1[a]	66.9 ± 1.8[a]	0.356	58 – 85
Band neutrophil	%	3 ± 0.5[a]	3.3 ± 0.6[a+]	0.750	0 – 3
Lymphocytes	%	28.8 ± 2.9[a+]	26.2 ± 1.7[a+]	0.410	8 – 21
Monocytes	%	3.8 ± 0.5[a]	3.2 ± 0.3[a]	0.247	2 – 10
Eosinophils	%	2.8 ± 0.9[a]	2.2 ± 0.2[a]	0.426	0 – 9
Basophils	%	0	0	---	0 – 1
Fibrinogen	mg/dL	287 ± 26.9[a]	288.9 ± 24[a]	0.960	150 – 300

Index: *Reference range (Fielder, 2015). +Values outside normal reference range; Rows with similar superscripts 'a' indicate that the mean difference in haematological parameters between non-survivors and survivors are not significant ($p>0.05$)

Table 4.11: Mean biochemical values of the patients at presentation

Parameter	Units	Mean ± SEM	Observed range	Reference Interval*
Total protein	g/dL	7.7 ± 0.1	6.8 – 7.8	5.4 – 7.5
Globulin (G)	g/dL	4.7 ± 0.1^{+}	4.5 – 4.8	2.7 – 4.4
Albumin (A)	g/dL	2.4 ± 0.1	2.2 – 2.6	2.3 – 3.1
A/G ratio	---	2.1 ± 0.1^{+}	2.0 – 2.3	0.8 – 1.7
Glucose	mg/dL	138.4 ± 0.7^{+}	136.9 – 139.8	76 – 119
Urea (BUN)	mg/dL	37.0 ± 0.7^{+}	35.6 – 38.4	8 – 28
Creatinine (Cr)	mg/dL	1.9 ± 0.0^{+}	1.8 – 1.9	0.5 – 1.7
Urea/Cr ratio	---	20.4 ± 0.5	19.4 – 21.4	4 – 27
ALP	u/L	118.4 ± 1.1^{+}	116.3 – 120.6	1 – 114
ALT	u/L	117.3 ± 1^{+}	115.3 – 119.2	10 – 109
AST	u/L	13.7 ± 0.1	13.4 – 13.9	13 – 15

Index: $^{+}$Values outside normal reference range; *Reference range (Fielder, 2015)

Table 4.12: Number and percentage of patients with biochemical alterations

Abnormality	**No. of patients (%)**	**Observed range**	**Reference interval**
Hypoproteinaemia (g/dL)	9 (8.7)	4.5 – 5	5.4 – 7.5
Hyperproteinaemia (g/dL)	35 (33.7)	8 – 8.3	5.4 – 7.5
Hypoalbuminaemia (g/dL)	39 (37.5)	1.6 – 1.8	2.3 – 3.1
Hyperalbuminemia (g/dL)	17 (16.3)	3.5 – 3.7	2.3 – 3.1
Hypoglobulinaemia (g/dL)	2 (1.9)	2.3 – 2.7	2.7 – 4.4
Hyperglobulinaemia (g/dL)	69 (66.3)	4.9 – 5	2.7 – 4.4
Increased A/G ratio	58 (55.8)	2.3 – 2.7	0.8 – 1.7
Hyperglycaemia (mg/dL)	29 (27.9)	136.9 – 139.8	76 – 119
Hypercreatinaemia (mg/dL)	47 (45.2)	2 – 2.2	0.5 – 1.7
Azotaemia (Urea; mg/dL)	78 (75)	37.4 – 39.8	8 – 28
Increased Urea/Creatinine ratio	6 (5.8)	26.4 – 34.7	4 – 27
Increased ALP (u/L)	57 (54.8)	122.8 – 126.2	1 – 114
Increased ALT (u/L)	75 (72.1)	118.4 – 121.4	10 – 109
Low AST (u/L)	10 (9.6)	11.4 – 12	13 – 15
Increased AST (u/L)	2 (1.9)	–	13 – 15

Index: *Reference range (Fielder, 2015)

Table 4.13: Comparative biochemical responses of survivors with non-survivors

Parameter	Unit	Non-survivors Mean ± SEM	Survivors Mean ± SEM	Reference Interval*
N	--	34	70	--
Plasma protein	g/dL	7.2 ± 0.2^{a}	7.2 ± 0.2^{a}	6.0 – 7.5
Total protein	g/dL	7.1 ± 0.2^{a}	7 ± 0.2^{a}	5.4 – 7.5
Globulin (G)	g/dL	$4.7 \pm 0.1^{a+}$	$4.6 \pm 0.1^{a+}$	2.7 – 4.4
Albumin (A)	g/dL	2.5 ± 0.12^{a}	2.4 ± 0.1^{a}	2.3 – 3.1
A/G ratio	-	$2 \pm 0.1^{a+}$	$2.2 \pm 0.1^{a+}$	0.8 – 1.7
Glucose	mg/dL	$138.4 \pm 0.5^{a+}$	$138.4 \pm 0.5^{a+}$	76 – 119
Urea (BUN)	mg/dL	$35.6 \pm 1.3^{a+}$	$37.7 \pm 0.9^{a+}$	8 – 28
Creatinine (Cr)	mg/dL	$1.8 \pm 0.1^{a+}$	1.9^{a+}	0.5 – 1.7
Urea/Cr ratio	-	19.8 ± 0.8^{a}	20.8 ± 0.7^{a}	4 – 27
ALP	u/L	$116.9 \pm 1.6^{a+}$	$119.3 \pm 1.4^{a+}$	1 – 114
ALT	u/L	$117 \pm 1.5^{a+}$	$117.4 \pm 1.3^{a+}$	10 – 109
AST	u/L	13.5 ± 0.2^{a}	13.8 ± 0.1^{a}	13 – 15

Index: * Reference range (Fielder, 2015); +Values outside normal reference range; Rows with similar superscripts 'a' indicates the mean difference in biochemical parameters between non-survivors and survivors are not significant ($p>0.05$)

Table 4.14: Mean electrolyte values of the dogs at first presentation

Parameter	Units	Mean ± SEM	Observed range	Reference interval*
Potassium(K)	mEq/L	4.1 ± 0.1	3.9 – 4.4	3.9 – 5.7
Sodium (Na)	mEq/L	137.7± 0.6+	136.5 – 139.9	142 – 152
Chloride	mEq/L	115.3 ± 0.7	114 – 116.7	110 – 124
Calcium	mg/dL	11.3 ± 0.1	11 – 11.5	9.1 – 11.7
Na/K ratio	---	34.5 ± 0.5	33.5 – 35.5	25 – 40

Index: +Values outside normal reference range; *Reference range (Fielder, 2015)

Table 4.15: Number and percentage of patients with electrolytes imbalances

Abnormality	N (%)	Observed range	Reference interval*
Hypokalaemia (mEq/L)	27 (26.0)	3.4 – 3.6	3.9 – 5.7
Hyperkalaemia (mEq/L)	1(0.9)	–	3.9 – 5.7
Hyponatraemia (mEq/L)	64 (61.5)	134.5 – 136.7	142 – 152
Hypercalcaemia (mEq/L)	26 (25.0)	12.5 – 12.8	9.1 – 11.7
Low sodium/potassium ratio	1 (0.9)	–	25 – 40
Increased Na/K ratio	8 (7.7)	42.4 – 43.6	25 – 40
Hypochloraemia (mEq/L)	19 (18.3)	104.7 – 107.4	110 – 124
Hyperchloraemia (mEq/L)	4 (3.8)	124.8 – 126.7	110 – 124

Index: *Reference range (Fielder, 2015)

Table 4.16: Electrolyte responses in survivors and non-survivor dogs

Parameter	**Unit**	**Non-survivors (n = 34) Mean ± SEM**	**Survivors (n = 70) Mean ± SEM**	**Reference* Interval**
Potassium (K)	mEq/L	4.2 ± 0.4[a]	4.1 ± 0.1[a]	3.9 – 5.7
Sodium (Na)	mEq/L	137.7 ± 1.1[a]	137.8 ± 0.7[a]	142 – 152
Chloride	mEq/L	115.2 ± 1.3[a]	115.4 ± 0.8[a]	110 – 124
Calcium (Ca)	mg/dL	11.1 ± 0.2[a]	11.3 ± 0.1[a]	9.1 – 11.7
Na/K ratio	---	35.1 ± 1.2[a]	34.2 ± 0.5[a]	25 – 40
Ca/K ratio	---	2.84 ±0.12	2.81 ± 0.05	--

Index: *Reference range (Fielder, 2015); Rows with similar superscripts 'a' indicates the mean difference in biochemical parameters between non-survivors and survivors are significant ($p>0.05$)

4.5 Predictors of duration of management and outcome in dogs with canine gastroenteritis

Several factors correlated with the clinical outcomes of gastroenteritis in this study. Total WBC count, mucosa pallor, depression, lethargy, colic, and anaemia had a significant positive correlation with clinical outcomes. Also, albumin and DOM significantly negatively correlated (Table 4.17). Logistic regression (Table 4.18) revealed that the significant predictors of the clinical outcomes for the studied patients were changes in albumin concentration, total WBC count, and presence of colic. Alterations in total albumin (b= 1.9, OR= 7.1, α =0.006), total WBC (b= -2.3, OR=0.1, α=0.002) and the presence of colic (b= -5.5, OR=0.01, α=0.001) had 99.4%, 99.8%, and 99.9% chances, respectively of negatively influencing clinical outcomes in dogs with gastroenteritis. Hosmer–Lemeshow statistic shows that the logistic regression model used adequately describes or fits the data and predicted correctly the actual outcome in 84.3% of the categories. The patients with abnormal serum albumin concentrations have 7.1 times odds to die than patients with apparently normal serum albumin concentration, every other thing being equal. The risk of poor prognosis/death was 0.1 times greater for patients with discordant total leukocytes count than those without, all other things being equal. Colic was 0.01 times more likely to result in poor prognosis or death. In this investigation, leukopaenia and hypoalbuminaemia were the major leukocytes ($\leq 4x10^3/\mu L$) and albumin ($\leq$1.8 g/dL) alterations respectively recorded in the patients studied.

There are limited reports available on prognostication in canine gastroenteritis. This study has identified hypoalbuminemia, leukopenia, and the presence of colic at first presentation as predictors for poor prognosis in canine patients presenting with acute gastroenteritis. The findings reported here are in consonant with the reports of several other authors but also contradict some existing reports. This may be due to differences in study designs, statistical analysis used, the sample size used and period of study. Most of the available reports have focused on specific diseases associated with gastroenteritis such as viral enteritis. For dogs with diarrhoea, a good prognosis is certain when total WBC and blood pH exceeds 2 x 10^1 mm^{-3} and 7.36 respectively, and anion gap below 25 mmol L^{-1} during admission (Haligur *et al*., 2009). In CPE, total leukocytes ≥4,500 cells/µL and differential counts of ≥1000 lymphocytes/µL, ≥150 monocytes/µL, ≥100 eosinophils/µL with a shift to the left seen in the blood prior to treatment and 24 hours

post-admission is prognostic for recovery. Total leukocytes and lymphocytes have a 100% positive predictive value of a lack of cytopaenias for recovery 24 hours following admission (Goddard *et al*., 2008).

This study and others have corroborated on leukopaenia as prognostic for poor outcomes (O'Sullivan *et al*., 1984; Potgieter *et al*., 1981). In a different scenario, leukopaenia was linked to neutropaenia with a 50.0% reduction in the normal lymphocytes value (Potgieter *et al*.,1981). Thus, the authors concluded that neutrophils are more prognostic contradicting the reports of McCaw *et al*. (1996), that says neutropaenia was not a significant prognostic biomarker even when severe. Therefore, Mason *et al*. (1987) advised that leukopaenia alone should not constitute a yardstick for prognostication in patients with gastroenteritis. Similarly, leukopaenia observed at admission had no effect on the clinical outcomes (Glickman *et al*., 1985) contrasting the reports that neutropenia, leukopaenia and/or lymphopaenia are associated with poor prognosis (Brunner and Swango, 1985; Mason *et al*., 1987; McCaw and Hoskins, 2006). In CPE, this progression of leukopaenia can help in setting a prognosis (Barr, 2009). The presence of band cells in smears is indicative of a markable improvement in prognosis. The prognosis is often good to excellent in puppies that have been treated and survived the initial three days. Most patients studied here were presented within the first three days of the clinical onset of the gastroenteritis.

Colic decreases the chances for survival in dogs with gastroenteritis studied. Colic indicates the severity of the gastroenteritis following inflammation of the GI mucosa accompanied by secondary infections.

The DOM has a positive correlation with PCV, haemoglobin concentration, ALP, pancytopaenia and diarrhoea duration. A positive correlation is indicative of an interaction between higher values of different variables. Total WBC count, total proteins, plasma protein, sex and lifestyle of the patients correlated negatively with the DOM (Table 4.19). A negative correlation implies that higher values for one variable are interacting with lower values for the other variable.

Logistic regression analysis revealed that a discordant total WBC count ($b = 1.2$, $OR=3.5$, $\alpha=0.01$) and pancytopaenia ($b = -1.6$, $OR=0.2$, $\alpha=0.002$) were the most significant predictors impacting DOM by increasing it by at least 0.9 days above the

median duration of 5 days used in managing the clinical cases studied. The Hosmer–Lemeshow test affirmed the regression model predicted correctly the actual outcome in 70.3% of the categories. Also, the null hypothesis showed the overall predictive ability of the model for a patient spending less than 5 days or longer for treatment was 62.4%.

Gastroenteritis dog patients with pancytopaenia and altered total WBC counts had 99.8% and 99.0% chances respectively of prolonging the DOM compared to those without. Leukopaenia than leukocytosis was the major leukocyte response observed in the dogs sampled. A one-unit change in normal blood parameters to pancytopaenic state increases the odds of prolonging the DOM by a factor of 0.19, all other things being equal. Similarly, the odds of prolonging the DOM for patients with deranged total leukocytes count were 250.0% (i.e. 3.5 - 1) higher than the odds of prolonging the DOM for a patient who was presented without alterations in total leukocytes count, all other things being equal (Table 4.20).

Pancytopaenia and leukopaenia were the only clinicopathological changes at the time of presentation that significantly influence DOM in the dogs with gastroenteritis studied as opposed to the findings that vomiting, depression, lymphopaenia (<1,000/μL) and hypoalbuminaemia at the time of admission prolong the DOH in CPE patients (Kalli *et al*., 2010). Hypoalbuminaemia below 20 g/L was associated with poor prognosis in dogs with CPE (Boag, 2013). Good and Otto (2006) reported that a combination of body mass with albumin, AST, glucose, and sodium concentrations are useful in predicting the DOH in CPE. In a different study, the DOH was increased significantly with the administration of antiemetic – metoclopramide in patients diagnosed with CPE (Mantione and Otto, 2005). Prolongation of DOM associated with vomiting in the studied patients could be linked to the severity of the gastroenteritis.

Leukopaenia was the only clinicopathologic alteration at initial presentation that significantly impacted both the DOM and clinical outcomes different from reports on CPE that it influences the DOH (Schoeman and van Schoor, 2008; Kalli *et al*., 2010). Pancytopaenia at presentation was identified in this study as a significant prognosticator for prolonged DOM. Pancytopaenia occurs in several infections including CPV infection (Frezoulis *et al*., 2017). The severity of pancytopaenia significantly affects the clinical outcomes but not the DOH (Frezoulis *et al*., 2017) as opposed to the findings in this

study in which pancytopaenia significantly influences the DOM rather than the clinical outcomes in canine gastroenteritis.

Non-specific clinicopathologic responses are common in canine gastroenteritis. The mean haematologic and biochemical values between survivors and non-survivors recorded at initial presentation were not significantly ($\alpha>0.05$) different; however, changes in the total WBC counts, particularly leukopaenia ($\leq 4x10^3/\mu L$), and pancytopaenia are prognostic for a prolonged DOM. Conversely, alterations in the total WBC counts ($\leq 4x10^3/\mu L$), albumin (≤ 1.8 g/dL), and colic influence the clinical outcomes in patients with gastroenteritis. These findings agree with that of Boag (2013) who also found out that hypoalbuminaemia <20 g/L is associated with poor outcome in dogs with CPE. It worthy to note that albumin is formed primarily in the liver and it constitutes 4 - 7% or 8% protein concentration in the plasma. It is responsible for much of the colloidal osmotic pressure of the blood, immune response and acid-base balance, and is an important factor in regulating the exchange of water between the plasma and interstitial compartment. Hence, any disturbance in albumin or proper functioning of the liver, in any disease condition, could lead to poor prognosis.

Regardless of the aetiologies involved, the identified clinicopathologic responses could be useful indicators of dismal prognosis and prolong DOM; may aid clinicians in taking decisions and targeted management plan. The various treatments administered to the dogs, their dosage, frequency, and routes are detailed in Appendix 7.

Table 4.17: Clinical signs and pathology variables correlating with clinical outcome

Variables	No. of patients	Pearson correlation	α-value
Clinical pathology			
White blood cells**	104	0.4	<0.001
Albumin**	104	-0.3	0.008
Anaemia*	104	0.2	0.04
Clinical signs			
Duration of management**	104	-0.4	<0.001
Mucosa pallor*	104	0.2	0.026
Depression*	104	0.2	0.023
Lethargy*	104	0.2	0.03
Colic**	104	0.3	0.001

Index: * and ** indicates correlation is significant at 0.05 and 0.01 level, respectively. The studied variables showed low to moderate correlations with the clinical outcome.

Table 4.18: Predictors of the clinical outcome for dogs with acute gastroenteritis

Variables	B	Sig.	OR	95% CI for Odds Ratios	
				Lower	Upper
Albumin (1)	1.9	0.006	7.1	1.7	28.6
Total WBC (1)	-2.3	0.002	0.1	0.0	0.4
Colic (1)	-5.5	0.001	0.01	0.0	0.1
Constant	2.7	0.060	14.7		

Index: Reference category (1) = died; CI = confidence interval; 83 (79.8%) cases were included in the analysis. The null hypothesis shows that the overall model predictive ability for a patient to survive or die = 65.1%; Omnibus tests of model coefficients ($\chi 2 = 51.9$, $\alpha = 0.000$); Nagelkerke R-square (0.677) which shows about 70% of the outcome variants was explained by the predictors; Hosmer and Lemeshow statistic ($\chi 2 = 1.6$; $\alpha = 0.978$); The observed number of deaths was 7 with the model predicting 6.8 of patients dying from a group of 7. Again, the Hosmer–Lemeshow classification statistic shows that the model predicted correctly the actual outcome in 84.3% of the categories. This was far better than the prediction of the null hypothesis.

Table 4.19: Variables correlating with the duration of management in canine gastroenteritis

Variables	No. of patients	Pearson correlation	α-value
PCV*	104	0.2	0.033
Haemoglobin*	104	0.2	0.039
White blood cells*	104	-0.2	0.015
Pancytopenia**	104	0.3	0.001
Total proteins*	104	-0.3	0.015
Plasma protein*	104	-0.3	0.033
ALP*	104	0.2	0.05
Sex*	104	-0.2	0.017
Lifestyle*	104	-0.2	0.038
Duration of diarrhoea**	104	0.3	0.009

Index: * and ** indicates correlation is significant at 0.05 and 0.01 level, respectively. The variables showed low correlations with the DOM

Table 4.20: Predictors of the duration of management in canine gastroenteritis

Variables	(B)	Sig.	Odds ratios	95 % CI for OR Lower	Upper
Pancytopenia (1)	-1.6	0.002	0.2	0.1	0.5
WBC counts (1)	1.2	0.01	3.5	1.3	9.1
Constant	-0.1	0.882	0.9		

Index: A total of 101 (97.1%) cases were included in this analysis. The null hypothesis shows the model's predictive ability for a patient to spend <5d or >5d in hospital at 62.4%; Omnibus tests of model coefficients ($\chi 2 = 16.9$, $\alpha < 0.001$); model summary (Nagelkerke R-Square = 0.210) which shows about 21% of the outcome variants are explained by the predictors; Hosmer–Lemeshow test ($\chi 2 = 0.1$; $\alpha = 0.952$); The observed DOM was 11 days while this model predicted 10.7 days as actually day a patient with gastroenteritis spent receiving treatment, indicating the model is pretty good. Again, Hosmer–Lemeshow statistics showed the model predicted correctly the actual DOM in 70.3% of the patients. This is better than the prediction of the null hypothesis which predicted only 63.9%. Reference category = 1.

4.6 Electrocardiographic responses in dogs with canine parvovirus enteritis

Electrocardiographic assessment of 40 dogs positive for CPV comprising nine breeds: Alsatian (22.5%), Boerboel (20.0%), Rottweiler (17.5%), Crossbreeds (17.5%), Caucasian (12.5%), Bullmastiff (2.5%), Chow-Chow (2.5%), Lhasa Apso (2.5%) and the Nigerian Indigenous dog (2.5%); which were all intact males (47.5%) and females (52.5%); aged 6.8 ± 1.2 months (95% CI = 4.4 months to 9.2 months); weight of 13.1 ± 1.1 kilograms (95 % CI = 11 kg to 15.2 kg) was carried out (Table 4.21). The result demonstrates that 70.0% out of the 40 dogs with CPE studied had cardiac involvement (Table 4.22); indicating cardiac involvement has a key effect on morbidity and death in the disease.

The heart rate of 141.3 ± 6.0 bpm recorded in the studied dogs (Table 4.23) is within the normal ECG reference limits published for dogs (Bolton, 1975; McFee and Parungao, 1961; Tilley and Burtnick, 1999). However, heart rate ranging from 60 bpm to 120 bpm has been documented for dogs under normal clinical conditions (Detweiler, 2011). Abnormal heart rate above 180 bpm was recorded in 15.0% of the patients (Table 4.22). Higher values obtained in a short ECG strip are influenced by some degree of ECG duress and are regularly atypical of the unperturbed heart rate, hence should be interpreted cautiously (Detweiler, 2011). Rhythm characteristics were normal sinus rhythms in 25.0% and arrhythmias in 75.0% of the studied dogs (Table 4.24)

The mean P-wave duration of 55.3 ± 4.6 milliseconds reported here is within the normal upper limits for healthy dogs (Table 4.23); corroborating existing findings in CPE patients (Areshkumar *et al*., 2018). Atrial enlargement characterised by tall or wide P-waves in lead II (French, 2008; Detweiler, 2011) was recorded in 22.5% of the cases (Table 4.22).

The mean PR-duration of 106.9 ± 4.8 milliseconds in the studied patients is within the normal limits (Table 4.23). This also corroborates the findings reported by Areshkumar *et al*. (2018). Prolonged PR durations suggestive of first-degree atrioventricular blocks or an AV conduction delay (Tilley and Burtnick, 1999; French, 2008) was seen in 7.5% of the cases (Table 4.22).

The mean QT-duration of 206.6 ± 4.3 milliseconds and mean QTc of 309.8 ± 5.5 milliseconds were within the upper limit for dogs (Table 4.23). The QT-interval

prolongation occurred in 25.0% of the cases (Table 4.22). In contrast, Areshkumar *et al*. (2018) reported QT-intervals shortening in their study. Prolonged QT-duration in the studied patients was attributed to hyponatraemia, hypokalaemia, and hypochloraemia. This finding agrees with the reports of French (2008) but disagreed with the reports that hypokalaemia and hypochloraemia caused QT-interval shortening in non-survivor dogs with CPE (Areshkumar *et al*., 2018). The values for sodium (136.9 ± 1.1 mEq/L), potassium (3.9 ± 0.1 mEq/L), chloride (114.9 ± 1.1 mEq/L), and calcium (11.3 ± 0.2 mg/dL) were recorded in the cases studied. About 81.0%, 41.9%, and 32.3% of the dog patients were hyponatraemic, hypokalaemia and hypochloraemic, respectively. Hypercalcaemia was the only electrolyte elevated in the dogs and was recorded in 38.7% of the cases (Table 4.25).

Also, the mean QT-duration (206.6 ± 4.3 milliseconds) in this study corroborates 0.2 ± 0.01 seconds interval reported in non-diarrhoeic dogs used as control group (Areshkumar *et al*., 2018). This could mean that CPE prolongs QT-interval owing to metabolic acid-base and electrolytes imbalances. Severe diarrhoea and vomiting lead to electrolytes losses and imbalances; affecting electrocardiogram measurements such as the heart rate, QT-duration, QRS duration, T-waves and ventricular changes (Soave, 1959; Hilwig and Hahn, 1976). Hypokalaemia reduces T-wave amplitude, prolongs the QT-interval, causes ST-segment depression, atrial and ventricular tachyarrhythmias (Dibartola and De Morais, 2012; Levis, 2012).

The mean QRS duration of 52.3 ± 2.0 milliseconds in the dogs with CPE is within the normal upper limit for healthy dogs (Table 4.23), agreeing with reports by Areshkumar *et al*. (2018), who also reported values within the normal limits. The QRS complexes widening above 70.0 milliseconds were detected in 10.0% of the studied patients. The QRS widening ≥80.0 milliseconds suggest bundle branch block (BB-block). Total of 10.0 % of the patients had an incomplete right bundle branch block (Table 4.22). Increase in R-wave or S-wave duration discriminates between left or right-sided bundle branch block (Tilley and Burtnick, 1999).

The dogs displayed variable R-waves in their ECGs. However, the mean R wave amplitude of 1.5 ± 0.1 mV is within normal limits (Table 4.23). This is consistent with the reports that variable R-waves are common in viral myocarditis (Robinson *et al*., 1979; Wood, 1983). Low amplitude R-wave suggestive of low ventricular conduction

was observed in one dog. A similar finding was observed in non-survivors of parvovirus enteritis (Areshkumar *et al*., 2018). Increase in R-wave amplitude suggests left ventricular hypertrophy (Tilley and Burtnick, 1999; Detweiler, 2011). Hypertrophy of the left ventricles is characterised by wide QRS complexes, deep negative T-waves, tall R-waves in leads II, III, aVF, and V_{10} and a mean QRS axis in the frontal plane directing toward the left (Detweiler, 2011).

Increased S wave amplitude is suggestive of right ventricular enlargement (Tilley and Burtnick, 1999; Detweiler, 2011). The S wave deepening was noted in 20.0 % of the CPE cases studied (Table 4.22).

As existing reports, variable T-wave patterns were noted in the studied CPE cases (Areshkumar *et al*., 2018). These include positive, negative, and flat T-waves. Typically, wide QRS complexes, P waves absence and tall, peaked T-waves are early ECG changes reported in dogs (Tilley, 1992). Clinically, tall spiked T-waves are seen with conduction disturbances, hyperkalaemia, and ventricular enlargement. Small T-waves imply hypokalaemia. Abnormalities in T waves are regularly associated with bradycardia, myocardial hypoxia, and myocardial infarction (French, 2008). A total of 27.5% of the cases had tall T waves greater than 25.0% of R on their ECGs (Table 4.22). Because T wave remains unstable in dogs until about 12 months old, the importance of control dogs for all studies is emphasised (Detweiler, 2011). Majority of the sampled dogs were puppies less than 12 months.

The elevation of ST segment, QRS notching, small R waves, and decreased QT-duration seen in the electrocardiogram of dogs with CPE are predictive of a poor prognosis (Wood, 1983; Areshkumar *et al*., 2018). ST depression indicative of myocardial infarction was recorded in 7.5% of the patients (Table 4.22).

Ultimately, the electrocardiogram of the dogs studied revealed an increase in the P–wave, widening of QRS, prolonged QT durations, and variable T wave patterns as reported previously (Robinson *et al*., 1979; Wood, 1983; Areshkumar *et al*., 2018).

The ECG changes reported herein show cardiac involvement in the enteric form of parvovirus infection. These findings support existing published works. Myocardial damage consisting of inflammation, myocarditis, and fibrosis was reported in non-surviving young adult dogs with CPE (Ford *et al*., 2017). Similarly, survivors of acute

CPV infection were diagnosed with some structural changes in their myocardial tissues (Lenghaus and Studdert, 1984). In other scenarios, changes in cardiac markers suggestive of cardiac involvement were described in CPE dogs (Bhat *et al*., 2012; Schoeman *et al*., 2013; Cenk and Mahmut, 2015). These findings are an affirmation that cardiac involvement is common in CPE and may contribute to the high rate of mortality often reported in CPV disease. Therefore, combining electrocardiography with biochemical assay are valuable tools for diagnosing, management and monitoring of patients presenting with CPE. By combining ECG with biochemical analyses, patients at risk of parvovirus myocarditis and other cardiovascular anomalies can be detected early for proper monitoring.

The ECG strips showing some of the abnormalities recorded in some of the dogs with parvovirus enteritis are presented in Plate 4.5 to 4.7.

Table 4.21: Demographic attributes of the parvo-positive dogs studied

Category	Frequency (n)	Percentage (%)
Breed		
Alsatian	9	22.5
Boerboel	8	20.0
Rottweiler	7	17.5
Crossbreeds	7	17.5
Caucasian	5	12.5
Bullmastiff	1	2.5
Indigenous breed	1	2.5
Chow-chow	1	2.5
Lhasa Apso	1	2.5
Sex		
Female	21	52.5
Male	19	47.5
Total	**40**	**100**

Index: The mean age of the dogs was 6.80 ± 1.23 S.E. months (95% CI = 4.40 to 9.21 months); the body weight is 13.07 ± 1.06 kilograms (95 % CI = 10.99 to 15.15 kg).

Table 4.22: Electrocardiographic responses in dogs with parvovirus enteritis

ECG changes	No. of dogs	Percentage (%)	Indication
Heart rate >180 bpm	6/40	15.0	Tachycardia
Prolonged P-wave duration	7/40	17.5	Atrial enlargement
Prolonged PR duration	3/40	7.5	1° AV-block
Prolonged QT-duration	10/40	25.0	Electrolytes imbalance
Prolonged QRS duration (≤ 80ms)	4/40	10.0	Incomplete right bundle branch block
Deep Q-wave	1/40	2.5	Septal or right ventricular hypertrophy
Tall P-wave amplitude (>0.4mV)	2/40	5.0	Atrial hypertrophy
Deep S-wave (>0.3mV)	8/40	20.0	Right ventricular hypertrophy
ST-depression (>0.2mV)	3/40	7.5	Myocardial infarction
T-wave (≥25% of R wave)	11/40	27.5	Hyperkalaemia
Variable R waves heights	2/40	5.0	Electrical alternans
Total with ECG changes	**28/40**	**70.0**	

Table 4.23: Mean electrocardiographic values of dogs with CPE

Parameter	**Mean ± SE**	**95% CI.**	**Reference values***
HR (bpm)	141.3 ± 6.0	129.2 – 153.5	60 – 180
P–wave duration (ms)	52.3 ± 4.6	43.0– 61.6	30 – 60
PR-duration (ms)	106.9 ± 4.8	97.1 – 116.6	60 – 140
QRS duration (ms)	52.3 ± 2.0	48.3 – 56.3	30 – 70
QT-duration (ms)	206.6 ± 4.3	197.8 – 215.3	150 – 230
QTc (ms)	309.8 ± 5.5	298.6 – 320.9	-
Ra wave (mV)	1.5 ± 0.1	1.3 – 1.7	0.45 – 3.0
Ta wave (mV)	0.2 ± 0.0	0.1 – 0.2	-

Table 4.24: Characteristics of rhythms in dogs with parvovirus enteritis

Rhythm abnormalities	**No. of dogs**	**Percentage (%)**
Normal sinus rhythms	10	25.0
Arrhythmias	30	75.0
1. Sinus arrhythmia	20	50.0
2. Atrial arrhythmia	2	5.0
3. Tachycardia	8	20.0
Total	**40**	**100**

Table 4.25: Electrolytes profile of 31 CPE dogs with ECG abnormalities

Parameter	Units	Mean ± S.E.	Observed range	Reference range*	Imbalances n (%)
Potassium	mEq/L	3.9 ± 0.1	3 – 5	3.9 – 5.7	13 (41.9) ↓
Sodium	mEq/L	136.9± 1.1	123 – 148	142 – 152	25 (80.7) ↓
Chloride	mEq/L	114.9 ± 1.1	105 – 125	110 – 124	10 (32.3) ↓
Calcium	mg/dL	11.3 ± 0.2	10 – 13	9.1 – 11.7	12 (38.7) ↑

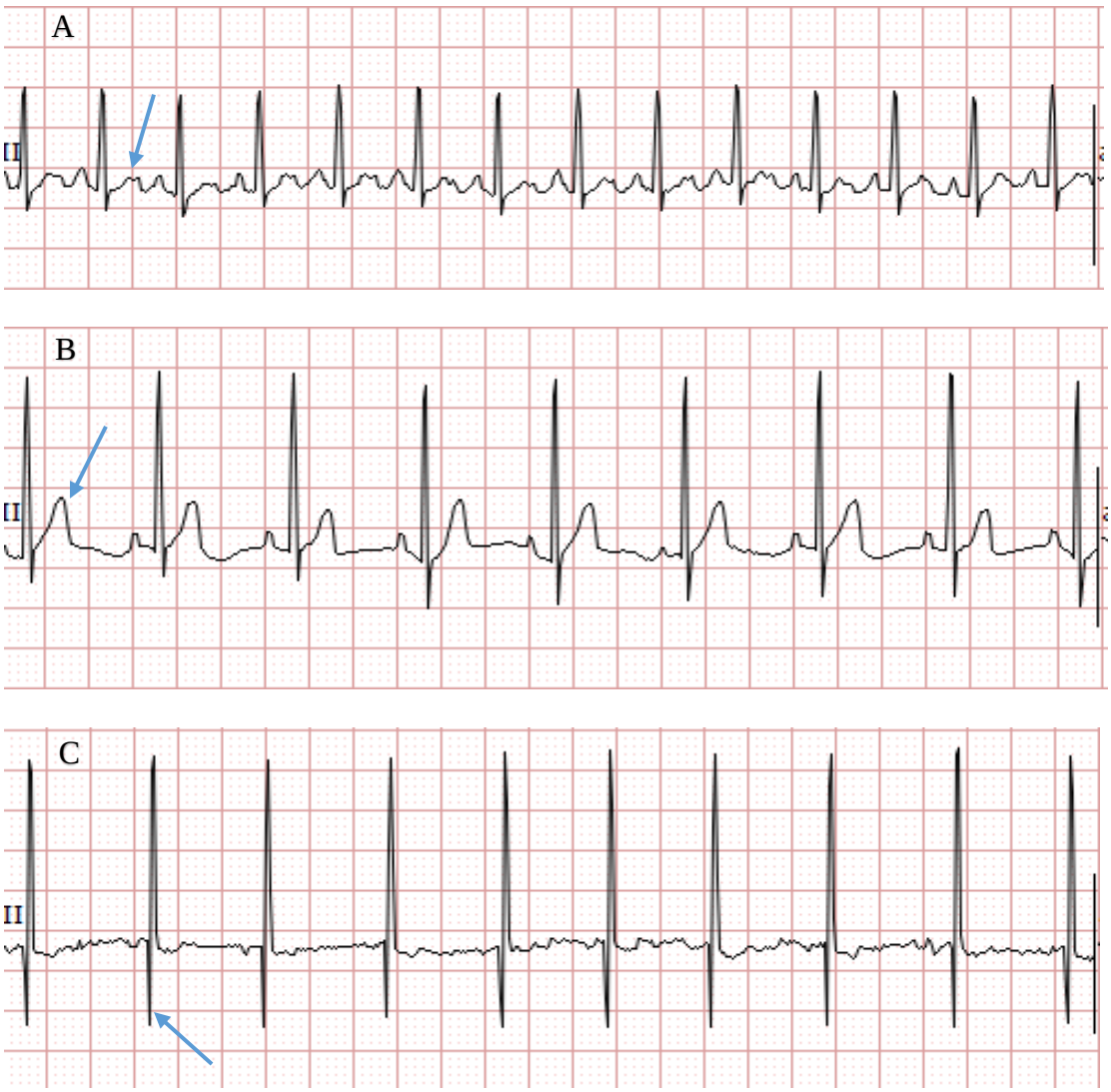

Plate 4.4: Some ECG changes in patients with canine parvovirus enteritis

Index: A: Bifid T wave with prolonged P–wave durations suggestive of atrial enlargement; **B:** Large T wave with prolonged P–wave durations suggestive of atrial enlargement; **C:** Deep Q-wave suggestive of either septal hypertrophy or right ventricular hypertrophy.

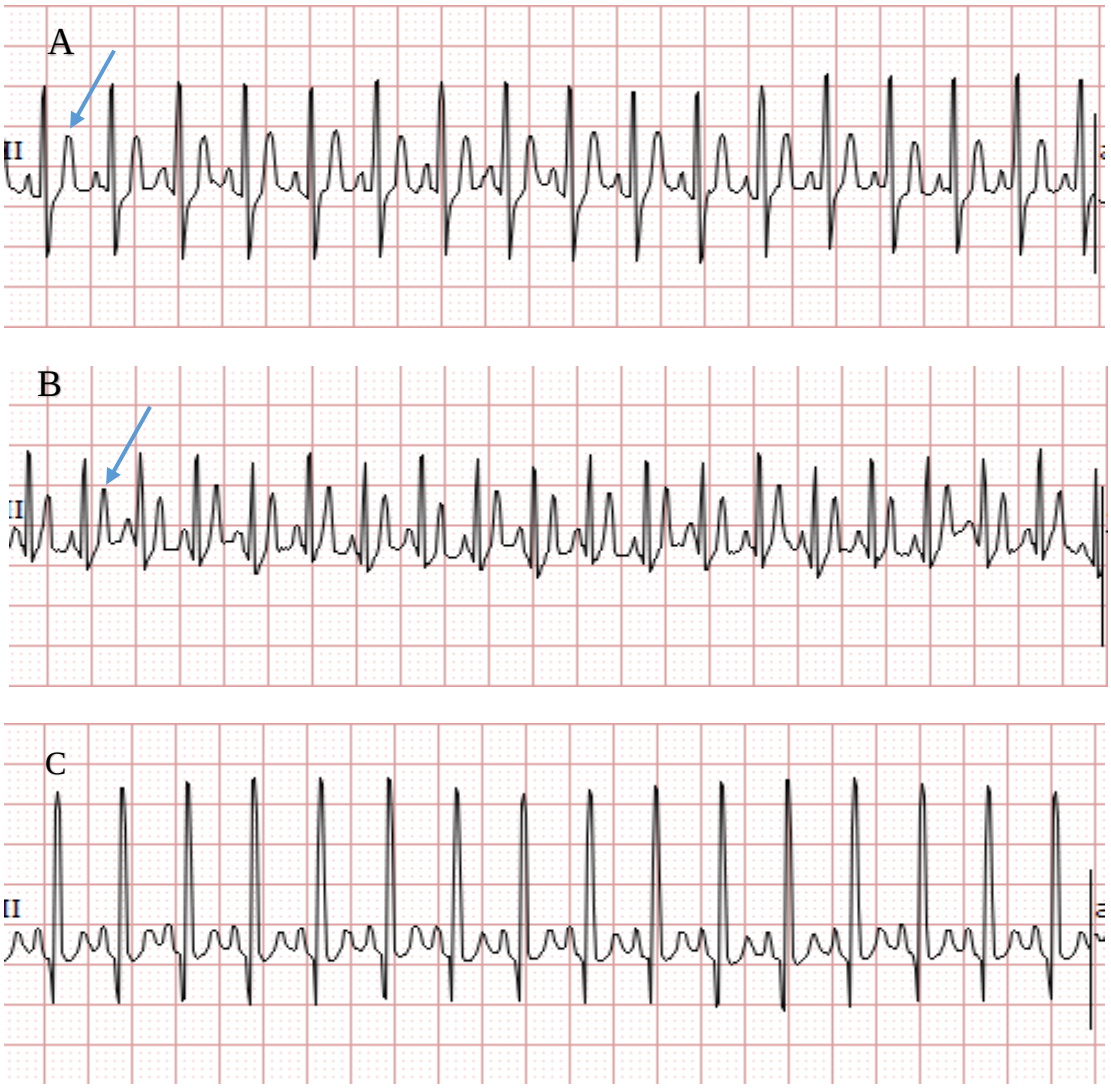

Plate 4.5: Tachycardia in dogs with canine parvovirus infection

Index: A: Tachycardia with very tall T waves, ST depression, deep S wave indicative of hyperkalaemia, myocardial infarction with right ventricular hypertrophy in a 7-month-old female Boerboel. **B:** Tachycardia with very tall T waves with QRS duration prolongation suggestive of hyperkalaemia in a 2-months-old male Alsatian **C:** Tachycardia with prolonged QRS duration indicative of incomplete right bundle branch block and electrolytes imbalance in a 9-month-old male Crossbred.

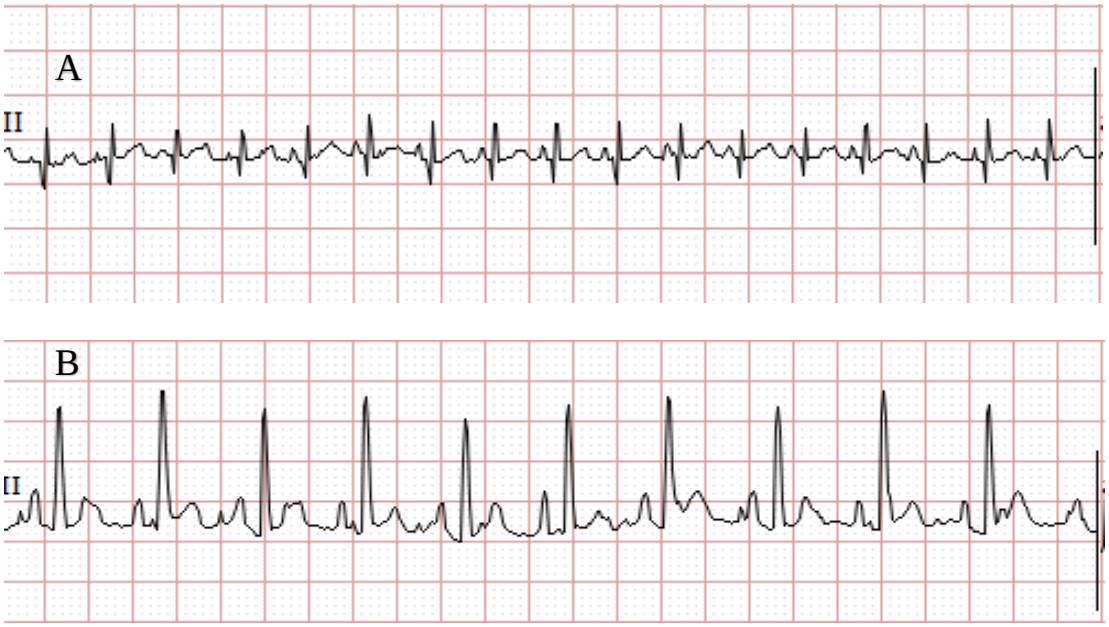

Plate 4.6: Conduction abnormalities in patients with canine parvovirus enteritis

Index: A: Low R waves amplitude in a 3-month-old female Crossbreed indicative of low ventricular conduction, **B:** Variable R wave heights suggestive of electrical alternans with tall P–wave indicative of Atrial enlargement.

4.7 Vaccine usage patterns of dog diagnosed with canine parvovirus enteritis

Prophylactic vaccination is accepted as a sure method of reducing CPV infection in dogs and is practised globally including Nigeria. The current vaccines licenced for vaccinating dogs confer adequate protection from parvovirus challenges in appropriately vaccinated dogs according to available reports (Cavalli *et al*., 2008; Eghafona *et al*., 2007; Waner *et al*., 2006; Babalola *et al*., 2016; Killey *et al*., 2017). CPV strain used in production of the vaccines does not influence vaccination failure (Altman *et al*., 2017). Nevertheless, there are confirmed and unconfirmed reports of CPV vaccination failures in Nigeria (Shima *et al*., 2015b; Apaa *et al*., 2016; Babalola *et al*., 2016). Vaccine usage patterns are rarely evaluated in Nigeria despite recurrent cases of vaccination failures.

The dog patients sampled for this analysis were administered multivalent vaccines to protect them from canine distemper, hepatitis, leptospirosis, parvovirus and para-influenza. Canine coronavirus vaccination is not practised in Nigeria. The vaccines are all imported with none manufactured locally. Hence, vaccine usage patterns for dogs diagnosed with CPE were also assessed in this study.

It was observed with these data that vaccination status was associated with CPE. About 40.1% of unvaccinated and 59.9% of vaccinated dogs constituted this study (Table 4.26). The 59.9% of CPV infection in vaccinated dogs is suggestive of occurrence of vaccination failure in Nigeria. This high rate of vaccination failure is consistent with reported infection rates of 62.0%, 64.3%, and 64.1% of CPV infection in vaccinated dogs from some parts of the world (Miranda and Thompson, 2016; Markovich *et al*., 2012; Decaro *et al*., 2007; Decaro *et al*., 2008b).

Table 4.27 shows DHLPP vaccination protocols, the number of doses, the mean age at which the puppy primary vaccination series were administered, intervals between vaccinations, and the number and percentage of cases of vaccination failure recorded. Out of the 94 vaccinated dogs with CPE, 48 (51.1%), 28 (28.7%), 18 (19.1%) and 1 (1.1%) were administered one, two, three, and four shots of puppy primary vaccines series, respectively. This is pertinent that infection rate decreased with the number of vaccine shots administered. The mean age at which the first dose of the puppy primary vaccination series was administered to the dogs sampled was 7.3 ± 2.6 weeks. However, this first dose was administered between the ages of 4 weeks and 24 weeks. The second dose was given at about 10.2 ± 2.8 weeks of age, ranging from 7 weeks to 18 weeks.

Similarly, the third booster was administered at 12.7 ± 2 weeks of age with a range of 10 weeks to 16 weeks. The average interval between vaccinations was 3.4 ± 1.8 weeks and ranged from 1 week to 8 weeks apart. Only one (1.1%) dog that was given a yearly booster dose of DHLPP had gastroenteritis. Canine parvovirus infection (93.0%) was the leading cause of gastroenteritis in this investigation.

The rate of vaccination failure reported here seems to have a significant relationship with vaccine usage patterns, such as incomplete puppy primary dose protocols. The detection rate of CPV was highest in dogs with a single-dose of DHLPP vaccination but diminishes with the higher number of doses a dog received. This stressed the need for full doses of puppy primary vaccination series. The vaccine failure perhaps was due to the inability of an incomplete puppy vaccination series to stimulate effective protective immunity. This implies that vaccine failure as observed here was associated with protocol adopted. The higher number of primary doses a puppy receives reduces cases of vaccination failure to minimal. Incomplete dose vaccination protocols reported here corroborates previous reports by some Nigerian veterinarians (Babalola *et al*., 2016, Apaa *et al*., 2016; Shima *et al*., 2015b). Several factors including cost concerns for the client could contribute to this. DHLPP vaccination costs between ₦2500 and ₦5000.

Seroconversion develops 14 days after completing the primary vaccination series and reaches its peak in about 21 days to 28 days (Decaro *et al*., 2014). Information gathered from the manuals of the vaccines also showed that the development of protective immunity commences from 3–14 days after vaccination. Most parvovirus-like enteritis during this lag phase of immunity development are triggered by field-virus (Decaro *et al*., 2007; Miranda and Thompson, 2016). This is demonstrated in studies where vaccination failures were recorded within 2 weeks post-vaccination (Miranda and Thompson, 2016; Altman *et al*., 2017). Vaccination failure most regularly occurs during this window period in dogs with immature/underdeveloped protective immunity or those with covert infections at the time of vaccination. Hence, immunologically immature puppies, those that are newly vaccinated and those that have completed their final serial puppy vaccination need to avoid dogs incubating the virus and other potential CPV-risk areas for at least 14 days. Therefore, it can be inferred from this data that the incomplete doses of puppy primary vaccine series, maternal immunity absence, and administration of “starting dose” earlier than six weeks of age are rather predisposing puppies to CPV.

Emphasis should be placed on starting vaccination at 6 weeks in puppies from unvaccinated bitches and at 8 weeks in those from vaccinated bitches.

Again, vaccination failure was associated with nonsynchronous vaccine timing. The age at which the first dose was administered to the dogs sampled was between 4 weeks and 24 weeks of age. However, the mean age (7.2±1.1 weeks) at which the initial dose was started agrees with standard recommendation (Jakel *et al*., 2012; Day *et al*., 2016). Records of over five puppies showed they were vaccinated below six weeks of age indicating inappropriate vaccine start time rather than recording error, agreeing with the report of Altman *et al*. (2017). Existing reports show that administering the start dose of the puppy primary vaccine series earlier than six weeks of age predisposes to vaccination failure (Altman *et al*., 2017). The initial puppy primary vaccination dose is administered as from six weeks or older irrespective of the vaccine brand (Jakel *et al*., 2012; Day *et al*., 2016). Administering the starting dose earlier than six weeks of age is interfered with by MDA, neutralising the vaccine, and amounting to vaccination failure (Decaro *et al*., 2005; Davis-Wurzler, 2014; Nandi *et al*., 2013). According to Iida *et al*. (1990), after birth, the MDA can protect puppies over a period of 40 to 69 days.

Furthermore, too early vaccine finish time was another factor related to vaccine timing in this study. The finished dose was given too early (12.7±1.1 wks.). This corroborates existing reports (Altman *et al*., 2017). Most vaccination failures with the current vaccines occurred when the finished dose is administered before the age of 16 weeks (Altman *et al*., 2017). Like earlier reports (Altman *et al*., 2017), some vaccine manufacturers recommend a two-dose protocol with the finished dose at 10 weeks or 12 weeks+, followed by an annual booster dose. This perhaps has contributed to the practice of a two-dose-protocol, especially by non-veterinary professionals who vaccinate their dogs themselves. The later a puppy receives the last dose of primary vaccination series, the minimal the chances of vaccination to fail (Day *et al*., 2016; Altman *et al*., 2017).

As report from the United States (Gorham, 2018), nonsynchronous intervals between vaccinations were common in this study. The recommended short period between doses (3-4 weeks) is crucial as it conforms with antibody production process and its rate of degradation in the body. Administering the vaccine too soon will not cause the immune system to generate a separate reaction as excess antibodies to the circulating strain will still be in the body, rendering the booster useless. The “booster” effect will be lost if the

subsequent doses are administered too long after the initial dose (Gorham, 2018). The current CPV vaccines are extremely effective when puppies are vaccinated in the early months of life, in a series of at least three shots starting at 6–8 weeks, with the last dose given not earlier than the 16th week of age, then followed by an annual booster (Jakel *et al*., 2012; Baker Institute for Animal Health, 2014; Day *et al*., 2016). This is because the maternal antibodies defence starts to fade as from 8 - 16 weeks of ages (Felsburg, 2002). When vaccinated at the time the MDAs are very active, the potency of the vaccine is neutralised (Nandi *et al*., 2013).

The explanation for the inconsistent intervals between vaccinations reported here could be linked to cost concerns for low-income earners who could not comply with vaccination schedules, in addition to the uncontrolled quackery in animal healthcare in Nigeria. Veterinarians should lay emphasis on strict adherence to dates of vaccination.

The vaccines licenced for use in Nigeria have different CPV tissue culture infective doses ($TCID_{50}$). Neither previous study nor this study have established evidences whether combining different brands of the vaccine triggers the same desired protective antibody titres or predisposes to vaccination failure. However, the minimum protective titre varies with the strain of the infective virus used in producing the vaccines (Truyen, 2006). Most of the vaccines will protect approximately 50% of puppies at 6 weeks of age (Friedrich and Truyen, 2000).

The incidence of CPE was lowest with a three-dose protocol and dogs that received annual booster doses compared to single or double-dose protocols. This emphasises the importance of the three-dose protocol administered appropriately for all CPV vaccines. The number of puppy primary vaccinations and post-primary vaccination has a significant relationship with the development of antibody titre against CPV (Babalola *et al*., 2016). How many doses of the primary vaccination series a puppy receives is dependent on the age, vaccine "start time", as well as, the preferred interval (Day *et al*., 2016).

The dogs that completed the full primary vaccination series and annual booster but came down with CPV infection may be poor seroconverts or absolute genetically non-responders (Killey *et al*., 2017). Genetic non-responders to CPV occur in one out of 1,000 dogs (Day *et al*., 2016). This was described in Rottweilers vaccinated with anti-

rabies and parvovirus vaccines (Kennedy *et al*., 2007; Houston *et al*., 1996). Also, too soon or too long intervals between vaccinations can only amount to vaccine wastage without making a dog mount adequate protective immunity. Again, delayed, or immature immune function, immunosuppression, poor immunogenicity, ineffective vaccine lots predispose to vaccination failure (Smith, 1995; Schultz, 2000; Heininger *et al*., 2012).

There were nine different brands of CPV vaccines identified in this study (Table 4.28). All the vaccines are multivalent. Biocan®, Pro-Vac® and Cavac® were the extensively used vaccine in this study and constituted 91.0% of the total vaccinations. Vanguard®, Megavac®, Canvac®, Romvac® and Primodog were moderately used. All the vaccines are attenuated. All the vaccines contain parvovirus strains of canine origin. The advertised maximum $TCID_{50}$ ranges from 10^3–10^7 per dose. The recommended vaccination protocol for the vaccines is a two-dose or three-dose regimen, followed by an annual booster dose. The advertised "vaccine start time" is from 6–9 weeks of age, whereas the "finish time" is between 10 weeks+ of age. The manufacturers of the DHLPP vaccines recommended vaccination interval is either 2 weeks or 3 weeks apart. Enough immunity develops over 2–4 weeks after the primary vaccination series and persists for at least one year.

Another factor that could contribute to vaccine failure in the dogs sampled is vaccination of parasites-infested dogs. Endoparasites were detected in the faeces of 12.1% of the studied patients (Table 4.5). Intestinal parasites burdens or vaccinating unhealthy puppies amounts to vaccine failure. Hence, this could justify why some of the dogs develop parvovirus-like gastroenteritis shortly after CPV vaccination. This stressed the need for deworming puppies first before CPV vaccine administration.

Vaccines may fail but before blaming it, it is pertinent to reassess the current vaccination protocol used in clinical practice to see if it is protecting or predisposing dogs to CPV infection. It seems the current vaccine protocols with a 10- or 12-week vaccine finish time is rather predisposing to vaccine failure. The incomplete doses of puppy primary vaccination series, too short or too long intervals between vaccinations are not protective. Incorporating antibody titre testing in canine practice is advised. The small sample size and study area coverage are the limitations of this study. A large prospective study is needed to unveil factors influencing CPV vaccination failure in Nigeria.

Table 4.26: Classification of the patients according to DHLPP vaccination history

Vaccination status	**Number of dogs (n)**	**Percentage (%)**
Unvaccinated	63	40.1
Vaccinated	94	59.9
Total	**157**	**100**

Table 4.27: DHLPP vaccination protocols, number of dosages, the mean age at which the vaccine series was administered, intervals between vaccinations, the number and percentage of vaccination failure for the dogs sampled

Category / Vaccination	Number of cases (n)	Percentage (%)	Mean age ± S.E. (wks.) for dose administered	Vaccination range (wks.)
a. One dose	49	51.1	7.2 ± 1.1	4–24
b. Two doses	27	28.7	10.2 ± 1.1	7–18
c. Three doses	18	19.1	12.7 ± 1.1	10–16
d. Four doses	1	1.1	52.0	-
Mean interval between vaccinations	-	-	3.4 ± 1.1	1–8
Total	**94**	**100.0**	-	-

Table 4.28: Multivalent canine vaccines currently available in Nigeria

Vaccine brand	DOI	Vaccine type	Vaccine strain	AST [weeks]	AFT	AVI (weeks)	Max TCID	Origin
Biocan DHPPi+L	One year	Modified-live	CPV-2	8	10 wks.+	2	$10^{5.5}$ $TCID_{50}$	Czech
Canvac DHPPi	One year	Modified-live	CPV-2	6	12 wks.+	3	$10^{3.6}$ HAU	Czech
CaniShot K5	One year	Modified-live	CPV-2	6	12 wks.+	2	10^{5} $TCID_{50}$	Korea
Megavac-7	One year	Modified-live	CPV-2	8	12 wks.	3	$10^{\geq 3}$ $TCID_{50}$	India
Pro-vac	One year	Modified-live	CPV FK-strain[#]	8	12 wks.+	4	15 %	Korea
Romvac	One year	Modified-live	CPV-N-1988	6	11 wks.+	3	10^{5} $TICP_{50}$	South Africa
Vanguard plus 5	One year	Modified-live	CPV-2	6	12 wks.+	3	10^{7} $TCID_{50}$	USA
Nobivac 1-DAPPv	One year*	Modified-live	CPV-2b	6	12 wks.	2-4	$10^{5.1}$ $FAID_{50}$	USA
Primodog	One year	Modified-live	CPV-2	6	12 wks.	2-3	$10^{5.5}$ $TCID_{50}$	Germany

Index: Advertised duration of immunity (ADOI), advertised vaccinations interval (AVI), Advertised start time (AST), Advertised finish time (AFT), Advertised maximum tissue culture infectious dose (Max $TCID_{50}$), Haemagglutination units (HAU), tissue culture infectious dose ($TCID_{50}$); Adequate immunity develops over 2 to 4 week following the initial primary vaccination series; Not stated (NS). [†] The information in the table is extracted from vaccine manufacturers' manual. [#]The strain of the virus used in the vaccine is not specified. *the DOI is 4yrs. but annual revaccination may be required for if the individual risk profile or immune status so determines.

4.8 Molecular characterisation canine parvoviruses from vaccines and clinical samples in Nigeria

Result of the on-the-spot immunochromatographic and molecular method of CPV detection corroborated that about 93.0% of the screened 157 dogs were infected (Table 4.29). This is an indication that the on-the-spot immunochromatographic test and presumptive clinical diagnosis to a certain extent are reliable. Higher CPV enteritis prevalence described herein tallies with previous reports (Fagbohun and Omobowale, 2018; Apaa *et al*., 2016; Shima *et al*., 2015b), indicating that CPV remains an issue in Nigeria even in this era of availability of potent vaccines. Furthermore, CPV antigens were detected in 23/25 (88.0%), 18/20 (90.0%), 20/22 (90.9%), 10/11 (90.9%), 69/72 (95.8%), 4/4 (100.0%), and 3/3 (100.0%) of the faecal samples screened from Warri, Jos, Abeokuta, Makurdi, Ibadan, Abuja, and Onitsha, correspondingly.

Most of the dogs (84.1%) screened were exotic/mixed breed (Alsatian, Bull Mastiff, Boerboel, Neapolitan Mastiff, Doberman, Caucasian, Rottweiler, Great Dane, Lhasa Apso, and Samoyed), with 73.2% of them puppies five weeks to six months old and 54.8% were male dogs, while more than half of them (60.5%) were vaccinated (Table 4.30). Higher CPV prevalence in non-indigenous dog breeds, puppies/young dogs, and male dogs as found in this study corroborates existing data (Castro *et al*., 2007b; Shima *et al*., 2015b; Apaa *et al*., 2016). Overrepresentation of non-indigenous breeds (84.1%) in this research is the likely explanation; agreeing with existing reports of that exotic breeds constitute the largest percentage (66.0%) of the caseload of Nigerian veterinary clinics (Otolorin *et al*., 2014). Also, puppies and young dogs are immunologically incompetent and are exposed to extended MDA window of interference making them vulnerable to CPV and other infections.

The electrophoresis gel picture shows that the vaccines licenced for parvovirus vaccination in Nigeria passed the identity test by containing CPV DNA. The vaccines and parvovirus positive samples tested amplified at 700 bp (Plate 4.8). CPV positive samples in the in-clinic assay and six parvovirus vaccines licenced for vaccinating dogs in Nigeria were further assayed by PCR. Subsequently, incomplete VP2 gene sequence of 700 bp amplicon size was done to identify the CPV subtypes. Of these, 112 random clinical samples and the 6 vaccines tested positive to CPV nucleic acid in the PCR assay. Five (5) vaccines and 11 of the randomly selected clinical samples amplicons were

successfully sequenced. The aligned sequences were checked for comparative sequences in the GenBank by submitting to nBLAST programme (http://www.ncbi.nlm.nih.gov/BLAST/). Nigerian CPV nucleotide (nt) sequences were 98.7% to 99.9% identical to canine parvoviruses from Asia and Europe. Alignment of the clinical and vaccine sequences against reference sequences, coupled with substitutions at 426 position of the amino acid indicated 4 (36.4%) samples were CPV-2a (N426N) and 7 (63.6%) CPV-2c (N426E) out of the 11 Nigerian sequences. On account of amino acid mutation D305Y, the 5 vaccines were typed as wild-type CPV. Mutation D305Y, A300G, and M87L in the amino acid sequences are used to distinguish wildtype CPV from the other biotypes (Truyen, 2006; Decaro *et al*., 2013b; Miranda and Thompson 2016), however the primers used did not include M87L and A300G regions. The use of wild-type strain alone in vaccines considering the continuous viral mutations has implications for failure of vaccination. The 10 and 5 of the sequences from the clinical samples and vaccines respectively submitted to NCBI GenBank repository have been assigned accession numbers from MT198664 – MT198678.

Regarding local distribution of the isolates, CPV-2c and CPV-2a were observed in isolates NGA-Wr3, NGA-Wr2, NGA-Wr1 from Warri, Delta State. For Onitsha representing Anambra State, the only sequence - NGA_Osh1 was mapped as CPV-2a. Furthermore, isolates NGA-Abk2, NGA-Abk1 from Abeokuta, Ogun State were mapped as CPV-2a and CPV-2c, respectively. Similarly, CPV-2c sequences were identified in samples from Ibadan, Oyo State (NGA-Ib9); Jos, Plateau State (NGA-Jos1); the two samples (NGA-Mkd1, NGA-Mkd2) from Makurdi, Benue State, and samples (NGA-Abj1) from FCT, Abuja (Figure 4.15).

The findings of a high prevalence of CPV-2c (63.6%) and its widespread compared to CPV-2a (36.4%), and the absence of CPV-2b contradict earlier reports that CPV-2a was the only biotype that was circulating in Nigeria a few years ago (Apaa *et al*., 2016; Dogonyaro *et al*., 2013). These findings also contrasted the report of Fagbohun and Omobowale (2018) who reported that CPV-2a, to some lesser extent CPV-2c and CPV-2b as the major biotype circulating in Nigeria. It is noteworthy that CPV-2c occurred in clinical samples from six of the seven states (Ogun, Oyo, Plateau, Delta, Benue, and FCT) sampled. On the contrary, the past studies in Nigeria were restricted to at most two study sites and sequenced only a few samples. Therefore, with these data it is inferred

that CPV-2a and CPV-2c are now the major subtypes circulation in Nigerian dog populations. This report reinforces the findings of Ogbu *et al*. (2019). None of the samples that were sequenced in this investigation was found to be CPV-2b.

The absence of vaccine-like virus among the clinical sequences analysed indicates the infection in vaccinated dogs emanates from the field-virus shortly after vaccination or puppies incubating infection rather than virus from the vaccine reverting to virulence (Decaro *et al*., 2007; Mittal *et al*., 2014). Recent evidence showed that vaccine-virus shedding rarely occurs with the contemporary CPV vaccines available (Miranda and Thompson, 2016). In addition, previous cases of parvovirus enteritis in some vaccinated dogs in Nigeria were due to a failure to vaccinate properly (Shima *et al*., 2015b; Babalola *et al*., 2016). Inappropriate vaccine regimens, including single-dose regimen, are inadequate to protect immature dogs, owing to interference by exposure to extended MDA window (Nandi *et al*., 2013; Ling *et al*., 2012; Davis-Wurzler, 2014). This could also be correlated with age of the dogs as they were mostly young.

Potential vital substitutions were noticed in the nucleotide (nt) sequences (Plate 4.9) and amino acid sequences of the DNA studied (Plate 4.10). At position 901 (ACT→TCT), position 913 (GAT→TAT), position 1123 (GAT→AAT), and position 1320 (ACA→GCA), transition affecting the 1st codons of the nt sequences culminated to mutations T301S, D305Y, N375D, and T440A respectively in the VP2 sequences. Similarly, a transmutation at positions 970 – 972 involving the 1st and 2nd codons (TAT→ATT) in the nt sequences resulted in substitution Y324I in the protein sequences. A switchover in the 2nd codons at positions 1109 (CAA→CGA), 1331 (TAT→TCT), 1352 (TAT→TGT) of the nt sequences tally with substitutions Q370R, Y444S, and Y451C, respectively; while transition at position 1341 (ATA→ATG) involving the last codon corresponds to mutation I447M in the protein sequences. At positions 1276 – 1278, transmutations involving the 1st codon (AAT→GAT) and the last codons (AAT→GAA) in the nt sequences tally with substitutions N426E and N426D, respectively in the protein sequences; which are CPV-2c and CPV-2/a, respectively. It appears natural selection and local differentiations due to infection pressure have contributed to mutations Y451C, I447M, Y444S, and T301S which are observed only in the Nigerian CPV biotypes.

Substitution Y324I was detected in all the CPV isolates that were sequenced. There was a T440A substitution in four CPV-2a isolates. In addition, one CPV-2c isolate has a Y444S amino acid substitution, while another CPV-2c isolate from Makurdi has important substitution in the amino acid at I447M. All the CPV-2c but not CPV-2a sequences displayed substitute Q370R. A D305Y change occurred in all the Nigerian sequences studied. Another substitution T301S occurred in the clinical isolates that were sequenced (Plate 4.10). A non-synonymous change Y324I which occurred in each of the CPV-2a and 2c biotypes studied reinforced the findings of Ogbu *et al*. (2019). This Y324I change was predominant in the recently sequenced Asian strains of CPV (Grecco *et al*., 2018; Zhou *et al*., 2017; Yi *et al*., 2016; Geng *et al*., 2015). Initially, change Y324I was thought to be an attribute of the Asian strains only (Li *et al*., 2017). However, is it now detected in some European CPV-2a and CPV-2c subtypes (Csagola *et al*., 2014; Mira *et al*., 2018). It was previously described in some Nigerian CPV-2a (Apaa *et al*., 2016); in Hungarian CPV-2a variants (Csagola *et al*., 2014), as well as, in Chinese CPV-2c isolates (Mira *et al*., 2018). Many other regions of the world are yet to observe this substitution Y324I in their canine parvoviruses.

Again, the CPV-2c variants revealed amino acid Q370R changes never observed in Nigerian strains until lately (Ogbu *et al*., 2019). This Q370R substitution has limited global distribution. It is commonly detected in Chinese canine parvoviruses among the Asian countries (Guo *et al*., 2013). This mutation was recently introduced from Asia through importation of dogs into Europe (Mira *et al*., 2018).

Furthermore, the T440A mutation detected in the CPV-2a Nigerian isolates studied was also present in those of previous reports (Fagbohun and Omobowale, 2018; Apaa *et al*., 2016). Three other mutations Y444S and Y451C, and I447M were displayed by two CPV-2c sequences analysed from Jos and Makurdi, respectively. The Y451C, I447M, Y444S, and T440A changes are all located within an exposed section of the VP2 βGH loop with the utmost mutations or variability in canine parvoviruses, comprising between amino acid 267 and 498 (Chapman and Rossmann, 1993; Battilani *et al*., 2002). The T440A change is widely distributed and frequently observed mutation in Chinese (Geng *et al*., 2015; Wang *et al*., 2016; Li *et al*., 2017), Italian (Battilani *et al*., 2002), Uruguayan (Maya *et al*., 2013), Korean (Kang *et al*., 2008), Indian (Mittal *et al*., 2014), Thai (Phromnoi *et al*., 2010) and lately in Argentine CPV strains (Calderón *et al*., 2015).

The D305Y mutation in the Nigerian isolates studied was not restricted to CPV-2a but was also observed CPV-2c isolates. This substitution was initially thought to be a characteristic shared among CPV-2a subtype only (Hoelzer & Parrish, 2010; Apaa *et al*., 2016). Similarly, change T301S was present in the Nigerian VP2 sequences studied. Its clinical and immunological relevance is elusive. But because it fell within the main antigenic region with highest antigenic variability, it also could contribute to the antigenicity of the CPVs and/or vaccination failure in Nigerian dogs.

The Maximum Likelihood Method was used in the phylogenetic tree construction with 1000 replicates bootstrapping by means of MEGA X software program (Kumar *et al*., 2018). The analysis grouped together the studied Nigerian CPV-2a sequences (NGA-Abk1, NGA-Osh1, NGA-Wr1, NGA-Wr2) with those from previous studies, China, USA, Italy, and CPV-2a reference variant (KF149978). The CPV-2c sequences (NGA-Abk2, NGA-Mkd2, NGA-Mkd1, NGA-Abj1, NGA-Jos1, NGA-Ib9, and NGA-Wr3) on the other hand fell within the same cluster with Brazilian, German, Chinese, and Italian CPV-2c. The vaccine sequences (NGA_Vacc1 to NGA_Vacc6) grouped with the wildtype CPV reference strain (EU659116). Canine adenovirus-2 (JX416842) acts as an outgroup (Figure 4.14).

Based on phylogeny and variability in amino acid affecting the capsids protein, the Nigerian parvovirus VP2 sequences studied bear genetic signatures homologous to those from distant geographical regions, particularly those of Asian CPV strains, diverging from earlier isolates studied pre-2015. Mutations Y451C, I447M, Y444S T301S, Q370R, Y324I, and D305Y in the Nigerian isolates have been noticed only recently; particularly in samples sequenced after 2015. The heterogeneous viral populations, genetic variability, and phylogenetic connection with sequences from overseas are suggestive that international migrations through dog trading and/or fomites, natural selection and local differentiations as a result of infection pressure are perhaps behind the continuous evolution of CPV witnessed in Nigeria lately. Viral migrations play a vital role in spreading CPV to several parts of the globe (Zhou *et al*. 2017; Grecco *et al*., 2018; Mira *et al*., 2018).

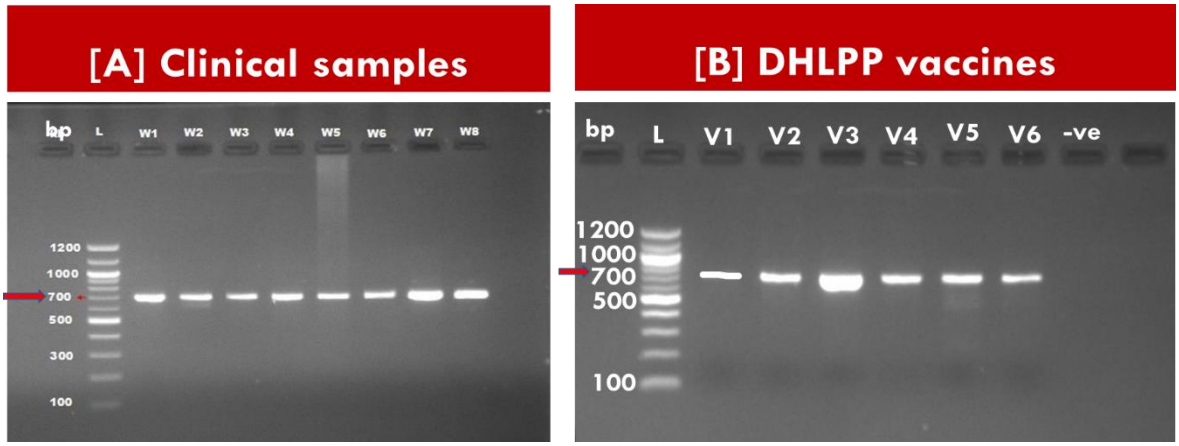

Plate 4.7: Gel picture of the PCR products

Table 4.29: Results obtained by the immunochromatographic and PCR assays

Assay	Result	Frequency (n)	Percentage (%)
Immunochromatography	Negative	11	7.1
	Positive	146	92.9
	Total	**157**	**100.0**
PCR	Negative	8	7.1
	Positive	104	92.9
	Total	**112**	**100.0**

Table 4.30: Characteristics of the screened dogs

Category	Tested positive		Total	
	n	**%**	**n**	**%**
Breed				
Native	14	8.9	14	8.9
Mixed	8	5.1	18	11.5
Exotic	124	79.0	125	79.6
Age (month)				
≥6	42	26.8	42	26.8
<6	104	66.2	115	73.2
Sex				
Female	65	41.4	71	45.2
Male	81	51.6	86	54.8
Vaccination history				
Unvaccinated	62	39.5	62	39.5
Vaccinated	84	53.5	95	60.5
Total	**146**	**93.0**	**157**	**100.0**

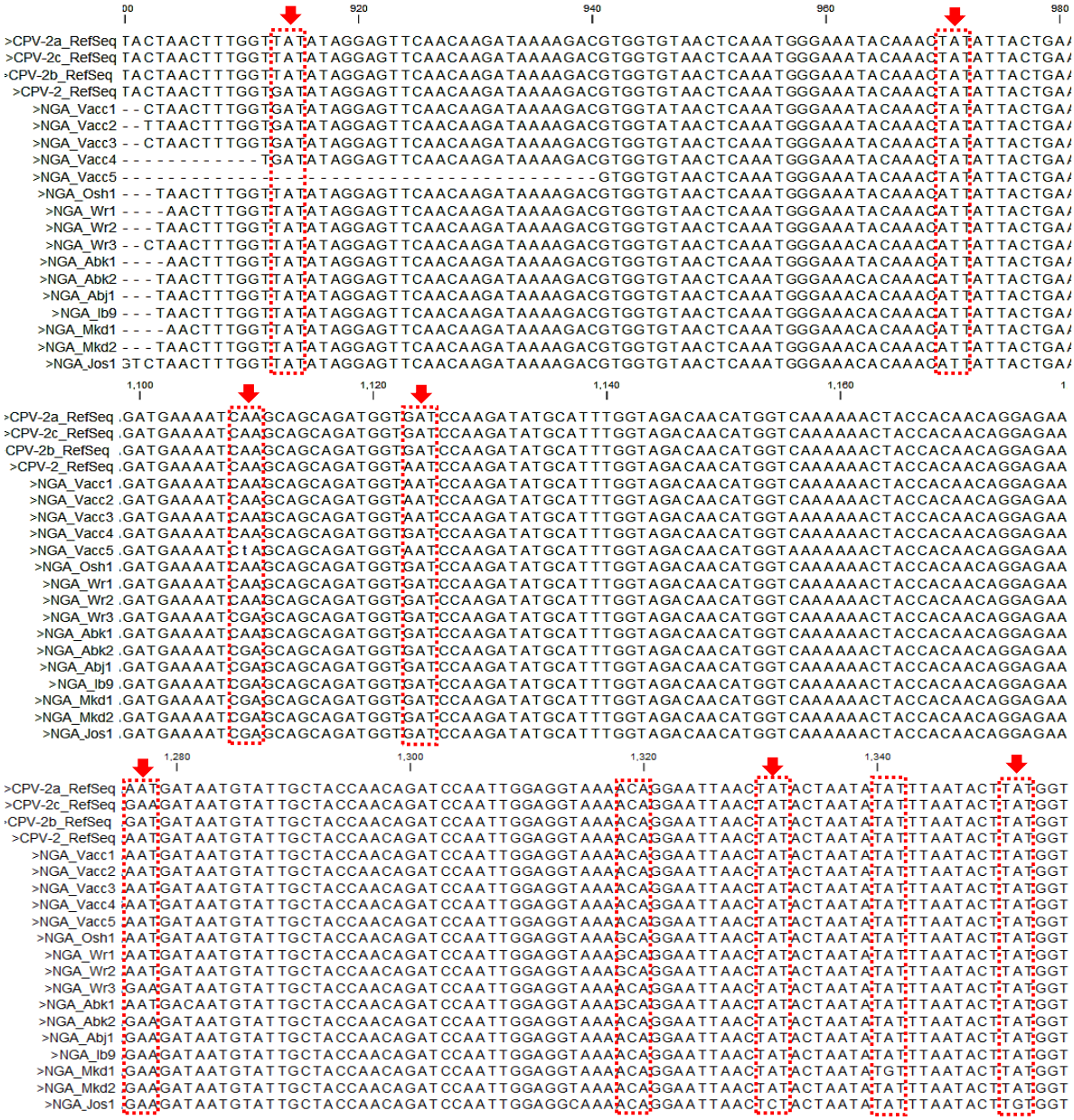

Plate 4.8: Multiple alignment of the nt sequences studied with reference sequences obtained from public domain. Clinical and vaccine isolates are indicated by NGA and location obtained. The positions of mutations in the nt sequences are marked by red boxes.

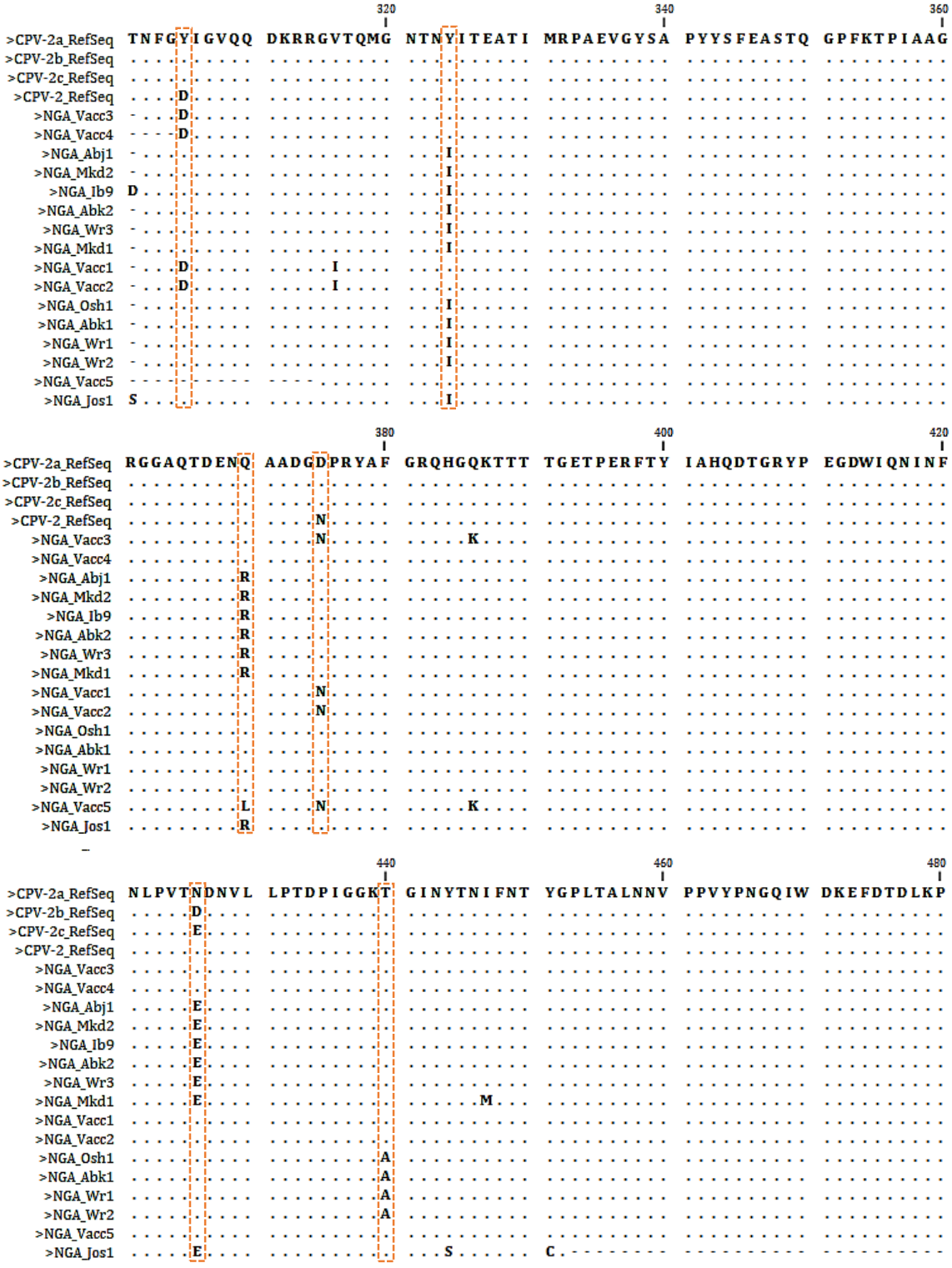

Plate 4.9: VP2 gene amino acid mutations displayed by the Nigerian parvoviruses

Index: Y = tyrosine, T = threonine, S = Serine, R= arginine, Q = glutamine, N = asparagine, M = methionine, I = isoleucine, E=glutamic acid, D=aspartic acid, A=Alanine, Reference strains: CPV2a (EU659118); CPV2b (KR559892) and CPV2c (M38245). Samples V1–V6 are vaccines licenced for use in Nigeria. The sites of mutations in the samples at positions 301, 305, 324, 370, 426, 440, 444, 447 and 451.

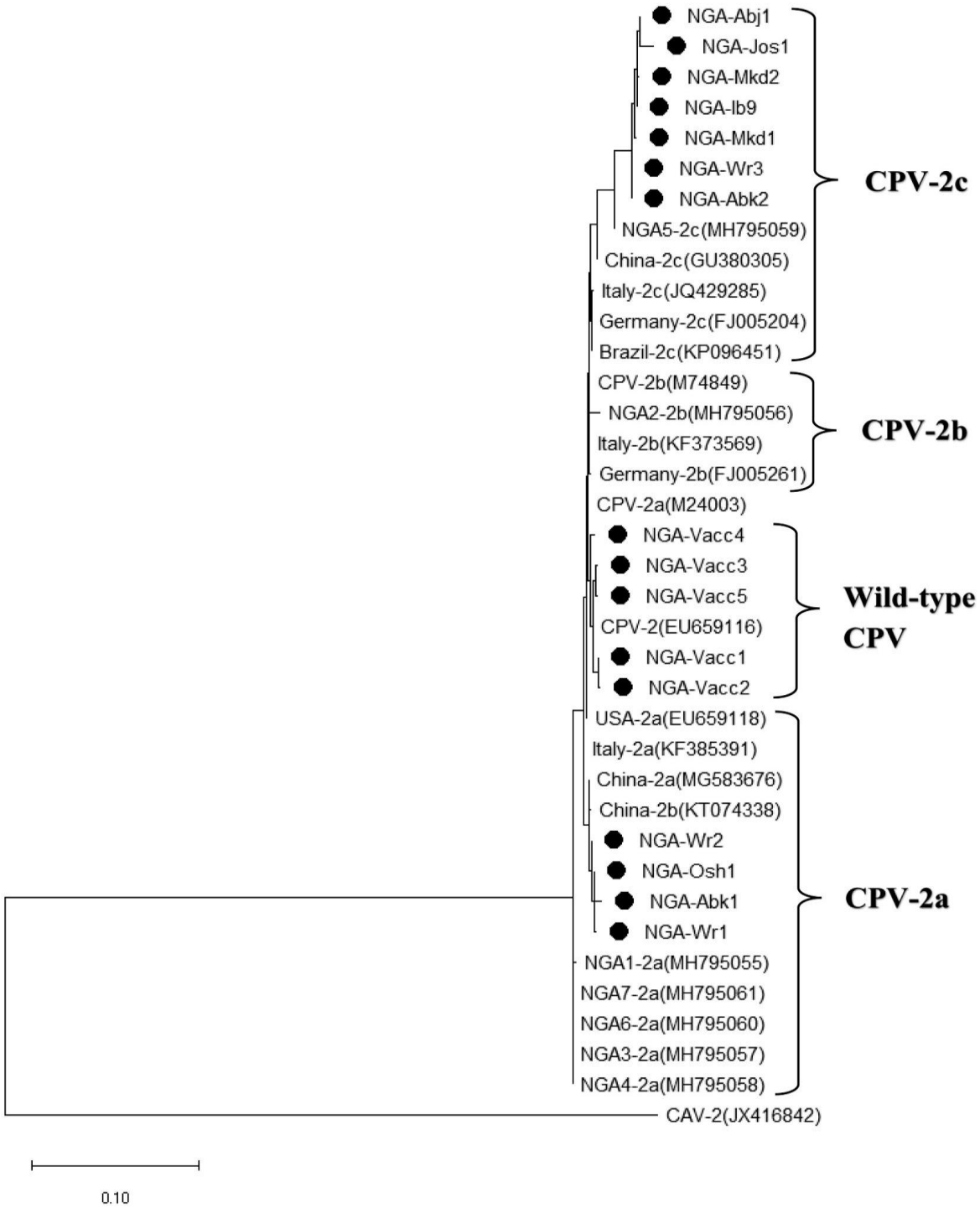

Figure 4.14: Phylogenetic classification of the studied CPV sequences

Index: The CPV isolates in this study are represented by black (•). The Reference strains, sequences from previous studies in Nigeria, and those from other countries have ascension numbers. For short branches, the sequences are very similar, with an increase in the length of the branch suggesting a decreasing set of similarities. The constructed phylogenetic tree involved 37 CPV nt sequences by means of the Maximum Likelihood method with 1000 replicates bootstrapping. The bar represents the average number of nt substitutions per site. Canine adenovirus-2 was used as outgroup.

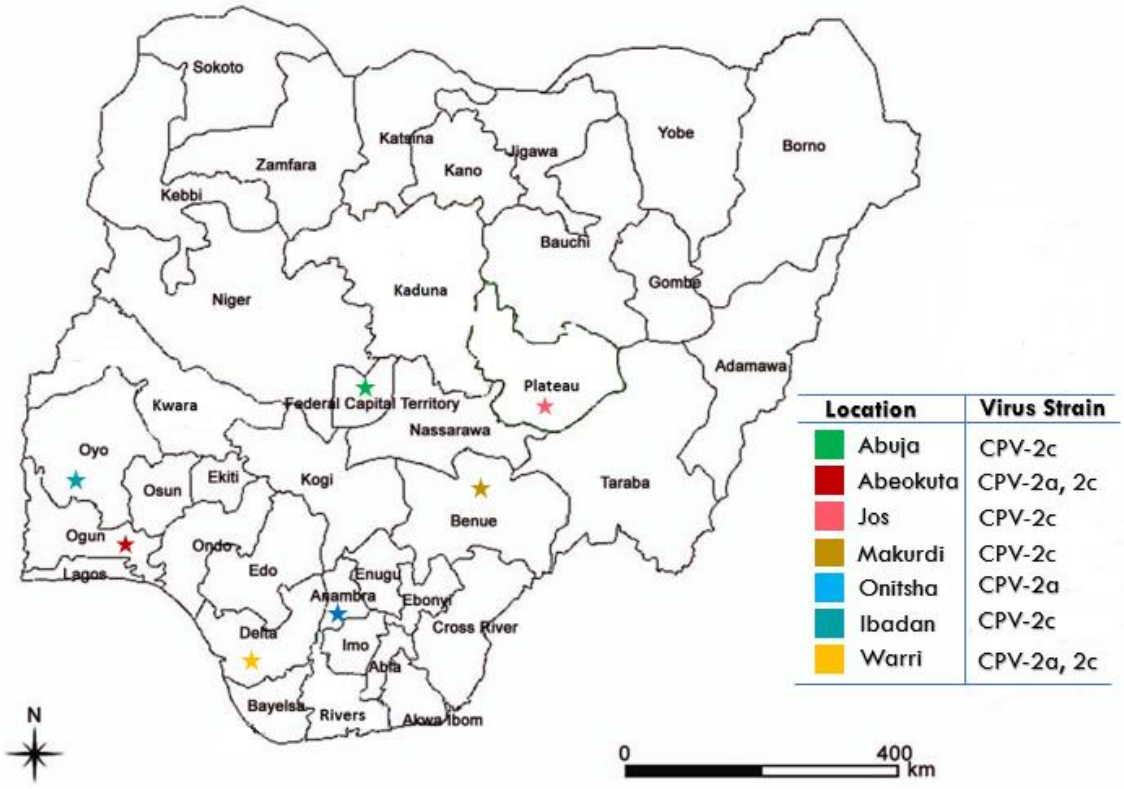

Figure 4.15: Regional distribution of analysed CPV biotypes in Nigeria

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This investigation has elucidated prevalence and drug usage patterns; biomarkers for duration of management and prognosis for dogs with gastroenteritis; characterise electrocardiographic changes in dogs with CPE; characterise CPVs circulating in canine population, and determine protocols influencing CPV vaccination failures in Nigeria.

This study demonstrated that gastroenteritis is prevalent in Nigeria, accounting for 41.2% of all clinical cases. Substantial evidence exists for prevalence variations in the period of the year, vaccination status, breed, and age of the dogs. Large breeds had higher odds for developing gastroenteritis compared to the giant, medium and small dog breeds. DHLPP unvaccinated status predisposes to canine gastroenteritis. The peak canine gastroenteritis prevalence was recorded in January and the lowest around August and September coinciding with the peak of the rainy season in Nigeria.

Regarding the drug prescription patterns, polypharmacy, antibacterial overuse and injudicious prescription are common practices in the management of several cases of canine gastroenteritis in Nigeria. An average of 5.4 different drugs was prescribed in a treatment regimen, with 45.8% of the dogs prescribed above four different drugs. Antibacterials were widely prescribed in 75.4% of the dogs even though only 10.1% of the diagnoses were ascribed to bacterial enteritis. An average of 1.5 antibacterial was prescribed, with 27.2% prescribed over two different antibacterials during a treatment lasting for a week. Furthermore, there is substantial evidence for imprudent prescription of NSAID, atropine, hyoscine, diphenoxylate and loperamide. The prescription patterns were significantly influenced by the clinical signs observed ($\alpha<0.001$). Dogs presented with severe bloody diarrhoea or concomitant diarrhoea with vomiting were more prone to polypharmacy.

Canine parvovirus singly (93.0%), GI parasites (12.1%) and to a lesser extent coronavirus (2.6%) and *Giardia* (0.6%) were associated with the clinical cases. The

endoparasites detected included a non-zoonotic helminth [*Isospora* – 4.5%] and zoonotic helminths [*Ancylostoma* – 5.7%; *Toxocara* – 0.6%] and protozoan [*Giardia* – 0.6%]. The dog patients showed mostly anorexia, diarrhoea, vomiting, dehydration, depression/lethargy, fever, and colic at the time of presentation.

The dogs with gastroenteritis demonstrated nonspecific clinicopathological changes which included anaemia, leukopaenia, lymphocytosis, monocytopaenia, pancytopaenia, thrombocytopaenia, neutropaenia, hyperfibrinogenaemia and hypofibrinogenaemia. Serum biochemical changes were azotaemia, hyperglycaemia, hyperproteinaemia, hyperalbuminaemia, hyperglobulinaemia, increased albumin/globulin ratio, hypercreatinaemia, increased serum Alanine transaminase, increased alkaline phosphatase, increased aspartate transaminase, hypercalcaemia, hypoalbuminaemia, hyponatraemia, hypokalaemia, hypochloraemia, and hypoproteinaemia. The mean differences in the haematological and biochemical values between survivors and non-survivors obtained prior to initiation of treatment were statistically not significant ($\alpha>0.05$), however, presentation with pancytopaenia and leukopaenia prolonged the duration of management. The presence of colic, leukopaenia and hypoalbuminaemia were associated with a poor prognosis. Leukopaenia was the only abnormality influencing both the duration of management and prognosis.

The electrocardiographic changes suggestive of cardiac involvement in the intestinal form of CPV were observed in 70.0% of the cases. The observed electrocardiographic changes reflect disturbances in metabolic acid-base, electrolytes and haemodynamic secondary to CPV disease rather than the direct effect of the virus.

The DHLPP vaccination status has a significant relationship with the incidence of canine gastroenteritis. Vaccination failure rate of 59.9% was recorded in the cases studied. Vaccine usage protocols associated with the failure rate were the incomplete puppy primary vaccination series, inappropriate vaccination timing - "vaccine start and finish time", and nonsynchronous intervals between vaccinations.

The in-clinic assay for the detection of canine parvovirus performed reasonably when compared with molecular technique. Genetic typing of the isolates in Nigeria divulged the absence of CPV-2b and vaccine-like strains and preponderance of CPV-2c over CPV-2a among the clinical samples. The vaccine viruses were characterised as the wild-

type CPV strain; with infection in vaccinated dogs attributed to natural infection. The present Nigerian isolates displayed high level of divergence and mutations (Y451C, I447M, Y444S, T440A, Q370R, Y324I, D305Y, T301S) in their amino acid sequences that were not present in isolates sequenced before 2015. They are now distantly related to the Asian and European strains by sharing 98.7 to 99.9 per cent nucleotide similarity. These findings suggest the introduction of CPVs from other countries into Nigeria through international viral migrations and mutations. The mutations probably lead to the failure of the CPV vaccine.

5.2 Recommendations

This study has established that canine gastroenteritis, polypharmacy, injudicious use of drugs, particularly antibacterials in the management of canine gastroenteritis is very common in veterinary practice in Nigeria. Also, the usefulness of clinicopathology in prognostication and management of canine gastroenteritis; the role of CPV and endoparasites in canine gastroenteritis; the effects of DHLPP vaccination protocol on the incidence of gastroenteritis and vaccine failure in Nigeria; the value of electrocardiogram and biochemical assay in the management of CPV enteritis are demonstrated in this report. Hence, it is recommended that:

1. Canine parvovirus, CCoV and GI parasites should be included in the list of differentials for dogs presenting with gastroenteritis.
2. Polypharmacy and injudicious drug prescriptions are emerging issues requiring a cogent and structured approach to addressing it in veterinary practice in Nigeria besides regular appraisal of drug usage patterns.
3. Colic, hypoalbuminaemia, leukopaenia or pancytopaenia observed at presentation are useful biomarkers for predicting the course of gastroenteritis in dogs.
4. In-clinic assays are relatively inexpensive and should be incorporated in canine practice for rapid detection of CPV, Coronavirus, and *Giardia*.
5. Assessment of cardiac function in patients with the intestinal form of CPV disease is recommended.
6. Clinicians should review and improve their vaccination protocols to effect changes to their vaccine usage patterns.
7. Education of the public on the importance of vaccinating their dogs from licenced veterinarians would help lower incidence of CPV vaccination failures is warranted.

8. Regular monitoring and genetic characterisation of CPV isolates will help identify mutations that can induce vaccination failure and viral evolution in Nigeria.
9. Canine parvovirus vaccines for vaccinating dogs in Nigeria should incorporate the Nigerian strain of the virus.
10. There is a need to evaluate the potency and seroconversion of the various brands of CPV vaccines marketed in Nigeria.

5.3 Contributions to knowledge

This investigation contributed substantially to the area of clinical and scientific body of knowledge as follows:

1. The first large study establishing the prevalence of canine gastroenteritis in Nigeria.
2. Documentation of injudicious drug prescriptions in the clinical management of canine gastroenteritis in Nigeria.
3. The first documentation of co-infection of CPV with CCoV, *Giardia*, *Isospora*, *Toxocara*, and *Ancylostoma* in clinical cases of canine gastroenteritis in Nigeria.
4. Demonstration that CPV is the leading cause of canine gastroenteritis in Nigeria.
5. Regardless of the causative agents involved, colic, hypoalbuminaemia (albumin ≤1.8 g/dL), leukopaenia (leukocytes ≤4 x 10^3/µL or reduction in the three blood cellular components (pancytopaenia) at initial presentation are predictive of a prolonged duration of management and poor prognosis in dogs with gastroenteritis.
6. Demonstration that cardiac involvement is common in the intestinal form of CPV but often underrecognised in clinical practice.
7. Demonstration of CPV-2c predominance over CPV-2a strains in Nigerian dog populations.
8. Demonstration of mutations (Y451C, I447M, Y444S, T440A, Q370R, Y324I, D305Y, T301S) and divergence of the recent Nigerian CPVs from previous ones.
9. The Nigerian CPVs shares genetic credentials with strains from other countries, particularly Asia and Europe.
10. Confirmation of wild-type CPV as a major strain present in the DHLPP vaccines licenced for vaccinating dogs against CPV in Nigeria.
11. No virus from the vaccines was detected in the clinical samples that were sequenced, indicating infection with field virus.
12. The first documentation of CPV vaccine usage patterns in Nigeria.
13. This is the first detection of CCoV in Nigerian dogs.

REFERENCES

Abere, T., Bogale, B., Melaku, A. 2013. Gastrointestinal helminth parasites of pet and stray dogs as a potential risk for human health in Bahir Dar Town north-western Ethiopia. Veterinary World 6:388-392.

Adamu, N.B., Adamu, J.Y., Salisu, L. 2012. Prevalence of ecto-endo and haemoparasites in slaughtered dogs in Maiduguri, Nigeria. Revue de Médecine Vétérinaire 163(4):178-182.

Adejinmi J.O., Osayomi J.O. 2010. Prevalence of intestinal protozoan parasites of dogs in Ibadan, southwestern Nigeria. Journal of Animal and Plant Sciences 7(2):783-788.

Adejumobi O.A., Omotosho O.O., Omobowale T.O., Akinrinmade J.F. 2017. Retrospective study of the prevalence and pattern of parvoviral enteritis presented at the Veterinary Teaching Hospital, University of Ibadan, Nigeria. European Journal of Pharmaceutical and Medical Research 4(3):109-113

Adimanyi, C.O., Omudu, E.A. 2016. Prevalence of gastrointestinal helminths of faeces from in-door dogs and stray-dogs in Makurdi, Nigeria. Nigerian Journal of Parasitology 37:23-27.

Agbandje, M., Parrish, C.R. Rossmann, M.G. 1995. The structure of parvoviruses. Seminars in Virology 6:299-309.

Ali, S.A., Akhtar, T., Mahmood, K., Safi, W.A. 2013. Spatial distribution of ancylostomiasis in soil of slums of Northern Lahore. Journal of Agriculture and Veterinary Science 4:20-25.

Allenspach, K., Rüfenacht, S., Sauter, S., Gröne, A., Steffan, J., Strehlau, G., Gaschen F. 2006. Pharmacokinetics and clinical efficacy of cyclosporine treatment of dogs with steroid-refractory inflammatory bowel disease. Journal of Veterinary Internal Medicine 20(2):239-244.

Allenspach, K., Wieland, B., Grone, A., Gaschen, F. 2007. Chronic enteropathies in dogs: evaluation of risk factors for negative outcome. Journal of Veterinary Internal Medicine 21:700-708.

Altman, K.D., Kelman, M., Ward, M.P. 2017. Are vaccine strain, type, or administration protocol risk factors for canine parvovirus vaccine failure? Veterinary Microbiology 210:8-16. https://doi.org/10.1016/j.vetmic.2017.08.019.

Amfim, A., Parvu, M., Bacescu, B., Simion, V.E. 2011. Estimation of canine intestinal parasites in Bucharest and their risk to public health. Bulletin of UASVM Veterinary Medicine 68(2):26-30.

Amrani, N., Desario, C., Kadiri, A., Cavalli, A., Berrada, J., Zro, K., Sebbar G., Colaianni M.L., Parisi A., Elia, G., Buonavoglia, C., Malik, J., Decaro, N. 2016. Molecular epidemiology of canine parvovirus in Morocco. Infection Genetics and Evolution 41:201-206.

Apaa, T.T., Daly, J.M., Tarlinton, R.E. 2016. Canine parvovirus (CPV-2) variants circulating in Nigerian dogs. Veterinary Record Open 3:e000198. https://doi.org/10.1136/ vetreco-2016-000198.

Appel, M.J., Meunier, P., Pollock, R. 1980. Canine viral enteritis, a report to practitioners. Canine practice 7(4):22-36.

Areshkumar, M., Vijayalakshmi, P., Selvi, D. 2018. Evaluation of prognostic indicator based on the electrical conductivity of the heart in canine parvovirus enteritis. International Journal of Livestock Research 8(12):41-46.

Argenzio, R.A. 1992. Pathophysiology of diarrhoea. In: Anderson N.V., Ed. Veterinary Gastroenterology. 2nd ed. Lea & Febiger, Philadelphia PA, 163-172 pp.

Armstrong, P.J. 2013a. GI intervention: Approach to diagnosis and therapy of the patient with acute diarrhoea. Today's Veterinary Practice May/June 2013:20-24, 54-56.

Armstrong, P.J. 2013b. GI intervention: Approach to diagnosis and therapy of the vomiting patient. Today's Veterinary Practice March/April 2013:18-27.

Arpaia, N., Campbell, C., Fan, X., Dikiy, S., van der Veeken, J., deRoos, P., Liu, H., Cross, J.R., Pfeffer, K., Coffer, P.J., Rudensky, A.Y. 2013. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. Nature 504(7480):451-455. https://doi.org/10.1038/nature12726.

Arrieta, M.C., Stiemsma, L.T., Dimitriu, P.A., Thorson, L., Russell, S., Yurist-Doutsch, S., Kuzeljevic, B., Gold, M.J., Britton, H.M., Lefebvre, D.L., Subbarao P., Mandhane, P., Becker, A., McNagny K.M., Sears, M.R., Kollmann, T; CHILD Study Investigators, Mohn, W.W., Turvey, S.E., Finlay, B.B. 2015. Early infancy microbial and metabolic alterations affect risk of childhood asthma. Science Translational Medicine 7(307):307ra152.

Atata, J.A., Esievo, K.A.N., Adamu, S., Abdulsalam, H. 2018. Baseline haematological, serum biochemical and some urine parameters in Nigerian indigenous dogs. Savannah Veterinary Journal, 1:48-52.

Atsbaha, G., Hussien, D., Cruz, C.R. 2014. Assessment of major health problems of dogs in Mekelle City, Ethiopia. Global Veterinaria 12(2):176-180.

Babalola E.T., Ijaopo O.K., Okonkwo I.O. 2016. Evaluation of immunity and seropositivity of IgG antibodies to canine parvoviruses in vaccinated and unvaccinated dogs in Abeokuta, Nigeria. Journal of Immunoassay and Immunochemistry 37:16-28.

Baker Institute for Animal Health. 2014. Canine parvovirus. Cornell University College of Veterinary Medicine, Retrieved February 20, 2019, from www.vet.cornell.edu/baker/about/articles/CanineParvovirus.cfm.

Ballweber, L.R., Xiao, L.H., Bowman, D.D., Khan, G., Cama, V.A. 2010. Giardiasis in dogs and cats; an update on epidemiology and public health significance. Trends Parasitology 26:180-189.

Banfield Pet Hospital, 2013. State of pet health report, Retrieved January 10, 2016, from https://www.banfield.com/Banfield/media/PDF/Downloads/soph/Banfield-State-of-Pet-Health-Report_2013.pdf

Bansal, T., Alaniz, R.C., Wood, T.K., Jayaraman, A. 2010. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proceedings of National Academy of Science, USA 107:228-233. https://doi.org/10.1073/pnas.0906112107

Bär, S., Daeffler, L., Rommelaere, J., Nüesch J.P.F. 2008. Vesicular egress of non-enveloped lytic parvoviruses depends on Gelsolin functioning, PLoS Pathogens 4(8):e1000126. doi:10.1371/journal.ppat.1000126

Bär, S., Rommelaere, J., Nüesch, J.P.F. 2013. Vesicular transport of progeny parvovirus particles through ER and Golgi regulates maturation and cytolysis, PLoS Pathogens 9(9):e1003605. https://doi.org/10.1371/journal.ppat.1003605.

Barr, S.C. 2009. Canine parvovirus: Are current vaccines effective against new strains? College of Veterinary Medicine, Cornell University Ithaca, NY. 4 pages.

Bastan, I., Kurtdede, A., Özen, D. 2013. Prognostic usefulness of some parameters in dogs with canine parvovirus. Veterinary Journal of Ankara University 60:53-58.

Batchelor, D.J., Noble, P.J., Taylor, R.H., Cripps, P.J., German, A.J. 2007. Prognostic factors in canine exocrine pancreatic insufficiency: prolonged survival is likely if clinical remission is achieved. Journal of Veterinary Internal Medicine 21:54-60.

Batchelor, D.J., Tzannes, S., Graham, P.A., Wastling, J.M., Pinchbeck, G.L., German, A.J. 2008. Detection of endoparasites with zoonotic potential in dogs with gastrointestinal disease in the UK. Transboundary Emerging Diseases 55:99-104.

Battersby, I., Harvey, A. 2006. Differential diagnosis and treatment of acute diarrhoea in the dog and cat. In Practice 28:480-488.

Battilani, M., Ciulli, S., Tisato, E., Prosperi, S. 2002. Genetic analysis of canine parvovirus isolates (CPV-2) from dogs in Italy. Virus Research 83:149-157

Baumgart, M., Dogan, B., Rishniw, M., Weitzman, G., Bosworth, B., Yantiss, R., Orsi, R.H., Wiedmann, M., McDonough, P., Kim, S.G., Berg, D., Schukken, Y., Scherl, E., Simpson, K.W. 2007. Culture-independent analysis of ileal mucosa reveals a selective increase in invasive *Escherichia coli* of novel phylogeny relative to depletion of *Clostridiales* in Crohn's disease involving the ileum. ISME Journal 1(5):403-418.

Baxia, S.L., Kumar, M., Jayachandran, C., Roy, B.K., Kumari, A. 2010. Pharmacokinetics of gentamicin and its interaction with paracetamol after I.V. administration in buffalo calves (*Bubalus bubalis*). Journal of Bioanalysis and Biomedicine 2(3):65-68.

Benchaoui, H.A., Siedek, E.M., de la Puente-Redondo, V.A., Tilt, N., Rowan, T.G., Clemence, R.G. 2007. Efficacy of maropitant for preventing vomiting associated with motion sickness in dogs. Veterinary Record 161:444-447.

Berghoff, N., Steiner, J.M. 2011. Laboratory tests for the diagnosis and management of chronic canine and feline enteropathies. Veterinary Clinics of Small Animal 41:311-328.

Berns, K., Parrish, C.R. 2007. Parvoviridae. In: Knipe, D.M., Howley, P.M. Eds. Fields' virology. 5th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins. 2437-2477 pp.

Berset-Istratescu, C.M., Glardona. O.J., Magouras, I., Frey, C.F., Gobeli, S., Burgener, I.A. 2014. Follow–up of 100 dogs with acute diarrhoea in a primary care practice. The Veterinary Journal 199:188-190.

Bhat, A.A., Imran, S., Chander, V. 2012. Plasma concentration of creatinine kinase-MB isoenzyme in dogs with intestinal form of canine parvovirus–2 infection. Comparative Clinical Pathology 21(6):3pages. https://doi.org/10.1007/s00580-012-1667-3.

Bhat, A.A., Wadhwa, D.R., Singh, S.P., Singh, I. 2013. Haematological and biochemical analysis in canine enteritis. Veterinary World 6(7):380–383.

Bigby, M. 2011. Rates of cutaneous reactions to drugs. Archive of Dermatology. 137:765-770.

Bjerrum, L., Gonzalez Lopez-Valcarcel, B., Petersen, G. 2008. Risk factors for potential drug interactions in general practice. The European Journal of General Practice, 14:23-29.

Black, J.W., Holscher, M.A., Powell, H.S. 1979. Experimentally- induced severe canine parvovirus enteritis. Australian Veterinary Journal 61:1-4.

Boag, A.K., Coe, R.J., Martinez, T.A., Hughes, D. 2005. Acid-base and electrolyte abnormalities in dogs with gastrointestinal foreign bodies. Journal of Veterinary Internal Medicine 19:816-821.

Boag, A. 2013. Managing parvovirus cases. 10th Emergency and Critical Care UK Annual Congress held in Harrogate, November 2013. 200-207 pp.

Bolton, G.R. 1975. Handbook of canine electrocardiography. WB Saunders Company. Philadelphia, London. 61 pp.

Boothe, D.M. 2012. Small Animal Clinical Pharmacology and Therapeutics. 2nd ed. Philadelphia, USA: Saunders. 1384 pp.

Boscan, P., Monnet, E., Mama, K., Twedt, D.C., Congdon, J., Steffey, E.P. 2011. Effect of maropitant, a neurokinin-1 receptor antagonist, on anaesthetic requirements during noxious visceral stimulation of the ovary in dogs. American Journal of Veterinary Research 72:1576-1579.

Bouzid, M., Halai. K., Jeffreys. D., Hunter, P.R. 2015. The prevalence of Giardia infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. Veterinary Parasitology 207:181-202.

Bradshaw, J.W.S., Lea, A.M. 1992. Dyadic interactions between domestic dogs. Anthropozoosis 5:245-253.

British Small Animal Veterinary Association (BSAVA) 2020. BSAVA Guide to the Use of Veterinary Medicines (2020 edn.) Nind, F., P. Mosedale. Eds. BSAVA: UK. 1-99 pp. http://dx.doi.org/10.22233/9781905319862.app2

British Veterinary Association (BVA) 2015. Responsible use of antimicrobials in veterinary practice. BVA: UK. Policy November 2015:5 pages.

Broek, A.H. 1990. Serum protein electrophoresis in canine parvovirus enteritis. British Veterinary Journal 146(3):255-259.

Broussard, J.D. 2003: Optimal faecal assessment. Clinical Techniques in Small Animal Practice 18:218-230.

Brown, A.J., Otto, C.M. 2008. Fluid therapy in vomiting and diarrhoea. Veterinary Clinics of North America: Small Animal Practice 38(3):653-675.

Brunner, C., Swango, L. 1985. Canine parvovirus infection: Effects on the immune system and factors that predispose to severe disease. Compendium on Continuing Education for the Practising Veterinarian 7:979-989.

Buonavoglia, C., Tollis, M., Buonavoglia, D. 1992. Response of pups with maternal derived antibody to modified-live canine parvovirus vaccine. Comparative Immunology and Microbiology of Infectious Diseases 15:281-283.

Bybee, S.N., Scorza, A.V., Lappin, M.R. 2011. Effect of the probiotic Enterococcus faecium SF68 on presence of diarrhoea in cats and dogs housed in an animal shelter. Journal of Veterinary Internal Medicine 25:856-860.

Caccio, S.M., Thompson, R.C., McLauchlin, J., Smith, H.V. 2005. Unravelling Cryptosporidium and Giardia epidemiology. Trends Parasitology 21:430-437.

Calderón, M.G., Romanutti, C., Wilda, M., D'Antuono A., Keller, L., Giacomodonato M.N., Mattion, N., La Torre, J. 2015. Resurgence of canine parvovirus 2a strain in the domestic dog population from Argentina. Journal of Virological Methods, http://dx.doi.org/10.1016/j.jviromet.2015.06.012.

Carattoli, A., Lovari, S., Franco, A., Cordaro, G., di Matteo, P., Battisti, A. 2005. Extended-spectrum beta-lactamases in Escherichia coli isolated from dogs and cats in Rome, Italy, from 2001 to 2003. Antimicrobial Agents and Chemotherapy 49:833-835.

Carlin, E.P., Bowman, D.D., Scarlett, J.M., Garrett, J., Lorentzen, L. 2006. Prevalence of Giardia in symptomatic dogs and cats throughout the United States as determined by the IDEXX SNAP Giardia test. Veterinary Theriogenology 3:199-206.

Carmichael, L.E. 2005. An annotated historical account of canine parvovirus. Journal of Medicine B: Infectious Diseases, Veterinary Public Health 52:303-311.

Carr-Smith, S., Macintire, D.K., Swango, L.J. 1997. Canine parvovirus. Part I. Pathogenesis and vaccination. Compendium on Continuing Education for the Practicing Veterinarian 19(2):125-133.

Castro, T.X., Uchoa, C.M.A., Albuquerque, M.C., Labarthe, N.V., Cubel Garcia, R.C.N. 2007a. Canine parvovirus and intestinal parasites: Laboratorial diagnosis and clinical signs from puppies with gastroenteritis. International Journal of Applied Research in Veterinary Medicine 5(2):72-76.

Castro, T.X., Miranda, S.C., Labarthe, N.V., Silva, L.E., Cubel Garcia, R.C.N. 2007b. Clinical and epidemiological aspects of canine parvovirus (CPV) Enteritis in the State of Rio de Janeiro: 1995-2004. Arquivo Brasileiro de Medicina Veterinariae Zootecnia, 59:333-339.

Castro, T.X., Cubel Garcia R.N., Gonçalves, L.P.S., Costa, E.M., Marcello, G.C.G., Labarthe, N.V., Mendes-de-Almeida, F. 2013. Clinical, haematological, and biochemical findings in puppies with coronavirus and parvovirus enteritis. The Canadian Veterinary Journal 54:885-888.

Cavalli, A., Martella, V., Desario, C., Camero, M., Bellacicco, A.L., De Palo, P., Decaro, N., Elia, G., Buonavoglia, C. 2008. Evaluation of the antigenic relationships among canine parvovirus type 2 variants. Clinical and Vaccine Immunology 15:534-539.

Cave, N.J., Marks, S.L., Kass, P.H., Melli, A.C., Brophy, M.A. 2002. Evaluation of a routine diagnostic faecal panel for dogs with diarrhoea. Journal of American Veterinary Medical Association 221:52-59.

Cenk, R.E., Mahmut, K.O. 2015. Levels of cardiac biomarkers and coagulation profiles in dogs with parvoviral enteritis. Kafkas Universitesi Veteriner Fakultesi Dergisi 21(3):383-388. https://doi.org/10.9775/kvfd.2014.12575.

Chaitman, J., Jergens, A.E., Gaschen, F., Garcia-Mazcorro, J.F., Marks, S.L., Marroquin-Cardona, A.G., Richter, K., Rossi, G., Suchodolski, J.S., Weese, J.S.

2016. Faecal microbiota transplantation in small animal practice. Veterinary Medicine: Research and Reports 2016:771-774.

Chakrabarty, A. 2005. Dogs, their care and treatment. 2nd edition, Kalyani Publishers, New Delhi, India, 143 pp.

Chan, D.L., Rozanski, E.A., Freeman, L.M., Rush, J.E. 2001. Colloid osmotic pressure in health and disease. Compendium on Continuing Education for Practicing Veterinarian 23:886-904.

Chang, S.F., Sgro, J.Y., Parrish, C.R. 1992. Multiple amino acid in the capsid structure of canine parvovirus co-ordinately determines the canine host range and specific antigenic and haemagglutination properties. Journal of Virology 66:6858-6867.

Chapman, M.S., Rossmann, M.G. 1993. Structure sequence and function correlations among parvoviruses. Virology 194:491-508

Chehade, M., Mayer, L. 2005. Oral tolerance and its relation to food hypersensitivities. Journal of Allergy and Clinical Immunology 115.1:3-12. https://doi.org/10.1016/j.jaci.2004.11.008.

Chollom, S.C., Fyaktu, E.J., Okwori, A.E.J., Agada, G.O.A., Hashimu, G., Akele, R.Y. Voumangai, E.I., Dashe, T., Egah, D.Z. 2013. Molecular detection of canine parvovirus in Jos, Nigeria. Journal of Veterinary Medicine and Animal Health 5(2):57-59.

Claerebout, E., Casaert, S., De Dalemans, A.C., Wilde, N., Levecke, B., Vercruysse, J., Guerden, T. 2009. Giardia and other intestinal parasites in different dog populations in Northern Belgium. Veterinary Parasitology 161:41-46.

Coles, E.H. (1986): Veterinary Clinical Pathology. 4th ed. W.B. Saunders Co. Philadelphia; 1986:10:360-730

Costa, A.P., Leite, J.P., Labarthe, N.V. 2005. Genomic typing of canine parvovirus circulation in the state of Rio de Janeiro, Brazil from 1995 to 2001 using polymerase chain reaction assay. Veterinary Research Communication 29:735-743.

Cotmore, S.F., Tattersall, P. 1987. The autonomously replicating parvoviruses of vertebrates. Advances in Virus Research 33:91-174.

Cotmore, S.F., Tattersall, P. 1994. An asymmetric nucleotide in the parvoviral 3′ hairpin directs segregation of a single active origin of DNA replication. EMBO Journal 13:4145-4152.

Cotmore, S.F. Agbandje-McKenna, M., Chiorini, J.A., Mukha, D.V., Pintel, D.J., Qiu, J., Soderlund-Venermo, M., Tattersall, P., Tijssen, P., Gatherer, D., Davison, A.J. 2014. The family Parvoviridae. Archive of Virology, 159:1239-1247. doi:10.1007/s00705-013-1914-1.

Cotmore, S.F., Agbandje-McKenna, M., Canuti, M., Chiorini, J.A., EisHubinger, A.-M., Hughes, J., … Tijssen, P. 2019. ICTV virus taxonomy profile: Parvoviridae. The Journal of General Virology, 100(3) :367-368.

Cotton, J.A., Beatty, J.K., Buret, A.G. 2011. Host-parasite interactions and pathophysiology in Giardia infections. International Journal of Parasitology 41:925-933.

Cranny, G.O., McCormick B.A. 2008. Bacteria in the intestine, helpful residents or enemies from within? Infection and Immunity 76(8):3360-3373.

Craven, M., Simpson, J.W., Ridyard, A.E., Chandler M.L. 2004. Canine inflammatory bowel disease: retrospective analysis of diagnosis and outcome in 80 cases (1995-2002). Journal of Small Animal Practice 45:336-342.

Craven, M., Mansfield, C.S. Simpson, K.W. 2011. Granulomatous colitis of boxer dogs. Veterinary Clinics of North America Small Animal Practice 41(2):433-445.

Crawford, P.C., Sellon, R.K. 2010. Canine viral diseases. Ettinger, S.J., Feldman, E.C. Eds. Textbook of Veterinary Internal Medicine, 7th ed. Elsevier-Saunders. St. Louis, Missouri. 958-971 pp.

Cságola, A., Varga, S., Lőrincz, M., Tuboly, T. 2014. Analysis of the full-length VP2 protein of canine parvoviruses circulating in Hungary. Archives of Virology 159(9):2441-2444. https://doi.org/10.1007/ s00705-014-2068-5 2014 Apr 3.

Cummings, J.H., Pomare, E.W., Branch, W.J., Naylor, C.P., Macfarlane, G.T. 1987. Short-chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 28(10):1221-1227.

Dagmawi, P., Mellonen, A., Abebe, F., Berhanu, M. 2012. Prevalence of gastrointestinal helminths among dogs and owners' perception about zoonotic dog parasites in Hawassa Town, Ethiopia. Journal of Public Health and Epidemiology 4(8):205-209.

Dahiru, T. 2008. P-value, a true test of statistical significance? A cautionary note. Annals of Ibadan Postgraduate Medicine 6(1):21-26.

Dandrieux, J.R.S., Noble, P.J., Halladay, L.J., McLean, L., German, A.J. 2010. Breed predisposition for severe hypocobalaminemia and relation to folate concentration in dogs with gastrointestinal disease. Journal of Veterinary Internal Medicine 24:722.

Davis-Wurzler, G.M. 2014. 2013 update on current vaccination strategies in puppies and kittens. Veterinary Clinics: Small Animal Practice 44:235-263.

Day M.J., Horzinek, M.C., Schultz, R.D., Squires R.A. 2016. Guidelines for the vaccination of dogs and cats. Journal of Small Animal Practice 57:E1-E45.

De, S., Gupta, M.P., Singla, L.D., Sood, N.K. 2017. Copro-prevalence of hookworm infection and associated risk factors in pet dogs in Ludhian. Journal of Animal Research 7(2):409-413.

De Laforcade, A.M., Freeman, L.M., Shaw, S.P., Brooks, M.B., Rozanski, E.A., Rush, J.E. 2003. Haemostatic changes in dogs with naturally occurring sepsis. Journal of Veterinary Internal Medicine 17:674-679.

De Ybanez, R.R., Vela, C., Cortes, E., Simarro, I., Casal, J.I. 1995. Identification of types of canine parvovirus circulating in Spain. Veterinary Record 136:174-175.

Decaro, N., Desario, C., Campolo, M., Cavalli, A., Ricci, D., Martella, V., Tempesta, M., Buonavoglia C. 2004. Evaluation of lactogenic immunity to canine parvovirus in pups. New Microbiology 27:375-379.

Decaro, N., Campolo, M., Desario, C., Elia, G., Martella, V., Lorusso, E., Buonavoglia, C. 2005. Maternally-derived antibodies in pups and protection from canine parvovirus infection, Biologicals 33:261-267.

Decaro, N., Martella, V., Desario, C., Bellacicco, A. L., Camero, M., Manna, L. Buonavogolia, C. 2006. First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. Journal of Medicine B: Infectious Diseases, Veterinary Public Health 53:468-472.

Decaro, N., Desario, C., Elia, G., Campolo, M., Lorusso, A., Mari, V., Martella, V. Buonavoglia, C. 2007. Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: a clinical and laboratory diagnostic dilemma. Vaccine 25:1161-1166.

Decaro, N., Buonavoglia, C. 2008. An update on canine coronaviruses: Viral evolution and pathobiology. Veterinary Microbiology 132:221-234.

Decaro, N., Campolo, M., Lorusso, A., Desario, C., Mari, V., Colaianni, M. L., Elia, G., Martella, V., Buonavoglia, C. 2008a. Experimental infection of dogs with a novel strain of canine coronavirus causing systemic disease and lymphopenia. Veterinary Microbiology 128:253-260.

Decaro, N., Desario, C., Elia, G., Martella, V., Mari, V., Lavazza, A., Nardi, M., Buonavoglia, C. 2008b. Evidence for immunisation failure in vaccinated adult dogs infected with canine parvovirus type 2c. New Microbiologica 31:125-130.

Decaro, N., Buonavoglia, C. 2012. Canine parvovirus—a review of epidemiological and diagnostic aspects, with emphasis on type 2c. Veterinary Microbiology 155:1-12.

Decaro, N., Cordonnier, N., Demeter, Z., Egberink, H., Elia, G., Grellet, A., Le Poder, S., Mari, V., Martella, V., Ntafis, V., von Reitzenstein, M., Rottier, P. J., Rusvai, M., Shields, S., …, Buonavoglia, C. 2013a. European surveillance for pantropic canine coronavirus. Journal of Clinical Microbiology 51:83-88.

Decaro, N., Desario, C., Amorisco, F., Losurdo, M., Elia, G., Parisi, A., Ventrella, G., Martella, V., Buonavoglia, C. 2013b. Detection of a canine parvovirus type 2c with a non-coding mutation and its implications for molecular characterisation. Veterinary Journal 196(3):555-557.

Decaro, N., Crescenzo, G., Desario, C., Cavalli, A., Losurdo, M., Colaianni, M.L., Ventrella, G., Rizzi, S., Aulicino, S., Lucente, M.S., Buonavoglia, C. 2014. Long-term viremia and faecal shedding in pups after modified-live canine parvovirus vaccination. Vaccine 32:3850-3853.

Deem, S.L., Spelman, L.H., Yates, R.A., Montali, R.J. 2000. Canine distemper in terrestrial carnivores: A review. Journal of Zoology and Wildlife Medicine 31(4):441-451.

Detweiler D.K. 2011. The dog electrocardiogram: A critical review. Chapter 41. In: Macfarlane, P.W., van Oosterom, A., Pahlm, O., Kligfield, P., Janse, M., Camm, J. (eds.), Comprehensive Electrocardiology, https://doi.org/10.1007/978-1-84882-046.3–41, © Springer-Verlag London Limited. 1863-1908 Pp.

Devrajani, B.R., Shah, S.Z.A., Shaikh, S., Shaikh, S., Essa, S. 2009. Hypocalcaemia in acute gastroenteritis. World Applied Sciences Journal 7(6):777-780.

DiBartola, S.P., Johnson, S.E., Davenport, D.J., Preuter, J.C., Chew, D.J., Sherding RG. 1985. Clinicopathologic findings resembling hypoadrenocorticism in dogs with

primary gastrointestinal disease. Journal of American Veterinary Medical Association 187:60-63.

DiBartola, S.P. ed. 2011. Fluid, electrolytes, and acid-base disorders in small animal practice. 4th ed. Elsevier, Canada. 1-768 pp.

Dibartola, S.P., De Morais, H.A. 2012 Disorders of potassium: hypokalemia and hyperkalemia. In: Dibartola S. P., ed. Fluid, Electrolyte and Acid-Base Disorders in Small Animal Practice, 4th ed. St. Louis: Elsevier; 92-119.

Dogonyaro, B.B., Bosman, A.M., Sibeko, K.P., Venter, E.H., van Vuuren, M. 2013. Genetic analysis of the VP2–encoding gene of canine parvovirus strains from Africa. Veterinary Microbiology 165:460-465.

Dossin, O. 2011. Laboratory tests for diagnosis of gastrointestinal and pancreatic diseases. Topics in Companion Animal Medicine 26(2):86-95.

Dossin, O., Lavour, R. 2011. Protein-losing enteropathies in dogs. Veterinary Clinics of Small Animal 41:399-418.

Dryden, M.W., Payne, P.A., Smith, V. 2006. Accurate diagnosis of Giardia spp. and proper examination procedures. Veterinary Theriogenology 7:4-14.

Duboc, H., Rajca, S., Rainteau, D., Benarous, D., Maubert, M.A., Quervain, E., Thomas, G., Barbu, V., Humbert, L., Despras, G., Bridonneau, C., Dumetz, F., Grill, J.P., Masliah, J., Beaugerie, L., Cosnes, J., Chazouillères, O., Poupon, R., Wolf, C., Mallet, J.M., Langella, P., Trugnan, G., Sokol, H., Seksik, P. 2013. Connecting dysbiosis, bile-acid dysmetabolism and gut inflammation in inflammatory bowel diseases. Gut 62(4):531-539.

Dudley, L.M., Johnny, D.H. 2006. Canine viral enteritis. Infectious diseases of the dog and cat. C. E. Greene. Ed. 3rd ed. Elsevier–Saunders, St. Louis, Missouri. 63-70

Dutta, P.R., Hafiz, A., Bora, S., Phukan, A., Baishya B.C., Kalita, D.N. 2013. Prevalence of canine diseases in Guwahati City. Indian Veterinary Journal, 90(6):103-104.

Edwards, D.S., Henley, W.E., Ely, E.R., Wood, J.L. 2004. Vaccination and ill–health in dogs: a lack of temporal association and evidence of equivalence. Vaccine 22:3270-3273.

Eghafona, N.O., Jacob, J., Yah, S.C. 2007. Evaluation of post-vaccination immunity to canine distemper and parvoviruses in Benin City, Nigeria. African Journal of Biotechnology 6(16):1898-1904.

Ekakoro, J.E., Okafor C.C. 2019. Antimicrobial use practices of veterinary clinicians at a veterinary teaching hospital in the United States. Veterinary and Animal Science, 7:100038. https://doi.org/10.1016/j.vas.2018.09.002.

Elson, C.O., Cong, Y., McCracken, V.J., Dimmitt, R.A., Lorenz, R.G., Weaver, C.T. 2005. Experimental models of inflammatory bowel disease reveal innate, adaptive, and regulatory mechanisms of host dialogue with the microbiota. Immunology Review 206:260-276.

Elwood, C., Devauchelle, P., Elliott, J., Freiche, V., German, A.J., Gualtieri, M., Hall, E., Den Hertog, E., Neiger, R., Peeters, D., Roura, X., Savary-Bataille, K. 2010. Vomiting in dogs: a review. Journal of Small Animal Practice 51:4-22.

Ettinger, J.S., Feldman, E.C. 2010. Text Book of Veterinary Internal Medicine: Diseases of dog and cat. WB Saunders Company, Philadelphia. 1310-1408 pp.

European Union (EU). 2015. Guidelines for the prudent use of antimicrobials in veterinary medicine. Official Journal of the European Union 2015/C:7-26.

Evermann, J. F., Abbott, J.R., Han, S. 2005. Canine coronavirus–associated puppy mortality without evidence of concurrent canine parvovirus infection. Journal of Veterinary Diagnostic Investigation 17:610-614.

Fagbohun, O.A., Omobowale, T.O. 2018. Sequence and phylogenetic analysis of canine parvovirus-2 isolates in dogs revealed the circulation of three subtypes in Nigeria. Virus Disease 6 pages. https://doi.org/10.1007/s13337-018-0475-z.

Fan, T.M. 2007. Hypercalcemia in dogs and cats. NAVC clinician's brief. May 2007:23-27. Retrieved July 10, 2018, from http://www.cliniciansbrief.com/

Feldman, E.C., Nelson R.W. 2004. Hypoadrenocorticism (Addison's disease). In Feldman, E. C. and Nelson, R. W. (eds): Canine and feline endocrinology and reproduction, 3rd edn, St Louis, Saunders Elsevier, 394-439 pp.

Felsburg, P.J. 2002. Overview of immune system development in the dog: Comparison with Humans. Human and Experimental Toxicology 21.9-10:487-492.

Fielder S.E. 2015. Reference guides. In: The Merck Veterinary Manual. Retrieved on June 11, 2017, from https://www.merckvetmanual.com/special-subjects/reference-guides.

Ford, J., McEndaffer, L., Renshaw, R., Molesan, A., Kelly, K. 2017. Parvovirus infection is associated with myocarditis and myocardial fibrosis in young dogs. Veterinary Pathology 54(6):964-971.

French, A. 2008. Introduction to electrocardiography. World Small Animal Veterinary Association World Congress Proceedings. Retrieved on June 12, 2017 from http://www.vin.com/doc/?id=3866623. 6 pages.

Frezoulis, P.S., Angelidou, E., Karnezi, D., Oikonomidis, I.L., Kritsepi-Konstantinou M, Kasabalis D., Melonakos, M.E. 2017. Canine pancytopenia in a Mediterranean region: a retrospective study of 119 cases (2005-2013). Journal of Small Animal Practice 8 pages. https://doi.org/10.1111/jsap.12647.

Friedrich, K., Truyen, U. 2000. Efficacy of parvovirus vaccines and effectiveness of two vaccination protocols. Praktische Tierarzt. 81:988-994.

Gaschen, F.P., Merchant, S.R. 2011. Adverse food reactions in dogs and cats. Veterinary Clinics of North America Small Animal Practice 41(2):361-79. https://doi.org/10.1016/j.cvsm.2011.02.005.

Gberindyer, F.A. 2016. Prescription pattern, pharmaceutical equivalence and population pharmacokinetics of gentamicin in dogs with parvovirus enteritis in Nigeria. PhD. Thesis. Department of Veterinary Pharmacology. University of Ibadan. i-245 pp.

Gberindyer, F.A., Abatan, M.O., Apaa, T.T., Tion, T.M. 2017. Drugs prescription pattern in dogs diagnosed with parvovirus enteritis in some veterinary clinics in Nigeria. Nigerian Veterinary Journal 38(3):250-259.

Gberindyer, F.A., Shima, F.K., Omotosho, O.O., Omobowale, T.O., Abatan, M.O. 2018. Adverse drug events in puppies following treatment with amoxicillin with or without clavulanate. Alexandria Journal of Veterinary Sciences 59:143. https://doi.org/10.5455/ajvs.293352.

Geetha, M. 2015. Epidemiology, pathogenesis, clinical findings, and diagnosis of canine parvoviral infection. International Journal of Scientific Engineering and Applied Science 1:21-27.

Geng, Y., Guo, D., Li, C., Wang, E., Wei, S., Wang, Z., Yao, S., Zhao, X., Su, M., Wang, X., Wang, J., Wu, R., Feng, L., Sun, D. 2015. Co-circulation of the rare CPV-2c with unique Gln370Arg substitution, New CPV-2b with unique Thr440Ala substitution, and New CPV-2a with high prevalence and variation in Heilongjiang province, Northeast China. PLoS ONE, 10.9: e0137288. https://doi.org/10.1371/journal.pone.0137288.

German, A.J., Day, M.J., Ruaux, C.G., Steiner J.M., Williams, D.A., Hall, E.J. 2003. Comparison of direct and indirect tests for small intestinal bacterial overgrowth and antibiotic-responsive diarrhoea in dogs. Journal of Veterinary Internal Medicine 17:33-43.

German, A.J., Halladay, L.J., Noble, P-J.M. 2010. First-choice therapy for dogs presenting with diarrhoea in clinical practice. Veterinary Record 167:810-814.

Gharekhani, J. 2014. Study on gastrointestinal zoonotic parasites in pet dogs in Western Iran. Turkiye Parazitoloji Dernegi 38:172-176.

Giaretta, P.R., Rech, R.R., Guard, B.C., Blake, A.B., Blick, A.K., Steiner, J.M., Lidbury, J.A., Cook, A.K., Hanifeh, M., Spillmann, T., Kilpinen, S., Syrjä, P., Suchodolski, J. 2018. Comparison of intestinal expression of the apical sodium-dependent bile acid transporter between dogs with and without chronic inflammatory enteropathy. Journal of Veterinary Internal Medicine 32(6):1918-1926.

Gill, M., Srinivas, J., Morozov, I., Smith, J., Anderson, C., Glover, S., Champ, D. Chu, H-J. 2004. Three-Year duration of immunity for canine distemper, adenovirus, and parvovirus after vaccination with a multivalent canine vaccine. International Journal of Applied Research in Veterinary Medicine 2(4):227-234.

Glickman, L.T., Domanski, L.M., Patronek, G.J., Visintainer F. 1985. Breed-related risk factors for canine parvovirus enteritis. Journal of American Veterinary Medical Association 187(6):589-594.

Glover, S., Anderson, C., Piontkowski, M., Ng, T. 2012. Canine parvovirus (CPV) type 2b vaccine protects puppies with maternal antibodies CPV when challenged with virulent CPV-2c virus. International Journal of Applied Research in Veterinary Medicine, 10(3):217-224.

Goddard, A., Leisewtz, A.L., Christopher, M.M., Duncan, N.M., Becker, P.J. 2008. Prognostic usefulness of blood leukocyte changes in canine parvoviral enteritis. Journal of Veterinary Internal Medicine 22(2):309-316.

Good, J.M., Otto, C.M. 2006. Routine biomarkers of sepsis in dogs with canine parvoviral enteritis: a retrospective study (abstract). Proceedings of International Veterinary Emergency and Critical Care Symposium 8-9 pp.

Gorham, A. 2018. Importance of vaccinating for canine parvovirus: Best practices for prevention. Honors Theses. 440. Retrieved June 16, 2019 from http://opensiuc.lib.siu.edu/uhp_theses/440.

Gough, A. Ed. 2007. Differential diagnosis in small animal medicine. Blackwell Publishing Ltd, UK. 21-31.

Graves, T.K., Schall, W.D., Refsal, K., Nachreiner R.F. 1994. Basal and ACTH-stimulated plasma aldosterone concentrations are normal or increased in dogs with *trichuriasis*-associated pseudohypoadrenocorticism. Journal of Veterinary Internal Medicine 8:287-289.

Grecco, S., Iraola, G., Decaro, N., Alfieri, A., Alfieri, A., Calderon, G.M, da Silva, P.A, Name, D., Aldaz, J., Calleros, L., Marandino, A., Tomas, G., Maya, L., Francia, L., Panzera, Y., Pérez, R. 2018. Inter- and intracontinental migrations and local differentiation have shaped the contemporary epidemiological landscape of canine parvovirus in South America. Virus Evolution 4:vey011. 10 pages. https://doi.org/10.1093/ve/vey011.

Greene, C.E., Appel, M.J. 2006. Canine distemper. Infectious diseases of the dogs and cats. C.E Greene Eds., 3rd ed. Saunders Elsevier, St. Louis, Missouri, 25-41 pp.

Greenwood, N.M., Chalmer, W.S.K., Baxendale, W., Tampson, H. 1996. Comparison of isolates of canine parvovirus by monoclonal antibody and restriction enzyme analysis. Veterinary Record 138:495-496.

Grellet, A., Chastant-Maillard, S., Robin, C., Feugier, A., Boogaert, C., Boucraut-Baralon, C., Grandjean, D., Polack, B. 2014. Risk factors of weaning diarrhoea in puppies housed in breeding kennels. Preventive Veterinary Medicine 117:260-265.

Grellet, A., 2016. Weaning diarrhoea in puppies. Veterinary Focus 26:14-21.

Grutzner, N., Bishop, M.A., Suchodolski, J.S., Steiner, J.M. 2010. Association study of cobalamin deficiency in the Chinese Shar Pei. Journal of Heredity 101:211-217.

Guard. C.B., Barr, W.J., Reddivari, L., Klemashevich, C., Jayaraman, A., Steiner, J.M., Vanamala, J., Suchodolski, J.S. 2015. Characterization of microbial dysbiosis and metabolomic changes in dogs with acute diarrhoea. PLoS One 10(5):e0127259. https://doi.org/10.1371/journal.pone.0127259.

Guo, L., Yang, S.L., Chen, S.J., Zhang, Z., Wang, C., Hou, R., Ren, Y., Wen, X., Cao, S., Guo, W., Hao, Z., Quan, Z., Zhang, M., Yan, Q.G. 2013. Identification of

canine parvovirus with the Q370R point mutation in the VP2 gene from a giant panda (*Ailuropoda melanoleuca*). Virology Journal 10:163.

Haligur, M., Ozmen, O., Sezer, K., Sahinduran, S. 2009. Clinical, pathological and immunohistochemical findings in diarrheic dogs and evaluation of canine parvoviral and coronaviral enteritis. Journal of Animal and Veterinary Advances 8(4):720-725.

Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series 41:95-98.

Hall, E.J., German, A.J. 2010. Diseases of the small intestine. Textbook of Veterinary Internal Medicine. 7th edn. Ettinger, S.J., E.C. Feldman. Eds. Saunders Elsevier. 1526-1572 pp.

Hamnes, I.S., Gjerde, B.K., Robertson, L.J. 2007. A longitudinal study on the occurrence of *Cryptosporidium* and *Giardia* in dogs during their first year of life. Acta Veterinaria Scandinavica 49:22.

Handl, S., Dowd, S.E., Garcia-Mazcorro, J.F., Steiner, J.M., Suchodolski, J.S. 2011. Massive parallel 16S rRNA gene pyrosequencing reveals highly diverse faecal bacterial and fungal communities in healthy dogs and cats. FEMS Microbiology and Ecology 76(2):301-310.

Hankes, G.H., Dillon, A.R., Ravis, W.R. 1992. Effects of lactated Ringer's solution and prednisolone sodium succinate on dogs with induced haemorrhagic shock. American Journal of Veterinary Research 53:26-33.

Heininger, U., Bachtiar, N.S., Bahri, P., Dana, A., Dodoo, A., Gidudu, J., Matos dos Santos, E. 2012. The concept of vaccine failure. Vaccine 30:1265-1268.

Herstad, H.K., Nesheim, B.B., L'Abée-Lund, T., Larsen, S., Skancke, E. 2010. Effects of a probiotic intervention in acute canine gastroenteritis – a controlled clinical trial. Journal of Small Animal Practice 51:34-38.

Hilwig, R.W., Hahn, A. 1976. Genesis of the electrocardiogram. Journal of American Veterinary Medical Association 169:1054-1055.

Himsworth, C.G., Skinner, S., Chaban, B., Jenkins, E.J., Wagner, B.A., Harms, N.J., Leighton, F.A., Thompson, R.C.A., Hill, J. E. 2010. Multiple zoonotic pathogens identified in canine faeces collected from a remote Canadian indigenous community. American Journal of Tropical Medicine and Hygiene 83:338-341

Hirasawa, T., Yono, K., Mikazuki, K. 1996. Detection and genomic analysis of canine parvovirus by the polymerase chain reaction. Journal of Veterinary Medicine, Series B. 43:545-554.

Hoelzer, K., Parrish, C. 2010. The emergence of parvoviruses of carnivores. Veterinary Research 41:39.

Hong, C., Decaro, N., Desario, C., Tanner, P., Pardo, M., Sanchez, S., Buonavoglia, C., Siliki, J. 2007. Occurrence of canine parvovirus type 2c in the United State. Journal of Veterinary Diagnostic Investigation 19:535-539.

Honneffer, J.B., Minamoto, Y., Suchodolski, J.S. 2014. Microbiota alterations in acute and chronic gastrointestinal inflammation of cats and dogs. World Journal of Gastroenterology 20:16489-16497.

Horiuchi, M., Yamaguchi, Y., Gojobori, T., Mochizuki, M., Nagasawa, H., Toyoda, Y., Ishiguro, N., Shinagawa, M. 1998. Differences in the evolutionary pattern of feline panleukopenia virus and canine parvovirus. Virology 249:440-452.

Hoskins, J.D. 1997. Update on canine parvoviral enteritis. Veterinary Medicine 92(8):694-709.

Hosmer, D.W.J., Lemeshow, S. 2000. Applied Logistic Regression, 2nd ed. New York: John Wiley and Sons. 1-202 pp. DOI:10.1002/0471722146.

Houston, D.M., Ribble, C.S., Head, L.L. 1996. Risk factors associated with parvovirus enteritis in dogs. 283 Cases (1982-1991). Journal of American Veterinary Medical Association 208:542-546.

Hubbard, K., Skelly, B.J., Mckelvie, J., Wood, J.L. 2007. Risk of vomiting and diarrhoea in dogs. Veterinary Record 161:755-757.

Humm, K.R., Hughes, D. 2009. Canine parvovirus infection. In: Small Animal Critical Care Medicine. Silverstein D.C. and K. Hopper (Eds.), St. Louis: Saunders. 482-485 pp.

Hunter, R.P., Isaza, R. 2017. Polypharmacy in zoological medicine. Pharmaceutics 9(10):1-8.

Iida, H., Fukuda, S., Kawashima, N., Yamazaki, T., Aoki, J., Tokita, K., Morioka, K., Takarada, N., Soeda, T. 1990. Effect of maternally derived antibody levels on antibody responses to canine parvovirus, canine distemper virus and infectious canine hepatitis virus after vaccinations in beagle puppies. Jikken Dobutsu 39: 9-19.

Iraguen, D., Urcelay, S., Martin, B.S. 2010. Pharmacovigilance in veterinary medicine in Chile: a pilot study. Journal of Veterinary Pharmacology and Therapeutics 34:108-115.

Isogai, E., Isogai, H., Onuma, M. 1989. *Escherichia coli* associated endotoxaemia in dogs with parvovirus infection. Japanese Journal of Veterinary Science 51(3): 597-606.

Jacobs, R.M., Weiser, M.G., Hall, R.L., Kowalski, J.J. 1980. Clinicopathologic features of canine parvoviral enteritis. Journal of the American Animal Hospital Association 16:809-814.

Jain, N.C. 1986. Schalm's Veterinary Haematology, 4th ed. Lea and Febiger Publication, Philadelphia, 274-349 pp.

Jakel, V., Cussler, K., Hanschmann, K.M., Truyen, U., König, M., Kamphuis, E., Duchow, K. 2012 Vaccination against Feline Panleukopenia: implications from a field study in kittens. BMC Veterinary Research 8:62

Janeczko, S., Atwater, D., Bogel, E., Greiter-Wilke, A., Gerold, A., Baumgart, M., Bender, H., McDonough, P.L., McDonough, S.P., Goldstein, R.E., Simpson, K.W. 2008. The relationship of mucosal bacteria to duodenal histopathology, cytokine mRNA, and clinical disease activity in cats with inflammatory bowel disease. Veterinary Microbiology 128(1–2):178-193.

Jani, R.G., Dave, M.R., Jani, B.M. 1992. Haematological and biochemical changes in dogs suffering from diarrhoea. Indian Journal of Veterinary Medicine 12:36.

Jarret, O., Ramsey, I. 2001. Vaccination. Manual of canine and feline infectious diseases. Ramsey, I., Gloucester, T.B. Eds. British Small Animal Veterinary Association. The UK. 41-50 pp.

Jernberg, C., Lofmark, S., Edlund C., Jansson, J.K. 2010. Long-term impacts of antibiotic exposure on the human intestinal microbiota. Microbiology 156(Pt 11): 216-223. https://doi.org/10. 1099/mic.0.040618-0

Johnson, C.C., Ownby, D.R., Alford, S.H., Havstad, S.L., Williams, L.K., Zoratti, E. M., Peterson, E.L., Joseph, C.L.M. 2005 Antibiotic exposure in early infancy and risk for childhood atopy. Journal of Allergy and Clinical Immunology 115(6):1218-1224. https://doi.org/10.1016/j.jaci.2005.04.020.

Johnson, S.A.M., Gakuyab, D.W., Mbuthiac, P.G., Mandeb, J.D., Maingic, N. 2015. Prevalence of gastrointestinal helminths and management practices for dogs in

the Greater Accra region of Ghana. Heliyon 2015.e00023:11 pages. http://dx.doi.org/10.1016/j.heliyon.2015.e00023.

Johnston, K.L. 1999. Small intestinal bacterial overgrowth. Veterinary Clinics of North American: Small Animal Practice 29:523-550.

Jones, A.E. 2018. Parvovirus myocarditis in young dogs. Clinician's Brief July 2018. Retrieved July 10, 2018, from https://www.cliniciansbrief.com/.../parvovirus-myocarditis-young-dogs. June 2018:2 pages.

Kahn, C.M. 2010. The Merck Veterinary Manual. 10th ed. New Jersey: Merck and Co. Inc. 2945 pp.

Kalli, I., Leontides, L.S., Mylonakis, M.E. 2010. Factors affecting the occurrence, duration of hospitalization and clinical outcome in canine parvovirus infection. Research in Veterinary Science 89:174-178.

Kang, B.K., Song, D.S., Lee, C.S., Jung, K.I., Park, S.J., Kim, E.M., Park, B.K. 2008. Prevalence and genetic characterization of canine parvoviruses in Korea. Virus Genes 36:127-133

Kapil, S., Cooper, E., Lamm, C., Murray, B., Rezabek, G., Johnston, L., Campbell, G., Johnson, B. 2007. Canine parvovirus types 2c and 2b circulating in North American dogs in 2006 and 2007. Journal of Clinical Microbiology 45(12):4044-4047.

Kathrani, A., Steiner, J.M., Suchodolski, J., Eastwood, J., Syme, H., Garden, O.A., Allenspach, K. 2009. Elevated canine pancreatic lipase immunoreactivity concentration in dogs with inflammatory bowel disease is associated with a negative outcome. Journal of Small Animal Practice 50:126-132.

Kaur, G., Chandra, M., Dwivedi, P.N., Narang, D. 2015. Current approaches in the diagnosis of canine parvovirus: An overview. Journal of Microbiology, Immunology and Biotechnology 2:1-4.

Kelley, R.L., Minikhiem, D., Kiely, B., O'Mahony, L., O'Sullivan, D., Boileau, T., Park, J.S. 2009. Clinical benefits of probiotic canine-derived *Bifidobacterium animalis* strain AHC7 in dogs with acute idiopathic diarrhoea. Veterinary Theriogenology 10(3):121-130.

Kelly, C.R., Kahn, S., Kashyap, P., Laine, L., Rubin, D., Atreja, A., Moore, T., Wu G. 2015. Update on faecal microbiota transplantation 2015: indications, methodologies, mechanisms, and outlook. Gastroenterology 149:223-237.

Kelly, D.M. 2016. Hemorrhagic gastroenteritis in small animals: Diseases of the stomach and intestines in small animals. The Merck Veterinary Manual. Retrieved on June 11, 2017, from http://www.merckvetmanual.com/

Kempf, C., Schulz, B., Strauch, C., Sauter–Louis, C., Truyen, U., Hartmann, K. 2010. Virus detection, clinical signs, and laboratory findings in dogs with acute haemorrhagic diarrhoea. A retrospective study of 935 cases. Tierärztl Prax 38(K):79-86.

Kennedy, L.J., Lunt, M., Barnes, A., McElhinney, L., Fooks, A.R., Baxter, D.N., Ollier, W.E.R. 2007. Factors influencing the antibody response of dogs vaccinated against rabies. Vaccine 25:8500-8507.

Kilian, E., Suchodolski, J.S., Hartmann, K., Mueller, R.S., Wess, G., Unterer, S..2018. Long-term effects of canine parvovirus infection in dogs. PLoS One 13(3):e0192198. https://doi.org/10.1371/ journal. pone.0192198.

Killey, R., Mynors, C., Pearce, R.., Nell, A., Prentis, A., Day, M.J. 2017. Long-lived immunity to canine core vaccine antigens in UK dogs as assessed by an in-practice test kit. Journal of Small Animal Practice 2017:12775, 5 pages. https://doi.org/10.1111/jsap.12775.

Kirsten, S., Henrik, M., Hans, O.B., Lars, O.H. 2010. Polypharmacy in general practice. Danish Medical Bulletin 57(7):1-5.

Klem, F., Wadhwa, A., Prokop, L.J., Sundt, W.J., Farrugia, G., Camilleri, M., Singh, S., Grover, M. 2017. Prevalence, risk factors, and outcomes of irritable bowel syndrome after infectious enteritis: A systematic review and meta-analysis. Gastroenterology 152(5):1042-1054. doi:10.1053/j.gastro.2016.12.039.

Kocatürk, M., Martinez, S., Eralp, O., Tvarijonaviciute, A., Ceron, J., Yılmaz, Z. 2010. Prognostic value of serum acute-phase proteins in dogs with parvoviral enteritis. Journal of Small Animal Practice 51:478-483.

Kogan, D.A., Johnson, L.R., Sturges, B.K., Jandrey, K.E., Pollard, R.E. 2008. Aetiology and clinical outcome in dogs with aspiration pneumonia: 88 cases (2004-2006). Journal of American Veterinary Medical Association 233:1748-1755.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35:1547-1549.

Kummeling, I., Stelma, F.F., Dagnelie, P.C., Snijders, B.E., Penders, J., Huber, M., van Ree, R., van den Brandt, P.A., Thijs, C. 2007. Early-life exposure to antibiotics and the subsequent development of eczema, wheeze, and allergic sensitization in the first 2 years of life: the KOALA Birth Cohort Study. Paediatrics 119:225-231. https://doi.org/10.1542/peds.2006-0896.

Kyffin, J. 2014. Probiotics and diarrhoea. Veterinary Practice June 2014:32. Retrieved February 12, 2020 from https://veterinary-practice.com/article/probiotics-and-diarrhoea.

Lamm, C.G., Rezabek, G.B. 2008. Parvovirus infection in domestic companion animals. Veterinary Clinics of North America: Small Animal Practice 38(4):837-850.

Latimer, K.S. 1995. Leukocytes in health and disease. In: Ettinger, S.J, Feldman, E.C., eds. Textbook of Veterinary Internal Medicine, 4th ed. WB Saunders Company, Philadelphia, PA, 1892-1929 pp.

Lee, J.A., Cohn, L.A. 2015. Canine paediatrics: the vomiting puppy. Today's Veterinary Practice September/October: 32-38. Retrieved on August 21, 2017, from http://www.tvpjournal.com.

Leekha, S., Terrell, C.L., Edson, R.S. 2011. General principles of antimicrobial therapy. Mayo Clinical Proceedings 86(2): 156–167. Retrieved September 21, 2016, from http://www.mayoclinicproceeding.com/

Leib, M.S. 2005. Acute diarrhoea. Manual of canine and feline gastroenterology. Hall, E.J., Simpson, J.W., Williams, D.A. Gloucester. Eds. 2nd ed. British Small Animal Veterinary Association. The UK. 78-81 pp.

Leib, M.S., Larson, M.M., Panciera, D.L., Troy, G.C., Monroe, W.E., Rossmeisl, J.H., Forrester, S.D., Herring, E.S. 2010. Diagnostic utility of abdominal ultrasonography in dogs with chronic vomiting. Journal of Veterinary Internal Medicine 24:803-808.

Lenghaus, C., Studdert, M.J. 1984. Acute and chronic viral myocarditis. Acute diffuse nonsuppurative myocarditis and residual myocardial scarring following infection with canine parvovirus. The American Journal of Pathology 11:316-319.

Leroy, G. 2011. Genetic diversity, inbreeding and breeding practices in dogs: results from pedigree analyses. The Veterinary Journal 189:177-182.

Levis J.T. 2012. ECG diagnosis: hypokalaemia. The Permanente Journal, 16(2):57. https://doi.org/10.7812/tpp/12-015.

Li, S.T., Grossman, D.C., Cummings, P. 2007. Loperamide therapy for acute diarrhoea in children: systematic review and meta-analysis. PLoS Medicine 4: e98.

Li, G., Ji, S., Zhai, X., Zhang, Y., Liu, J., Zhu, M., Zhou, J., Su, S. 2017. Evolutionary and genetic analysis of the VP2 gene of canine parvovirus. BMC Genomics 18:534. https://doi.org/10.1186/s12864-017-3935-8

Ling, M., Norris, J.M., Kelman, M., Ward, M.P. 2012. Risk factors for death from canine parvoviral-related disease in Australia, Veterinary Microbiology, 158:280-290.

Linklater, A. 2016. Fluid therapy: Maintenance fluid plan. The Merck Veterinary Manual. Retrieved on July 7, 2017, from http://www.merckvetmanual.com/

Little, S.E., Johnson, E.M., Lewis, D. 2009. Prevalence of intestinal parasites in pet dogs in the United States. Veterinary Parasitology 166:144-152.

Lloyd, D.H. 2007. Reservoirs of antimicrobial resistance in pet animals. Clinical Infectious Diseases 45(Suppl 2):148-152.

Lund, E.M., Armstrong, P.J., Kirk, C.A., Kolar, L.M., Klausner, J.S. 1999. Health status and population characteristics of dogs and cats examined at private veterinary practices in the United States. Journal of American Veterinary Medical Association 214:1336-1341.

Maarschalkerweerd, R.J., Endenburg, N., Kirpensteijn, J., Knol, B.W. 1997. Influence of orchiectomy on canine behaviour. Veterinary Record 140:617-619.

Macartney, L., McCandlish, I.A., Thompson, H. 1984. Canine parvovirus enteritis 1: clinical, haematological and pathological features of experimental infection. Veterinary Record 115(9):201-210.

Macintire, D.K., Smith-Carr, S. 1997. Canine parvovirus. Part II. Clinical signs, diagnosis, and treatment. Compendium on Continuing Education for the Practicing Veterinarian 19(3):291-302.

Mahdy, A.K., Yvonne, A.L., Romano, N., Siti, F., Choy, S.H., Ibrahim, J., Surin, J. 2012. Prevalence and zoonotic potential of canine hookworms in Malaysia. Parasites and Vectors 5:88. https://doi.org/10.1186/1756-3305-5-88.

Mahmuda, A., Magaji, A.A., Yakubu, Y., Salihu, M.D., Lawal, M.D., Mahmud, U., Suleiman, N., Danmaigoro, A. 2012. Prevalence of intestinal parasites of dogs slaughtered at Mami market area, Sokoto, Nigeria. Scientific Journal of Animal Science 1:126-130.

Mallesh, K., Shepur, T.A. 2015. Hyperglycemia in grade III and grade IV malnutrition with dehydrating gastroenteritis. Journal of Evidence-based Medicine and Healthcare 2(31):4623-4629. https://doi.org/10.18410/jebmh/2015/650.

Mandigers, P.J., Biourge, V., van den Ingh T.S., Ankringa, N., German, A.J. 2010. A randomized, open-label, positively-controlled field trial of a hydrolysed protein diet in dogs with chronic small bowel enteropathy. Journal of Veterinary Internal Medicine 24:1350-1357.

Mantione, N.L., Otto, C.M. 2005. Characterization of the use of antiemetic agents in dogs with parvovirus enteritis treated at Veterinary Teaching Hospital: 77 cases (1997–2000). Journal of American Veterinary Medical Association 227(11):1787-1793.

Markovich, J.E., Stucker, K.M., Carr, A.H., Harbison, C.E., Scarlett, J.M., Parrish, C.R. 2012. Effects of canine parvovirus strain variations on diagnostic test results and clinical management of enteritis in dogs. Journal of American Veterinary Medical Association 241:66-72.

Marks, S.L., Melli, A.C., Kass, P.H., Jang, S.S., Barkhoodarian, A., Hirsh, D.C. 1999. Evaluation of methods to diagnose *Clostridium perfringens*–associated diarrhoea in dogs. Journal of American Veterinary Medical Association 214(3):357-360.

Marks, S.L., Laflamme, D., McCandlish, A.P. 2002a. Dietary trial using a commercial hypoallergenic diet containing hydrolysed protein for dogs with inflammatory bowel disease. Veterinary Theriogenology 3:109-118.

Marks, S.L., Kather, E., Kass, P., Melli, A.C. 2002b. Genotypic and phenotypic characterization of *Clostridium perfringens* and *Clostridium difficile* in diarrheic and healthy dogs. Journal of Veterinary Internal Medicine 16(5):533-540.

Marks, S.L., Kather, E.J. 2003: Bacterial–associated diarrhoea in the dog: a critical appraisal. Veterinary Clinics of North American: Small Animal Practice 33:1029-1060.

Marks, S.L., Rankin, S.C., Byrne, B.A., Weese, J.S. 2011. Enteropathogenic bacteria in dogs and cats: Diagnosis, epidemiology, treatment, and control. Journal of Veterinary Internal Medicine 25:1195-1208.

Marks, S.L. 2013. Diarrhoea. In: Canine and feline gastroenterology Washabau, R.W., Day, M.J. (Eds.), St. Louis: Elsevier, 99-108 pp.

Marks, S.L., 2015. The Mysteries of the GI Tract: Demystifying chronic diarrhoea in dogs. Critical updates on canine and feline health. North American Veterinary Congress/World Veterinary Congress Proceedings held on January 17-21, 2015 at Orlando, FL. 1-9 pp.

Martella, V., Cavalli, A., Pratelli, A., Bozzo, G., Camero, M., Buonavoglia, D., Narcisi, D., Tempesta, M., Buonavoglia C. 2004. A parvovirus mutants is spreading in Italy. Journal of Clinical Microbiology 42:1333-1336.

Martínez-Carrasco, C., Berriatua, E., Garijo, M., Martínez, J., Alonso F.D., Ruiz de Ybáñez, R. 2007. Epidemiological study of nonsystematic parasitism in dogs in southeast Mediterranean Spain assessed by coprological and post-mortem examination. Zoonoses Public Health 54:195-203.

Martyn, J.C., Davidson, B.E., Studdert, M.J. 1990. Nucleotide sequence of feline panleukopenia virus: comparison with canine parvovirus identifies host-specific differences. Journal of General Virology 71(Pt 11):2747-2753.

Mason, M.J., Gillett, N.A., Muggenburg, B.A. 1987. Clinical, pathological, and epidemiological aspects of canine parvoviral enteritis in an unvaccinated closed beagle colony:1978–1985. Journal of the American Animal Hospital Association 23:183-192.

Maxie, M.G., Jubb, K.V.F. 2007. Jubb, Kennedy, and Palmer's Pathology of Domestic Animals. Edinburgh, UK: Elsevier Saunders.

Maya, L., Calleros, L., Francia, L., Hernandez, M., Iraola, G., Panzera, Y., Sosa, K., Perez, R. 2013. Phylodynamics analysis of canine parvovirus in Uruguay: evidence of two successive invasions by different variants. Archives of Virology 158:1133-1141.

Mazzaferro, E.M., Rudloff, E., Kirby, R. 2002. The role of albumin replacement in the critically ill veterinary patient. Journal of Veterinary Emergency Critical Care 12(2):113-124.

Mazzaferro, E.M. 2008. Complications of fluid therapy. Veterinary Clinics of North America: Small Animal Practice 38(3):607-619.

Mazzaferro, E.M., Powell, L.L. 2013. Fluid therapy for the emergent small animal patient: Crystalloids, colloids, and albumin products. Veterinary Clinics of North America: Small Animal Practice 43(4):721-374.

McCaw, D.L., Hoskins, J.D. 2006. Canine viral enteritis. In: Green CE, ed. Infectious diseases of the dog and cat. 4th ed. St Louis, MO: Saunders, 63-73 pp.

McFee, R., Parungao, A. 1961. An orthogonal lead system for clinical electrocardiography. American Heart Journal 62:93-100.

Mensack, S. 2008. Fluid therapy: Options and rational administration. Veterinary Clinics of North America: Small Animal Practice 38(3):575-586.

Merck, 2010. Vomiting. The Merck Veterinary Manual, 10th ed. Merck & Co., Inc. New Jersey, USA. 443-445 pp.

Metsala, J., Lundqvist, A., Virta, L.J., Kaila, M., Gissler, M., Virtanen, S.M. 2015. Prenatal and postnatal exposure to antibiotics and the risk of asthma in childhood. Clinical and Experimental Allergy 45:137-145.

Meunier, P.C., Cooper, B.J., Appel, M.J., Slauson, D.O. 1984. Experimental viral myocarditis: parvoviral infection of neonatal pups. Veterinary Pathology 21(5):509-515.

Mila, H., Grellet, A., Desario, C., Feugier, A., Decaro, N., Buonsvoglia, C., Chastan-Maillard, S. 2014. Protection against canine parvovirus type 2 infection in puppies by colostrum-derived antibodies. Journal of Nutritional Science 3(e54): 4pages. https://doi.org/10.1017/jns.2014.57.

Minakshi, P., Basanti, B., Sunderisen, K., Jiju, V.T., Savi, J., Ikbal, Koushlesh, R., Upendera, L., Madhusudan, G., Nitish, B., Pawan, K., Vinay, G.J., Rahul, K., Hari, M., Pundir, C.S., Sandip, K.K., Gaya, P. 2016. Canine parvovirus- an insight into diagnostic aspect. Journal of Experimental Biology and Agricultural Sciences 4(3S):279-290.

Minamoto, Y., Dhanani, N., Markel, M.E., Steiner, J.M., Suchodolski, J.S. 2014. Prevalence of *Clostridium perfringens*, *Clostridium perfringens* enterotoxin and dysbiosis in faecal samples of dogs with diarrhoea. Veterinary Microbiology 174(3-4):463-473.

Mira, F., Purpari, G., Lorusso, E., Di Bella, S., Gucciardi, F., Desario, C., Macaluso, G., Decaro, N., Guercio, A., 2018. Introduction of Asian canine parvovirus in Europe through dog importation. Transboundary and Emerging Diseases 65:16-21. https://doi.org/10.1111/tbed.12747

Miranda, C., Thompson, G. 2016. Canine parvovirus in vaccinated dogs: a field study. Veterinary Record, 178:397, https://doi.org/10.1136/vr.103508.

Mittal, M., Chakravarti, S., Mohapatra, J.K., Chug, P.K., Dubey, R., Upmanuyu, V., Narwal, P.S., Kumar, A., Churamani, C.P., Kanwar, N.S. 2014. Molecular typing of canine parvovirus strains circulating from 2008 to 2012 in an organized kennel in India reveals the possibility of vaccination failure. Infection and Genetics Evolution 23:1-6.

Mochizuki, M., Hashimoto, M., Ishida, T. 2001. Recent epidemiological status of canine viral enteric infections and Giardia infection in Japan. Journal of Veterinary Medical Science 63:573-575.

Mohamed, A.S., Glickman, L.T., Camp, Jr., J.W., Lund, E., Moore, G.E. 2013. Prevalence and risk factors for *Giardia spp*. infection in a large national sample of pet dogs visiting veterinary hospitals in the United States (2003-2009). Veterinary Parasitology 195:35-41.

Mohammed, J.G., Ogbe, A.O., Zwandor, N.J., Umoh, J.U. 2005. Risk factors associated with canine parvovirus enteritis in Vom and environs. Animal Research International 2:366-368.

Mohr, A.J., Leisewitz, A.L., Jacobson, L.S., Steiner, J.M., Ruaux, C.G., Williams, D.A. 2003. Effect of early enteral nutrition on intestinal permeability, intestinal protein loss, and outcome in dogs with severe parvoviral enteritis. Journal of Veterinary Internal Medicine 17:791-798.

Moore, F.M., Bender, H.S. 2000. Neutropenia. In: Feldman, B.F., Zinkl, J.G, Jain, N.C., Eds. Schalm's Veterinary Haematology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, PA. 350-355 pp.

Muhairwa, A.P., Nonga, H.E., Kusiluka, L.J. 2008. A retrospective study of intestinal helminthosis as a cause of clinical disease in dogs. Tanzania Veterinary Journal 25:24-30.

Murphy, T., Chaitman, J., Han, E. 2014. Use of faecal transplant in eight dogs with refractory *Clostridium perfringens* associated diarrhoea. Journal of Veterinary Internal Medicine 28:976-1134.

Mylonakis, M.E., Kalli, I., & Rallis, T.S. 2016. Canine parvoviral enteritis: an update on the clinical diagnosis, treatment, and prevention. Veterinary medicine (Auckland, N.Z.), 7:91-100. https://doi.org/10.2147/VMRR.S80971

Nakamura, M., Tohya, Y., Miyazawa, T., Mochizuki, M., Phung, H.T., Nguyen, N.H., Huynh, L.M., Nguyen, L.T., Nguyen, N.P., Akashi, H. 2004. A novel antigenic

variant of canine parvovirus from a Vietnamese dog. Archives of Virology 149:2261-2269.

Nandi, S., Kumar, M. 2010. Canine parvovirus: Current perspective. Indian Journal of Virology 21:31-44.

Nandi, S., Kumar, M., Mohapatra, T.K., Ravishankar, C. 2013. Emergence of canine parvovirus-2 variants and its impact on vaccination. World Applied Sciences Journal 23(10):1366-1376.

Neuerer, F.F., Horlacher, K., Truyen, U., Hartmann, K. 2008. Comparison of different in-house test systems to detect parvovirus in faeces of cats. Journal of Feline Medicine and Surgery 10(3):247-251.

Nielsen, L., Bell, R., Zoia, A., Mellor, D.J., Neiger, R., Ramsey, I. 2008. Low ratios of sodium to potassium in the serum of 238 dogs. Veterinary Record 162(14):431.

Niyom, S., Boscan, P., Twedt, D.C., Monnet, E., Eickhoff, J.C. 2013. Effect of maropitant, a neurokinin-1 receptor antagonist, on the minimum alveolar concentration of sevoflurane during stimulation of the ovarian ligament in cats. Veterinary Anaesthesia and Analgesia 40:425-431.

Normand, E.H., Gibson, N.R., Reid, S.W., Carmichael, S., Taylor, D.J. 2000. Antimicrobial-resistance trends in bacterial isolates from companion animal community practice in the UK. Preventive Veterinary Medicine 46:267-278.

Nüesch, J.P.F., Rommelaere, J. 2006. NS1 interaction with CKII α: a novel protein complex mediating parvovirus-induced cytotoxicity, Journal of Virology 80: 4729-4739.

O'Brien, S.E. 1994. Serologic response of pups to the low-passage modified-live canine parvovirus-2 component in a compound vaccine. Journal of American Veterinary Medical Association 204:1207-1209.

O'Sullivan G, Durham PJK, Smith JR, Campbell RSF. 1984. Experimentally induced severe canine parvoviral enteritis. Australian Veterinary Journal 61:1-4.

Odunayo, A. 2018. Fluid Therapy. Clinicians Brief, October 2018:71-75. Retrieved February 22, 2019, from https://www.cliniciansbrief.com/article/fluid-therapy.

Ogbaje, C.I., Ofukwu, R.A., Ajogi, I.A. 2015. Zoonotic Gastrointestinal parasite burden of local dogs in Zaria, Northern Nigeria: Implications for human health. International Journal of One Health 1:32-36.

Ogbu, K.I., Mira, F., Purpari, G., Nwosuh, C., Loria, G.R., Schirò, G., … Guercio, A., 2019. Nearly full-length genome characterization of canine parvovirus strains circulating in Nigeria. Transboundary and Emerging Diseases, 00:1-13.

Ohneiser, S.A. 2013. Canine parvovirus in New Zealand. Master Thesis, Massey University, Turitea, New Zealand, pp. ii-65.

O'Neill, D.G., Church, D.B, McGreevy, P.D., Thomson, P.C., Brodbelt, D.C., 2014. Prevalence of disorders recorded in dogs attending primary-care veterinary practices in England. PLoS One 9(3):1-16.

Ola-Fadunsin, S.D., Gimba, F.I., Abdullah, D.A., Sunil R., Sharma, K., Firdaus F.J., Abdullah, Sani, R.A. 2018. Epidemiology and risk factors associated with Anaplasma marginale infection of cattle in Peninsular Malaysia, Parasitology International, 67(6): 659-665. https://doi.org/10.1016/j.parint.2018.06.013.

Oswald, H., Sharkey, M., Pade, D., Martinez, M.N. 2015. Canine gastrointestinal physiology: breed variation that can influence drug absorption. European Journal of Pharmaceutics and Biopharmaceutics 97:192-203.

Otolorin, G.R., Umoh, J.U., Dzikwi, A.A. 2014. Demographic and ecological survey of dog population in Aba, Abia State, Nigeria. ISRN Veterinary Science 2014:806-849.

Otto, C.M., Drobatz, K.J. 1997. Endotoxaemia and tumour necrosis factor activity in dogs with naturally occurring parvovirus enteritis. Journal of Veterinary Internal Medicine 11(2):65-70.

Otto, C. M., Rieser, T. M., Brooks, M. B. and Russell, M. W. 2000. Evidence of hypercoagulability in dogs with parvoviral enteritis. *Journal of the American Veterinary Medical Association* 217(10):1500-1504.

Overgaauw, P.A.M., van Knapen, F., 2013. Veterinary and public health aspects of Toxocara spp. Veterinary Parasitology 193:398-403.

Pak, S.I. 2000. The clinical implication of sodium-potassium ratios in dogs. Journal of Veterinary Science 1:61.

Panda, D., Patra, R. C., Nandi, S., Swarup, D. 2009. Antigenic characterization of canine parvovirus by polymerase chain reaction. Indian Journal of Animal Science 79:876-879.

Papazahariadou, M., Founta, A., Papadopoulos, E., Chliounakis, S., Antoniadou, S.K. Theodorides, Y. 2007. Gastrointestinal parasites of shepherd and hunting dogs in the Serres Prefecture, Northern Greece. Veterinary Parasitology 148:170-173.

Papich, M.G. 2010. Antibacterial drug therapy. In Textbook of Veterinary Internal Medicine. S. J. Ettinger and E. C. Feldman. Eds. 7th ed. Saunders Elsevier. 589-595 pp.

Parrish, C., O'Connell, P., Evermann, J., Carmichael, L. 1985. Natural variation of canine parvovirus. Science 230:1046-1048.

Parrish, C.R., Have, P., Foreyt, W.J., Evermann, J.F., Senda, M., Carmichael, L.E. 1988. The global spread and replacement of canine parvovirus strains. Journal of General Virology 69(5):1111-1116.

Patterson, EV, Reese MJ, Tucker SJ, Dubovi EJ, Crawford PC, Levy JK. 2007. Effect of vaccination on parvovirus antigen testing in kittens. Journal of American Veterinary Medical Association 230:359-363.

Payne, R.A., Avery, A.J. 2011. Polypharmacy: one of the greatest prescribing challenges in general practice. Editorials. British Journal of General Practice 83-84. https://doi.org/10.3399/bjgp11X556146. Retrieved June 1, 2017.

Payne, R.A. 2016. The epidemiology of polypharmacy. Clinical Medicine 16:465–469.

Penninck, D.G., Nyland, T.G., Kerr, L.Y., Fisher, P.E. 1990. Ultrasonographic evaluation of gastrointestinal diseases in small animals. Veterinary Radiology 31:134-141.

Pereira, C.A., Monezi, T.A., Mehnert, D.U., D'Angelo, M., Durigon, E.L. 2000. Molecular characterization of canine parvovirus in Brazil by polymerase chain reaction assay. Veterinary Microbiology 75:127-133.

Philip, R.J. 2015. Management of the patient with canine parvovirus enteritis. Proceedings of the New Zealand Veterinary Nursing Association Annual Conference held Friday 15, 2015 at Rydges Hotel, Auckland. 5-11 Pp

Phromnoi, S., Sirinarumitr, K., Sirinarumitr, T. 2010. Sequence analysis of VP2 gene of canine parvovirus isolates in Thailand. Virus Genes 41:23-29.

Plumb, D.C. 2008. Plumb’s Veterinary Drug Handbook, 6th ed. Iowa: Blackwell. 310-311, 545-546 pp.

Pollock, R.V., Carmichael, L.E. 1982. Maternally derived immunity to canine parvovirus infection: Transfer, decline, and interference with vaccination. Journal of American Veterinary Medical Association 180:37-42.

Pollock, R.V., Coyne, M.J. 1993. Canine parvovirus. Veterinary Clinic North American Small Animal Practice 23(3):555-568.

Potgieter, L.N.D., Jones, J.B., Patton, C.S., Webb-Martin, T.A. 1981. Experimental parvovirus infection in dogs. Canadian Journal of Comparative Medicine 45:212-216.

Pramil, T., Rajiv, A., Gaurav, G. 2012. Pattern of prescribing at a paediatric outpatient setting in Northern India. Indian Journal of Pharmacy Practice 5: 40-44.

Pratelli, A., Tempesta, M., Roperto, F.P., Sagazio, P., Carmichael, L. and Buonavoglia, C. 1999. Fatal coronavirus infection in puppies following canine parvovirus 2b infection. Journal of Veterinary Diagnostic Investigation 11:550-553.

Pratelli, A., 2011. Canine distemper virus the emergence of new variants. Veterinary Journal 187:290-291.

Prittie, J. 2004. Canine parvoviral enteritis: a review of diagnosis, management and prevention. Journal of Veterinary Emergency Critical Care 14.3:167-176.

Proschowsky, H.F., Rugbjerg, H., Ersboll A.K. 2003. Morbidity of purebred dogs in Denmark. Preventive Veterinary Medicine 58:53-62.

Puotinen C.J. 2009. Hemorrhagic gastroenteritis in dogs. Whole Dog Journal. Retrieved February, 2, 2020 from, https://www.whole-dog-journal.com/health/hemorrhagic-gastroenteritis-in-dogs/

Purdy, C., Bytzer, P.M., Pimentel, M., Magar, R. 2015. A Novel Ibs diagnostic blood panel can enhance a positive diagnostic strategy versus a strategy of exclusion for patients with diarrhoea predominant irritable bowel syndrome (Ibs-D): Cost implications for Denmark. Value in Health 18(7):A350.

Queen, E.V., Marks, S.L., Farver, T.B. 2012. Prevalence of selected bacterial and parasitic agents in faeces from diarrheic and healthy control cats from Northern California. Journal of Veterinary Internal Medicine 26:54-60.

Rakha, G.M.H., Abdul-Haleem, M.M., Farghali, H.A.M., Abdel-Saeed, H. 2015. Prevalence of common canine digestive problems compared with other health problems at teaching veterinary hospital, Faculty of Veterinary Medicine, Cairo University, Egypt. Veterinary World 8(3):403-411.

Rantala, M., Hölsö, K., Lillas, A., Huovinen, P., Kaartinen, L. 2004. Survey of condition-based prescribing of antimicrobial drugs for dogs at a veterinary teaching hospital. Veterinary Record 155:259-262.

Reves, R., Baas, P., DuPont, H.L., Sullivan, P., Mendiola, J. 1983. Failure to demonstrate effectiveness of an anticholinergic drug in the symptomatic treatment of acute travellers' diarrhoea. Journal of Clinical Gastroenterology 5:223-227.

Ricardo Izurieta S.G., Clem, A. 2008. Leptospirosis: The "mysterious" mimic. Journal of Emergency and Trauma Shock 1:21-33.

Robinson, W.F., Huxtable, C.R., Pass, D.A. and Howell, J.M. 1979. Clinical and electrocardiographic findings in suspected viral myocarditis of pups. Australia Veterinary Journal 55(8):351-355. PMID:533485.

Rosé, A., Neiger, R. 2013. Causes of vomiting in dogs and usefulness of clinical investigations. Tierarztl Prax Ausg K Kleintiere Heimtiere 41:16-22.

Rossi, M., Hanninen, M.L., Revez, J., Hannula, M., Zanoni, R.G. 2008. Occurrence and species level diagnostics of *Campylobacter spp*., enteric *Helicobacter spp*. and *Anaerobiospirillum spp*. in healthy and diarrheic dogs and cats. Veterinary Microbiology 129:304-314.

Roth, L., Tyler, R.D. 1999. Evaluation of low sodium: potassium ratios in dogs. Journal of Veterinary Diagnostic Investigation 11:60-64.

Rozanski, E.A., Rush, J.E. 2007. Small Animal Emergency and Critical Care Medicine. Manson Publishing Ltd. London. The UK. 104-118 Pp.

Rudloff, E., Kirby, R. 2013. Colloid fluid therapy. In: Bonagura J.D., Twedt D.C., eds. Current Veterinary Therapy XIV. WB Saunders, Philadelphia, 16-28.

Russell, S.L., Gold, M.J., Hartmann, M., Willing, B.P., Thorson, L., Wlodarska, M., Gill, N., Blanchet, M.R., Mohn, W.W., McNagny, K.M., Finlay, B.B. 2012. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. EMBO Reports. 13(5):440-447.

Rutgers, H.C., Batt, R.M., Elwood, C.M., Lamport, A. 1995. Small intestinal bacterial overgrowth in dogs with chronic intestinal disease. Journal of American Veterinary Medical Association 206:187-193.

Saari, A., Virta, L.J., Sankilampi, U., Dunkel, L., Saxen, H. 2015. Antibiotic exposure in infancy and risk of being overweight in the first 24 months of life. Paediatrics. 135(4):617-626. https://doi.org/10. 1542/peds.2014-3407.

Sævik, B.K., Skancke, E.M., Trangerud, C.A. 2012. Longitudinal study on diarrhoea and vomiting in young dogs of four large breeds. Acta Veterinaria Scandinavica 54:8-19.

Sakulwira, K., Vanapongtipagorn, P., Theamboonlers, A., Oraveerakul, K., Poovorawan, Y. 2003. Prevalence of canine coronavirus and parvovirus infections in dogs with gastroenteritis in Thailand. Veterinary Medicine-Czech 48(6):163-167.

Salem, N.Y. 2014. Canine viral diarrhoea: clinical, hematologic and biochemical alterations with reference to in-clinic rapid diagnosis. Global Veterinaria 13(3):302-307.

Sanz, E.J., Boada, J.N. 1988. Drug utilization by children in Tenerife Island, Spain. European Journal of Clinical Pharmacology 34:495-99.

Schaer, M. 1994. Hyperkalaemia and hypernatremia. In: Textbook of veterinary internal medicine (Ed. S.J. Ettinger). 4th edition, WB Saunders, Philadelphia. 46-49 pp.

Schar, F., Inpankaew, T., Traub, R.J., Khieu, V., Dalsgaard, A., Chimnoi, W., Chhoun, C., Sok, D., Marti, H., Muth, S., Odermatt, P. 2014. The prevalence and diversity of intestinal parasitic infections in humans and domestic animals in a rural Cambodian village. Parasitology International 63:597-603.

Schatzberg, S.J., Haley, N.J.B.S., Bar, S.C., De La Hunta, A., Kornegay, J.N., Sharp, N.J.H. 2003. Polymerase chain reaction (PCR) amplification of parvoviral DNA from the brains of dogs and cats with cerebellar hypoplasia. Journal of Veterinary Internal Medicine 17:538-544.

Schmitz, S., Coenen, C., Konig, M., Thiel, H., Neiger, R. 2009. Comparison of three rapid commercial canine parvovirus antigen detection tests with electron microscopy and polymerase chain reaction. Journal of Veterinary Diagnostic Investigation 21:344-345.

Schoeman, J.P., van Schoor, M. 2008. Admission serum biochemical parameters are not associated with mortality in puppies with parvoviral diarrhoea (abstract). In: Proceedings 18th European College of Veterinary Internal Medicine-Companion Animal Congress, 4th-6th September 2008, Ghent, Belgium. 227-228 Pp.

Schoeman, J.P. Goddard, A., Leisewitz, A.L. 2013. Biomarkers in canine parvovirus enteritis. New Zealand Veterinary Journal 61(4):217-222.

Schultz, R.D. 2000. Considerations in designing effective and safe vaccination programs for dogs. In: Recent advances in canine infectious diseases, Carmichael L.E. (ed). 9 Pages. Retrieved on June 11, 2017, from www.ivis.org. Document No. A0110.0500.

Schwille-Kiuntke, J., Frick, J.S., Zanger, P., Enck, P. 2011. Post-infectious irritable bowel syndrome - a review of the literature. Zeitschrift Fur Gastroenterologie 49(8):997-1003. https://doi.org/10.1055/s0031-1281581.

Scorza, A.V., Ballweber, L.R., Tangtrongsup, S., Panuska, C., Lappin, M.R. 2012. Comparisons of mammalian *Giardia duodenalis* assemblages based on the β–giardin, glutamate dehydrogenase and triosephosphate isomerase genes. Veterinary Parasitology 189:182-188.

Sen, C., Sharma, A.K., Dhaliwal, P.S. 2014. Comparative efficacy of crystalloid and colloid-crystalloid combination for the management of haemorrhagic gastroenteritis in dogs. Veterinary World 7(12):1108-1112.

Seth, A., Aneja, S. 1995. Hyperglycemia in malnourished children with dehydrating gastroenteritis. Indian Journal of Paediatrics 62(3):353-355. PMID: 10829889.

Shah, S.A., Sood, N.K., Wani, N., Gupta, K., Singh, A. 2013. Haemato-biochemical changes in canine parvoviral infection. Indian Journal of Veterinary Pathology 37(2):131-133.

Shakespeare, A.S. 1999. The incidence of gastroenteritis diagnosis among sick dogs presented to the Onderstepoort Veterinary Academic Hospital correlated with meteorological data. Journal of South African Veterinary Association 70:95-97.

Sharp, C.R., Declue, A.E., Haak, C.K., Honaker, A.R., Reinero, C.R. 2010. Evaluation of the anti-endotoxin effects of polymyxin B in a feline model of endotoxaemia. Journal of Feline Medicine and Surgery 12(4):278-285.

Shea, A., McCarthy, R., Lindenmayer, J. 2011. Therapeutic antibiotic use patterns in dogs: observations from a veterinary teaching hospital. Journal of Small Animal Practice 52(6):310-318.

Shima, F.K., Mosugu, J.I.T., Apaa, T.T. 2014. Causes of mortality in dogs in and around Effurun/Warri Municipality of Delta State, Nigeria. Bulletin of Animal Health and Production in Africa 62(4):387-396.

Shima, F.K., Tion, M.T., Mosugu, J.I., Apaa, T.T. 2015a. Retrospective study of disease incidence and other clinical conditions diagnosed in owned dogs in Delta State, Nigeria. Journal of Advanced Veterinary and Animal Research 2(4):435-449.

Shima, F.K., Mosugu, J.I., Apaa, T.T. 2015b. Epidemiology of canine parvovirus enteritis among hospitalized dogs in Effurun/Warri Metropolitan region of Delta State, Nigeria. Open Access Library Journal 2:e1208. 7 pages, https://doi.org/10.4236/oalib.1101208.

Shima, F.K., Gberindyer, F.A., Apaa, T.T., Mosugu, J.I. 2015c. Clinico-epidemiological study on canine toxicosis in Effurun/Warri Municipality region of Delta State, Nigeria. Journal of Advanced Veterinary and Animal Research 2(3):357-361. https://doi.org/10.5455/javar.2015.b91

Shinde, S.S., Rajguru, D.N., Anantwar, L.G., Mohd, S., Ambore, B.N. 2000. Clinicopathology and therapeutic management of canine gastroenteritis. The blue cross book 15:26-29.

Sime, T.A., Powell, L.L., Schildt, J.C., Olson, E.J. 2015. Parvoviral myocarditis in a 5-week-old Dachshund. Journal of Veterinary Emergency and Critical Care 25(6):765-769. https://doi.org/10.1111/vec.12347.

Simpson, K.W., Fyfe, J., Cornetta, A., Sachs, A., Strauss-Ayali, D., Lamb, S.V., Reimers, T.J. 2001. Subnormal concentrations of serum cobalamin (vitamin B12) in cats with gastrointestinal disease. Journal of Veterinary Internal Medicine 15:26-32.

Simpson, K.W., Dogan, B., Rishniw, M., Goldstein, R.E., Klaessig, S., McDonough, P.L., German, A.J., Yates, R.M., Russell, D.G., Johnson, S.E., Berg, D.E., Harel, J., Bruant, G., McDonough, S.P., Schukken, Y.H. 2006. Adherent and invasive *Escherichia coli* is associated with granulomatous colitis in boxer dogs. Infection and Immunology 74(8):4778-4792.

Smith, C.A. 1995. Current concepts: Are we vaccinating too much? Journal of American Veterinary Medical Association 207:421-425.

Smith, G.S. 2000. Neutrophils. In: Feldman, B.F., Zinkl, J.G., Jain, N.C., eds. Schalm's Veterinary Hematology, 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins. 281-296 pp.

Soave, O.A. 1959. Clinical application of the electrocardiograph. Veterinary Medicine 54:193-198.

Sodikoff, C.H. 1995. Laboratory profile of small animal diseases – a guide to laboratory diagnosis. 2nd ed. Mosby, St. Louis. 435pp.

Sousby, E.J.L. 1982. Helminths, arthropods and protozoa of domesticated animals, 7th ed. Lea & Lebiger, Philadelphia PA, 203-206 pp.

Spencer, S, Tappin, S. 2014. Recommendations for treating and preventing canine parvovirus. Vet Times 2014:1-12.Veterinary Times July 21, 2014. Retrieved February 4, 2016, from https://www.vettimes.co.uk/

Spibey N., Greenwood, N., Sutton, D., Chalmers, W., Tarpey, I. 2008. Canine parvovirus type 2 vaccine protects against virulent challenge with type 2c virus. Veterinary Microbiology 128:48-55.

Spielman, B.L., Garvey, M.S. 1993. Haemorrhagic gastroenteritis in 15 dogs. The Journal of the American Animal Hospital Association 29(4):341-344.

Stavisky, J., Radford, A.D, Gaskell, R., Dawson, S., German, A., Parsons, B., Clegg, S., Newman, J., Pinchbeck G. 2011. A case-control study of pathogen and lifestyle risk factors for diarrhoea in dogs. Preventive Veterinary Medicine 99:185-192.

Steinel, A., Venter, E.H., Van Vuuren, M., Parrish, A., Truyen., A. 1998. Antigenic and genetic analysis of canine parvoviruses in Southern Africa. Onderstepoort Journal of Veterinary Research 65:239-242.

Stockwell, J.A., Huang, Y.C., Su, Y.F., Piantadosi, C.A. 1994. Bactericidal antibiotics increase tumour necrosis factor-alpha and cardiac output in rats after caecal ligation and puncture. Circulatory Shock 42:68-75.

Streck, A.F., Souza C.K., Gonçalves, K.R., Zang, L. Pinto, L.D., Cana C.W. 2009. First detection of canine parvovirus type 2c in Brazil. Brazilian Journal of Microbiology 40(3):465-469.

Suchodolski, J.S., Ruaux, C.G., Steiner, J.M., Fetz, K., Williams, D.A. 2005. Assessment of the qualitative variation in bacterial microflora among compartments of the intestinal tract of dogs by use of a molecular fingerprinting technique. American Journal of Veterinary Research 66(9):1556-1562.

Suchodolski, J.S., Xenoulis, P.G., Paddock, C.G., Steiner J.M., Jergens A.E. 2010. Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. Veterinary Microbiology 142:394-400.

Suchodolski, J.S., Markel, M.E., Garcia–Mazcorro, J.F., Unterer, S., Heilmann, R.M., Dowd, S.E., Kachroo, P., Ivanov, I., Minamoto, Y., Dillman, E.M., Steiner, J. M., Cook, A.K., Toresson, L. 2012. The faecal microbiome in dogs with acute diarrhoea and idiopathic inflammatory bowel disease. PLoS One 7(12):e51907.

Suchodolski, J.S., Whittemore, J.C. 2016. The role of probiotics in the management of intestinal dysbiosis. Clinical Notes July 2016:4 pages.

Suikkanen, S., Antilaa, M., Jaatinena, A., Vihinen-Rantaa, M., Vuentoa, M. 2003. Release of canine parvovirus from endocytic vesicles. Virology 316:267-280. https://doi.org/10.1016/j.virol.2003.08.031.

Sunvold, G.D., Fahey, G.C., Merchen, N.R., Reinhart, G.A., 1995. In vitro fermentation of selected fibrous substrates by dogs and cat faecal inoculum: influence of diet composition on substrate organic matter disappearance and short-chain fatty acid production. Journal of Animal Science 73(4):1110-1122.

Sykes, J.E. 2014. Canine parvovirus infections and other viral enteritides. In: Sykes, J.E, Ed. Canine and Feline Infectious Diseases. 1st ed. St Louis, MO: Elsevier; 141-151.

Szajewska, H., Dziechciarz, P., Mrukowicz, J. 2006. Meta-analysis: Smectite in the treatment of acute infectious diarrhoea in children. Alimentary Pharmacology and Therapeutics 23:217-227.

Tams, T.R. 2000. Diarrhoea. In: Textbook of Veterinary Internal Medicine. 5th ed. Ettinger S.J., E.C. Feldman (Eds.). Philadelphia: WB Saunders. 121-126 Pp.

Tams, T.R. 2003. Gastrointestinal symptoms. Handbook of Small Animal Gastroenterology. T.R. Tams. Ed. 2nd ed. Elsevier, USA. 1-60 pp

Tams, T.R. 2014. Gastroenterology. Chicago Veterinary Medical Association symposium held in Chicago, USA– September 3, 2014:1-56.

Tamuno, I., Fadare, J.O. 2012. Drug prescription pattern in Nigerian Tertiary Hospital. Tropical Journal of Pharmaceutical Research 11(1):146-152.

Tamura, K., Nei, M. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10: 512-526.

Tarafder, M., Samad, M.A. 2010. Prevalence of clinical diseases of pet dogs and risk perception of zoonotic infection by dog owners in Bangladesh. Bangladesh Journal of Veterinary Medicine 8(2):163-174.

Tennant, B. 2001. The alimentary tract. Manual of Canine and Feline Infectious Diseases. Ramsey, I. Gloucester, T.B., Eds. British Small Animal Veterinary Association: UK. 129-150 pp.

Thabane, M., Kottachchi D.T., Marshall J.K. 2007. Systematic review and meta-analysis: The incidence and prognosis of post-infectious irritable bowel syndrome. Alimentary Pharmacology and Theriogenology 26(4):535-544.

Thabane, M., Marshall, J.K. 2009. Post-infectious irritable bowel syndrome. World Journal of Gastroenterology 15(29):3591-3596.

Thisted, R.A. 2010. What is a P-value? Departments of Statistics and Health Studies, The University of Chicago. 6 pages. Retrieved February 28, 2019, from http://www.stat.uchicago.edu/~thisted.

Tilley, L.P. 1992. Essentials of canine and feline electrocardiography, 3rd ed. IDBC, Philadelphia. 120-121 pp.

Tilley, L.P. 1995. Essentials of canine and feline electrocardiography, 3rd ed. Philadelphia, PA: Lea & Febiger. 1-470 pp

Tilley, L.P., Burtnick, N.L. 1999. Electrocardiography for small animal practitioner. Teton NewMedia, Wyoming, USA. 10-94 pp

Tion T.M., Ikurior J.S., Orbunde, K.D. 2016. The prevalence of gastrointestinal helminths infection of dogs in Makurdi metropolis. Imperial Journal of Interdisciplinary Research 2(8):1042-1049.

Toresson, L., Takashima, G.K., Burns, K.M. 2018. Nutritional assessment in a dog with chronic enteropathy. Clinician's Brief April 2018. Retrieved April 12, 2018, http://www.cliniciansbrief.com/article/nutritional-assessment-dog-chronic-enteropathy/

Touihri L., Bouzid I., Daoud R., Desario C., El Goulli A.F., Decaro N., Ghorbel A., Buonavoglia C., Bahloul C. 2009. Molecular characterisation of canine parvovirus-2 variants circulating in Tunisia. Virus Genes 38:249-258.

Traub, R.J., Inpankaew, T., Sutthikornchai, C., Sukthana, Y., Thompson, R.C. 2008. PCR-based coprodiagnostic tools reveal dogs as reservoirs of zoonotic ancylostomiasis caused by *Ancylostoma ceylanicum* in temple communities in Bangkok. Veterinary Parasitology 155(1-2):67-73.

Tripathi, K.D. 2008. Chapter 47: Drugs for vomiting, reflux and digestive disorders. In: Essentials of Medical Pharmacology, 6th edn. Jaypee. New Delhi. India. 639-650 pp.

Truyen, U., Gruenberg, A., Chang, S., Obermaier, B., Veijalainen, P., Parrish, C. 1995. Evolution of feline sub-group parvoviruses and the control of canine host-range *in-vivo*. Journal of virology 69:4702-4710.

Truyen, U., Steinel, A., Bruckner, L., Lutz H., Mostl, K. 2000. Distribution of antigen types of canine parvovirus in Switzerland, Austria and Germany. Schweizer Archiv für Tierheilkunde 142:115-119.

Truyen, U. 2006. Evolution of canine parvovirus–a need for new vaccines? Veterinary Microbiology 117:9-13.

Tsukada, K., Katoh, H., Shiojima, M., Takenoshita, S-I., Nagamachi, Y. 1993. Mortality rate and bacteraemia, endotoxin and endothelin–1 levels in antibiotic therapy for *E. coli* septic peritonitis. Acta Pathologica, Microbiologica et Immunologica Scandinavica 101:97-100.

Tupler, T., Levy, J.K., Sabshin, S.J., Tucker, S.J., Greiner, E.C., Leutenegger, C.M. 2012. Enteropathogens identified in dogs entering a Florida animal shelter with normal faeces or diarrhoea. Journal of American Veterinary Medical Association 241:338-343.

Turk, J., Miller, M., Brown, T., Fales, W., Fischer, J., Gosser, H., Nelson, S., Shaw, D., Solorzano, R. 1990. Coliform septicaemia and pulmonary disease associated with canine parvoviral enteritis: 88cases (1987–1988). Journal of American Veterinary Medical Association 196(5):771-773.

Turk, J., Fales, W., Miller, M., Pace, L., Fischer, J., Johnson, G., Kreeger, J., Turnquist, S., Pittman, L., Rottinghaus, A. 1992. Enteric *Clostridium perfringens* infection associated with parvoviral enteritis in dogs: 74 cases (1987-1990). Journal of American Veterinary Medical Association 200:991-994.

Twedt, D.C. 2000. Vomiting. In: Textbook of Veterinary Internal Medicine. 5th ed. Ettinger S.J., E.C. Feldman (Eds.). Philadelphia: WB Saunders 117-121 pp.

Ungaro, R., Bernstein, C.N., Gearry, R., Hviid, A., Kolho, K.L., Kronman, M.P., Shaw, S., Van Kruiningen, H., Colombel, J.F., Atreja, A. 2014. Antibiotics associated with increased risk of new-onset Crohn's disease but not ulcerative colitis: a

meta-analysis. American Journal of Gastroenterology 109(11):1728-38. https://doi.org/10.1038/ajg.2014.246.

Unterer, S., Strohmeyer, K., Kruse, B.D., Sauter-Louis, C., Hartmann, K. 2011. Treatment of aseptic dogs with haemorrhagic gastroenteritis with amoxicillin/clavulanic acid: A prospective blinded study. Journal of Veterinary Internal Medicine 25:973-979.

Urquhart, G.M., Duncan, J.L., Armour, J., Dunn, A.M., Jennings, F.W. 1996. Veterinary Parasitology. 2nd edn. Blackwell Science Ltd, Oxford. 53-73 pp.

Vail, D.M., Rodabaugh, H.S., Conder, G.A. 2007. Efficacy of injectable maropitant (Cerenia^{TM}) in a randomised clinical trial for prevention and treatment of cisplatin-induced vomiting in dogs presented as veterinary patients. Veterinary and Comparative Oncology 5:38-46.

Valverde, A., Cantwell, S., Hernandez, J., Brotherson, C. 2004. Effects of acepromazine on the incidence of vomiting associated with opioid administration in dogs. Veterinary Anaesthesia and Analgesia 31:40-45.

Veehof, L., Stewart, R.E., Haaijer-Ruskamp, F.M. 2000. The development of polypharmacy. Family Practice17:261–267.

Verlinden, A., Hesta, M., Millet, S., Janssens, G.P. 2006. Food allergy in dogs and cats: a review. Critical Reviews in Food Science and Nutrition 46(3):259-73. https://doi.org/10.1080/10408390591001117.

Vesy, C.J., Peterson, W.L. 1999. Review article: the management of Giardiasis. Alimentary Pharmacology and Therapeutics 13:843-850.

Voie, K.L., Lavergne, S.N. 2012. Drug hypersensitivity reactions targeting the skin in dogs and cats. Journal of Veterinary Internal Medicine 26:863-874.

Walker, N.J., Croese, J., Clouston, A.D., Parry, M., Loukas, A., Prociv, P. 1995. Eosinophilic enteritis in north-eastern Australia–Pathology, association with *Ancylostoma caninum* and implications. American Journal of Surgery and Pathology 19:328-337.

Waner, T. 2004. A review of current international vaccination trends for dogs and cats. Are we up to date and in line with contemporary thinking? Journal of Israeli Veterinary Medical Association 59(3):1.

Waner, T., Mazar, S., Keren-Kornblatt, E. 2006. Application of a dot enzyme-linked immunosorbent assay for evaluation of the immune status to canine parvovirus

and distemper virus in adult dogs before revaccination. Journal of Veterinary Diagnostic Investigation 18:267-270.

Wang, C.R., Qiu, J.H., Zhao, J.P., Xu, L.M., Yu, W.C., Zhu, X.Q. 2006. Prevalence of helminthes in adult dogs in Heilongjiang Province, the People's Republic of China. Parasitology Research 99(5):627-630. doi:10.1007/s00436-006-0219-7.

Wang, H., Jin, H., Li, Q., Zhao, G., Cheng, N., Feng, N., Zheng, X., Wang, J., Zhao, Y., Li, L., Cao, Z., Yan, F., Wang, L., Wang, T., Gao, Y., Yang, S., Xia, X. 2016. Isolation and sequence analysis of the complete NS1 and VP2 genes of canine parvovirus from domestic dogs in 2013 and 2014 in China. Archives of Virology 161:385-393.

Webb, C.B., Suchodolski, J.S. 2014. Probiotic therapy. Kirk's Current Veterinary Therapy XV. Bonagura, J.D. and Twedt, D.C. ed. Elsevier. St. Louis, Missouri, 525-528 pp.

Webb, C.B. 2016. Chronic diarrhoea in the dog and cat. The RVT Journal, article #3:24-28. Retrieved on April 8, 2017, from http://www.thervtsquizzes.ca/

Webb, C.B. 2018. Top five gastrointestinal and hepatobiliary antibiotics. Clinician's Brief March 2018. Retrieved March 05, 2018, from http//:www.cliniciansbrief.com/

Weese, J.S., Staempfli, H.R., Prescott, J.F., Kruth, S.A., Greenwood, S.J., Weese, H. 2001. The roles of *Clostridium difficile* and enterotoxigenic *Clostridium perfringens* in diarrhoea in dogs. Journal of Veterinary Internal Medicine 15:374-378.

Weese, J.S. 2011. Bacterial enteritis in dogs and cats: Diagnosis, therapy, and zoonotic potential. Veterinary Clinics of Small Animal 41:287-309.

Weiss, D.J., Evanson, O.A., Sykes, J. 1999. A retrospective study of canine pancytopenia. Veterinary Clinical Pathology 28:83-88.

Wells, D.L., Hepper P.G. 1999. Prevalence of disease in dogs purchased from an animal rescue shelter. Veterinary Records 144:35-38.

Westermarck, E., Skrzypczak, T., Harmoinen J., Steiner, J.M., Ruaux, C.G., Williams, D.A., Eerola, E., Sundbäck, P., Rinkinen, M. 2005. Tylosin-responsive chronic diarrhoea in dogs. Journal of Veterinary Internal Medicine 19(2):177-186.

Westgarth, C., Pinchbeck, G.L., Bradshaw, J.W.S., Dawson, S., Gaskell, R.M., Christley, R.M. 2008. Dog-human and dog-dog interactions of 260 dog-owning households in a community in Cheshire. Veterinary Record 162:436-442.

Whitley, N.T., Day, M.J. 2011. Immunomodulatory drugs and their application to the management of canine immune-mediated disease. Journal of Small Animal Practice 52:70–85.

Whyte, L.A., Jenkins, H.R. 2012. Pathophysiology of diarrhoea. Paediatrics and Child Health 22(10):443-447. http://dx.doi.org/10.1016/j.paed.2012.05.006.

Will, K., Nolte, I., Zentek, J. 2005. Early enteral nutrition in young dogs suffering from haemorrhagic gastroenteritis. Journal of Veterinary Medicine A: Physiology, Pathology and Clinical Medicine 52:371-376.

Willard, M.D. 2009. Digestive system disorders. Small Animals Internal Medicine. R. W. Nelson and Couto, C. C. Eds. 4th ed. Mosby, Elsevier, St. Louis, Missouri. 351-486 pp.

Wood, G.L. 1983. Canine myocardial diseases. Current Veterinary Therapy VIII. Small animal practice (Ed.) Kirk. WB Saunders Company Philadelphia, London, Toronto, 325 p.

Woolford, L., Crocker, P., Bobrowski, H., Baker, T., Hemmatzadeh, F. 2017. Detection of the canine parvovirus 2c subtype in Australian dogs. Viral Immunology 30(5):1-5. https://doi.org/10.1089/vim.2017.0019.

Xenoulis, P.G., Palculict, B., Allenspach, K., Steiner, J.M., Van House, A.M., Suchodolski, J.S. 2008. Molecular-phylogenetic characterisation of microbial communities imbalances in the small intestine of dogs with inflammatory bowel disease. FEMS Microbiology and Ecology 66:579-589.

Yi, L., Tong, M., Cheng, Y., Song, W., Cheng, S. 2016. Phylogenetic analysis of canine parvovirus VP2 gene in China. Transboundary and Emerging Diseases, 63(2):e262-e269. https://doi.org/10.1111/tbed.12268.

Yilmaz, Z., Kennerma, E., Senturk, S., Temizel, M., Aytug, N. 2002. The evaluation of cats and dogs referred to the clinics of companion animals internal medicine, Veterinary Faculty, University of Uludag (1999–2000). Veterinary Fakultesi Derg. Uludag University 21(1&3):23-31.

Youn, Y., Park, S., Park, C., Lim, H., Kim, J., Park, H., Lee, S. 2012. The clinical course of postinfectious irritable bowel syndrome (IBS) after *shigellosis*: A 10-year follow-up study. Neurogastroenterology and Motility. 24:102.

Younas, A., Khan, M.S., Ijaz, M., Ashraf, K., Ali, M.M., Khan, I., Rehman, A. 2014. Prevalence and chemotherapy of *coccidiosis* in dogs in and around Lahore-Pakistan. The Journal of Animal and Plant Sciences 24(1):350-353.

Youngster, I., Russell, G.H., Pindar, C., Ziv-Baran, T., Sauk, J., Hohmann, E.L. 2014. Oral, capsulized, frozen faecal microbiota transplantation for relapsing *Clostridium difficile* infection. Journal of the American Medical Association 312(17):1772-1778.

Yuan, W., Parrish, C.R. 2001. Canine parvovirus capsid assembly and differences in mammalian and insect cells, Virology 279:546-557.

Zanini, B., Ricci, C., Bandera, F., Caselani, F., Magni, A., Laronga, A. M., Lanzini, A., & San Felice del Benaco Study Investigators (2012). Incidence of post-infectious irritable bowel syndrome and functional intestinal disorders following a water-borne viral gastroenteritis outbreak. The American journal of gastroenterology, 107(6), 891-899. https://doi.org/10.1038/ajg.2012.102.

Zhou, P., Zeng, W., Zhang, X., Li, S. 2017. The genetic evolution of canine parvovirus - A new perspective. PLoS ONE, 12(3):e0175035.

Zicola, A., Jolly, S., Mathijs, E., Ziant, D., Decaro, N., Mari, V., Thiry, E. 2012. Fatal outbreaks in dogs associated with pantropic canine coronavirus in France and Belgium. Journal of Small Animal Practice 53:297-300.

Ziese, A.L., Suchodolski, J.S. 2019. Intestinal dysbiosis. Clinicians Brief, January 2019:33-37. Retrieved February 12, 2019, from http//:www.cliniciansbrief.com/

APPENDICES

Appendix 1: List of publications resulting from this thesis

Infection, Genetics and Evolution 85 (2020) 104553

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Molecular characterisation of canine parvoviruses from clinical samples and vaccines in Nigeria

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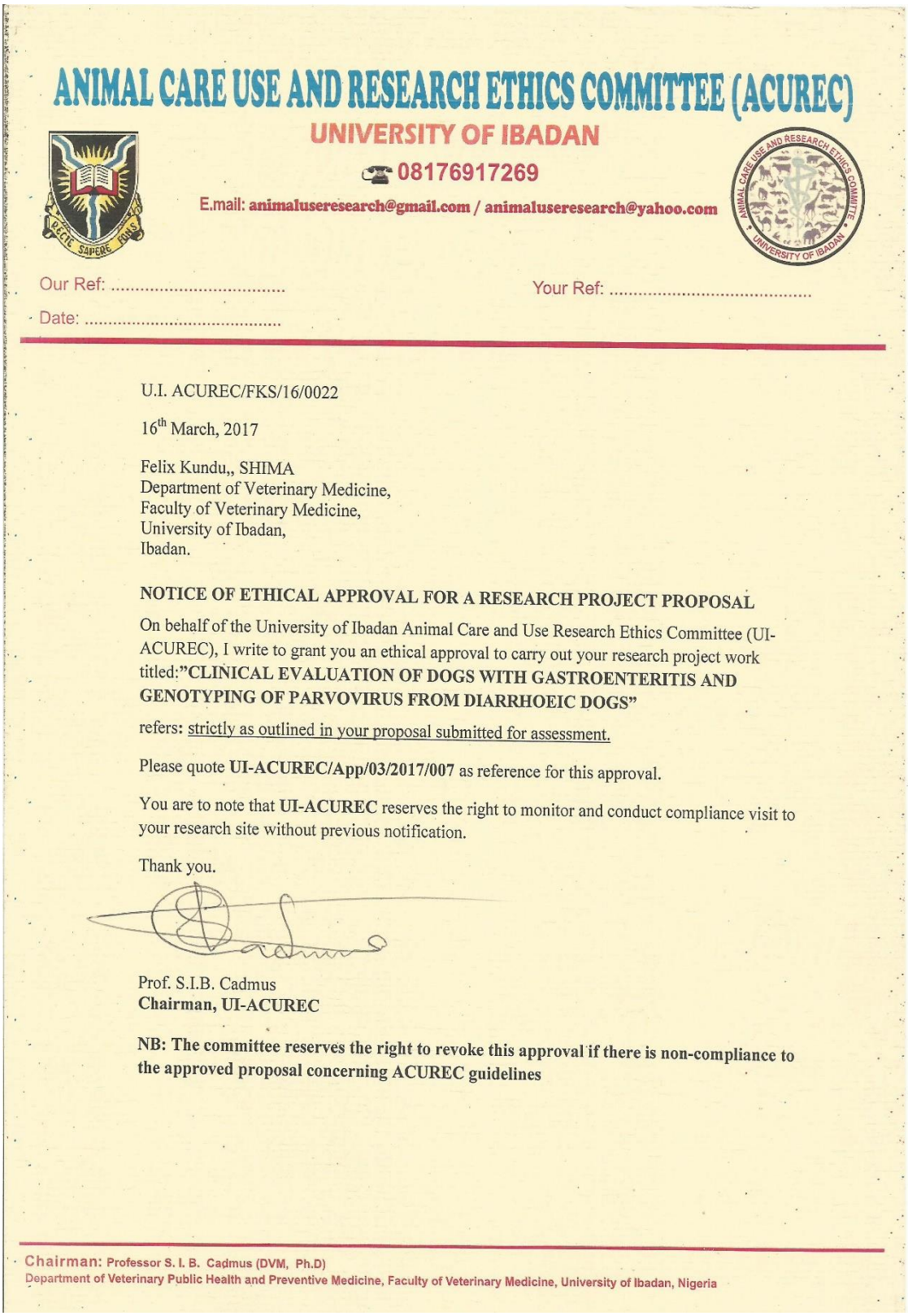
ABSTRACT

Canine parvovirus (CPV) the causative agent of canine parvovirus enteritis is an intractable pathogen of dogs characterised by mutations, evolutionary changes and eventual vaccine failure. The disease is a serious problem in dogs with limited studies conducted in Nigeria. Therefore, this study was designed to characterise the subtypes of CPV isolates in six commonly used vaccines and 157 clinical samples collected from seven states in Nigeria from June 2016 to March 2018. Faecal samples collected from the clinical cases were subjected to in-clinic immunoassay to detect viral antigens. Polymerase chain reaction (PCR) was used to amplify viral VP2 gene in the samples and commonly used vaccines in Nigeria. Thereafter, PCR products were sequenced and analysed. The result showed that 93.0% of the dogs tested positive for CPV in both assays; 72.8% were puppies less than six months old, with 58.3% of them vaccinated. Partial VP2 gene sequence and phylogenetic analysis of 11 random clinical samples showed that CPV-2c 7(63.6%) and CPV-2a 4(36.4%) were the predominant subtypes in Nigeria; with genetic signatures that are 98.7% to 99.9% closely related to Asian and European strains, respectively. No CPV-2b was detected. Amino acid mutation analysis divulged some imperative transmutation sites: D305Y, Y324I, Q370R, N375D, T440A, Y444S, I447M and Y451C in the isolates. The viruses in the vaccines were characterised as the wild-type CPV. The genetic variability, viral population heterogeneity and phylogenetic linkage with isolates from other countries probably suggest transboundary migrations and local differentiations are contributing to continuous CPV evolution and vaccine failure in Nigeria.

Appendix 2: Ethical approval for the research



ANIMAL CARE USE AND RESEARCH ETHICS COMMITTEE (ACUREC)

UNIVERSITY OF IBADAN

☎ 08176917269

E.mail: animaluseresearch@gmail.com / animaluseresearch@yahoo.com

Our Ref: ..... Your Ref: .....

Date: .....

U.I. ACUREC/FKS/16/0022

16th March, 2017

Felix Kundu,, SHIMA
Department of Veterinary Medicine,
Faculty of Veterinary Medicine,
University of Ibadan,
Ibadan.

NOTICE OF ETHICAL APPROVAL FOR A RESEARCH PROJECT PROPOSAL

On behalf of the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC), I write to grant you an ethical approval to carry out your research project work titled:**"CLINICAL EVALUATION OF DOGS WITH GASTROENTERITIS AND GENOTYPING OF PARVOVIRUS FROM DIARRHOEIC DOGS"**

refers: strictly as outlined in your proposal submitted for assessment.

Please quote **UI-ACUREC/App/03/2017/007** as reference for this approval.

You are to note that **UI-ACUREC** reserves the right to monitor and conduct compliance visit to your research site without previous notification.

Thank you.

Prof. S.I.B. Cadmus
Chairman, UI-ACUREC

NB: The committee reserves the right to revoke this approval if there is non-compliance to the approved proposal concerning ACUREC guidelines

Chairman: Professor S. I. B. Cadmus (DVM, Ph.D)
Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria

Appendix 3: Data collection form used for the retrospective study

A SURVEY OF COMMON DIGESTIVE PROBLEMS IN OWNED DOGS												
S/N	Date Admitted	Breed	Sex (M/F)	Age	Weight (kg)	Rectal Temp.	Vaccination (Yes/No/ Unknown)	Apparently Healthy	Diseased		Causes/ Diagnoses	Treatment/Drugs Used
									Digestive Problems	Other Health Problems		
1	2/01/2016	Caucasian	M	6wks	4	39.7	No	-	vomiting	-	Ancylostomosis	Albendazole
												Metronidazole
												Oxytetracycline
												Dextrose saline 5% (DS)
												Ringers Lactate (RL)
												metocloperamide
2	3/01/2016	Mixed	F	12Mo	19	38.9	unknown	-	Diarrhoea	-	Parvo infection	Albendazole
												Metronidazole
												Amoxicillin
												Gentamycin
												Dextrose saline 5% (DS)
												Ringers Lactate (RL)
												metocloperamide
												Atropine
3	3/01/2016	Rottweiler	F	3mo	6	40.0	Yes	-	Diarrhoea + vomiting		Parvo infection	Levamisol
												Metronidazole
												Amoxicillin
												Gentamycin
												Dextrose normal saline 5% (DNS)
												Ringers Lactate (RL)
												metocloperamide
4	3/01/2016	Local	M	8 wks	3.5	37.8	no	Routine vaccination	-	-	--	DHLPP + Antirabies
5	2/01/2016	Alsatian	M	5mo	12	38.1	Yes	Deworming	-	-	--	Prazisam
6	2/01/2016	Boerboele	F	7mo	15	39.0	Unknown	-	-	Mange	Demodecosis	Ivermectin, vitamin B co.
7	1/01/2016	Alsatian	F	2yrs	25	40	Yes	-	Vomiting	Ocular infection	Oestrus, septicaemia	Dextrose normal saline (DNS)
8	1/01/2016	Mixed	M	9yrs	28.6	37.9	Yes	-	-	Otitis external	Ear mites	Ivermec, amitraz, vitamins

Appendix 4: Sample submission form used for the prospective study

University of Ibadan, Department of Veterinary Medicine, Small Animal Unit

Sample Submission Form

Project: Clinical evaluation of gastroenteritis in clients-owned dogs

Patient's ID_____

A. Veterinary practice _____Location _____ Patient's file no_____ Date _____

B. Client's contact address _____ Phone _____

C. **Patient details:**

Breed _____ Age _____ **Sex**: Intact male ☐ Castrate ☐ Intact female ☐ Spayed ☐

Movement: Restricted☐ Stray/mix with other dogs in the neighborhood ☐

D. **Complaints** (*a brief summary*): _____

_____

_____

_____

E. **Physical examination:**

Body weight_____ Rectal Temp_____ Respiratory rate_____ Heart rate_____ M/membrane color_____

Body score (BCS): 1☐ 2☐ 3☐ 4☐ 5☐ (*See attached BCS chart*).

F. **DHLPP vaccination history:** Vaccinated? Yes☐ No☐ Not known☐ No of doses received_____

G. Age/date at first vaccination_____ 2nd Shot date_____ 3rd Shot date_____

H. **Vaccine brand:** Biocan ☐ Megavac ☐ Vanguard☐ Pro-Vac☐ Not known ☐ Others (specify)_____

I. (i) Litter size/number of dogs in the house_____ (ii) How many came down with the disease? _____

(iii) How many died from the illness? _____ (iv) Onset of clinical illness (days)_____

J. **Faecal consistency:** Watery and no solid pieces☐ Sloppy and mushy☐ Soft☐ Shaped and relatively firm☐

Solid and uncompressible☐

K. **Clinical signs observed:** (*Please tick only the ones observed in the patient*)

i.	Vomiting only	☐	ix.	Anorexia	☐
ii.	Vomiting and watery diarrhoea	☐	x.	Lethergy	☐
iii.	Vomiting and bloody diarrhoea	☐	xi.	Fever	☐
iv.	Watery diarrhoea only	☐	xii.	Depression	☐
v.	Bloody diarrhoea only	☐	xiii.	Dehydration	☐
vi.	Bloody & foul smelling diarrhoea	☐	xiv.	Abdominal pains	☐
vii.	Foul smelling diarrhoea only	☐	xv.	Anaemia	☐
viii.	No clinical signs observed	☐			

Other clinical signs observed (specify)_____

L. Onset of diarrhoea_____ Duration before the diarrhoea ceased (days)_____

M. How many episodes of diarrhoea observed since onset of the illness? _____; on admission? _____

N. How many episodes of vomiting before presentation? _____; During admission? _____

O. **Primary Screening**

i. **CPV test:** Positive ☐ Negative☐ Invalid ☐

ii. **CCV test:** Positive ☐ Negative☐ Invalid ☐

iii. **Giardia test:** Positive☐ Negative☐ Invalid ☐

*[*Please refer to the attached manuals for test procedure for color change and details*].

iv. If **ECG** was performed on the patient indicate ID _____

P. Is the patient suffering from more than one illness? If 'yes' *specify* _____

Q. Has the dog been dewormed within the past 3 months? Yes ☐ No☐

R. Please, indicate the treatment instituted for managing the patient with gastroenteritis.

1. _____ 6. _____
2. _____ 7. _____
3. _____ 8. _____
4. _____ 9. _____

S. Indicate the final treatment outcome: Recovered ☐ Died ☐ Euthanized due to poor prognosis ☐

T. Duration of recovery post–admission (days)_____

U. Duration before the patient died on admission (days) _____

V. Samples collected: Fecal_____ Blood _____

Appendix 5: **OWNER INFORMATION SHEET**

The University of Ibadan,
Department of Veterinary Medicine,
Small Animal Unit

Information for Owner

Sir/Madam:

I am Felix Shima a postgraduate student under the supervision of Professor Helen O. Nottidge and Dr. T.O. Omobowale. I am working on *gastroenteritis in owned-dogs* as my area of research. Gastroenteritis is the inflammation of the stomach and intestines, characterised by loss of appetite, feeling of vomiting, vomiting, diarrhoea, and abdominal discomfort. The causes of this condition are many making its diagnosis and management sometimes challenging for clinicians; resulting in high death rate in dogs.

This research involves collecting blood, faeces, and other relevant information about your pet, and monitoring of your dog's ECG, for the purposes of identifying the possible cause/s or generate data that would help clinicians in managing dogs with gastroenteritis.

Your dog has gastroenteritis hence we would like you to allow us included it in this investigation. We shall take about 4mL of blood once after examining your dog. Faeces will also be taken for laboratory examination. As an incentive for allowing your dog to participate in this study, it will be screened for suspected diseases and ECG will be monitored free of charge as to aid identify the health problem of your dog.

If you agree with our request to support this research, you are then requested to sign a written consent overleaf. Taking blood samples from a dog at the rate of 0.6mL/kg bodyweight/24h or about 10% of the total circulatory volume (average 85mL for dog) per kilogram body weight per 24h is safe.

This consent is voluntary and may be withdrawn after reading and understanding the scope of this research. The University of Ibadan animal welfare committee has reviewed the research protocols and gave approval.

Thank you for your cooperation.

Shima, Felix Kundu (DVM, MSc.)
Matric #: 166428
E-mail: Kshimaelyx@yahoo.co.uk
Phone: +2347083649744

Appendix 6: **OWNER CONSENT FORM**

The University of Ibadan,
Department of Veterinary Medicine,
Small Animal Unit

Owner Consent Form

I, ……..., the owner of:
Dog/s' Name/s…….
Breed …….
Age …….
Sex …….
Colour …….
permitted my dog to be included in the clinical study.

I have been briefed on the scope of this research and read the *Owner Information Sheet.* I understand that blood, faeces, and ECG data will be collected from my dog(s). I also understand that these are routine and safe clinical diagnostic procedures. I understand that participation is voluntary; may withdraw my dog in case I change my mind; but my dog would be accorded the desired veterinary care.

I have granted this permission in good faith, for this research is targeted at improving the health of dogs with gastroenteritis. Hence, I will not hold the researcher accountable in the event of death except proven otherwise that the procedures are the cause.

……...
Name, signature, and date

Appendix 7: Treatment options used in the management of the canine gastroenteritis cases studied.

Prescriptions	Dose, frequency, and routes of administration	Notes
Antibacterials		
Metronidazole[¥]	7.5mg/kg/daily, IV; Max.65mg/kg/day	Used in >80% of the cases
Gentamicin[£]	6mg/kg/day, IV	Used in >80% of the cases
Amoxicillin[β]	22mg/kg/day, IV	Used in >80% of the cases
Oxytetracycline 5%	1ml/5kg/day, IV, IM	Used in 19% of the cases
Sulphadimidine (333mg/100ml)	0.6mL/kg first day, subsequently 0.3mL daily for 3-4 days.	Used in 8% of the cases
Enrofloxacin	5-10mg/kg/day, IV	Used in 4% of the cases
Phthalylsulfathiazole (Thalazole®)	1tablet 3 times daily for at least 2 days, PO	Used in 3% of the cases
Antiemetics		
Metoclopramide[†]	1-2mg/kg/day IV, IM	Used in 50% of the cases
Antihemorrhagics		
Dicynone [Etamsylate 125mg/ml]	0.5-1mL stat, IM	Used in 20% of the cases
Vitamin K1	0.25-5mg/kg stat, IM	Used in 3% of the cases
Anthelmintics		
Ivermectin	0.2mg/kg stat, SC	Used in 14% of the cases
*Prazisam®, Caniverm®	1 tablet/10kg stat, PO	Used in 9% of the cases
Pyrantel pamoate [62.5 mg Tablet]	5-10mg/kg stat, PO	Used in 4% of the cases
Fluid therapy[Þ]		
5% Dextrose saline	44mL/kg/day, IV	Administered to all case
Lactated Ringer's solution	65mL/kg/day, IV	Administered to all case
Gastroprotectants		
Cimetidine	5-10mL/day, PO	Used in 3% of the cases
Immunomodulators		
Dexamethasone	0.2mg/kg/day, IM	Used in 2% of the cases
Prednisolone	2mg/kg/day, PO	Used in 1% of the cases
Vitamins and Minerals[ϕ]		
B-complex	1-5mL/day, IM	Used in 90% of the cases
Multivitamins	1-5mL/day, IM	Used in 10% of the cases

***NOTE** - **Prazisam®:** Fixed-dose formulary of anthelminthics containing praziquantel USP 50 mg, fenbendazole BP 500 mg, and pyrantel pamoate 144 mg; **Caniverm®:** pyrantel pamoate 144 mg, Praziquantel 50 mg, and fenbendazole 150 mg. The dogs were prescribed [¥, £, β, †, Þ, ϕ] extensively. The treatment options were decided by the clinicians based on the need of individual case. The median duration of treatment from reporting to discharge was 5 days.

Appendix 8: Set of CPV primers used in the genetic analysis

inqaba biotec™

Pg 371

Africa's Genomics Company

Inqaba Biotec West Africa Ltd.

Co. Reg. No: RC1232028
VAT No: 17949735-0001

Name: VP2850F	**Barcode:** S4365	**Manufacturing Date:**	
Sequence: GAGCATTGGGCTTACCA	**Length:** 17		**PAGE QC Image**
OD: 10.9	**MW min \ max:** 5210.45	**5' Mod:** None	
nmoles: 64.1	**GC % min \ max:** 52.94	**3' Mod:** None	
Tm min \ max: 47.05	**Purification:** Standard		
For a 100 µM stock solution add 640.97 **µl water or buffer**			
Comments:			

Name: VP21550R	**Barcode:** S4366	**Manufacturing Date:**	
Sequence: GCAAGATGCATCAGGATC	**Length:** 18		**PAGE QC Image**
OD: 10.78	**MW min \ max:** 5532.67	**5' Mod:** None	
nmoles: 59.89	**GC % min \ max:** 50.0	**3' Mod:** None	
Tm min \ max: 48.04	**Purification:** Standard		
For a 100 µM stock solution add 598.92 **µl water or buffer**			
Comments:			

RECOMMENDATIONS FOR HANDLING AND STORAGE OF OLIGOS

- Lyophilized oligo pellets might become displaced from the bottom of the tube during shipment. Briefly centrifuge each tube before opening to prevent the loss of the pellet.
- Prepare stock solution of oligos (e.g. 100 uM = 100 pmole per ul) preferably with a sterile buffered solution such as TE (10 mM Tris, pH 7.5 to 8.0, 1 mM EDTA). If sterile distilled water used, make sure that the pH is above 7.0 since acidic solutions favours oligo depurination and subsequent loss of activity.
- Working solutions might be diluted from the stock solution with sterile, nuclease-free water to prevent inhibition of enzymatic reactions (e.g. PCR) by EDTA.
- Store the oligos as concentrated stock solution or lyophilized at -20° C.
- Avoid frequent freeze-thaw cycles by dividing the stock solution into smaller aliquots for long term storage and to prevent accidental contamination.
- Dye-modified oligos are light sensitive and should always be stored in the dark.
- Resuspend modified oligos preferably in a slightly basic solution (i.e., TE at pH 8.0). However, Cy dye modified oligos are best kept at pH 7.0 at -20° C.
- Preferably store the modified oligos as dried aliquots at -20° C.

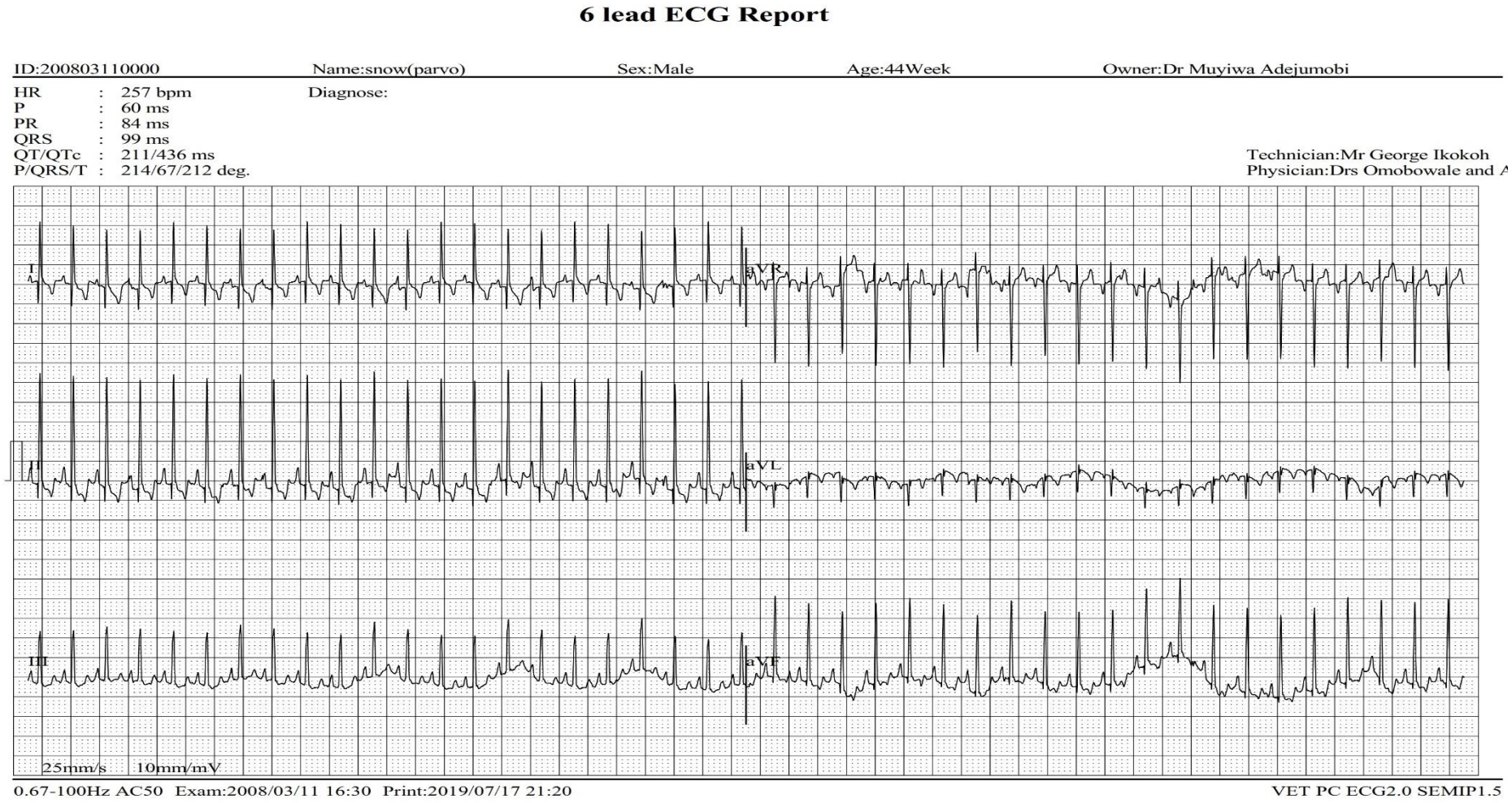
PRODUCTS FOR RESEARCH USE ONLY!

PMB 5320, Oyo Road, Ibadan 200001, Oyo State, , • Phone: +234 805 8827272 • Fax: +27 86 677 8409
Email: lukman.aroworamimo@inqababiotec.ng • Website: http://www.inqababiotec.ng

Appendix 9: ECG values of the CPV-positive dogs sampled

ECG VALUES OF CANINE PARVOVIRUS ENTERITIS PATIENTS EXAMINED													
S/N	Animal	Breed	Sex	Age (mo)	Wt (Kg)	HR (bpm)	P (ms)	PR (ms)	QRS (ms)	QT (ms)	QTC	Ra (mV)	Ta (mV)
1	Willea	ROT	F	4	10	120	36	91	44	218	308	1.989	0.182
2	Rocket	ALS	M	2	7	238	62	75	44	137	272	0.266	0.576
3	Chika	BBL	F	8	21	153	61	95	58	224	357	1.371	0.428
4	Puppy B	CRS	F	3	7	209	34	60	44	179	334	0.402	0.103
5	King	BBL	M	6	23	102	68	114	53	240	312	2.164	0.609
6	Sparkle	ROT	F	3	8	168	38	81	42	233	389	2.399	0.156
7	Bella	CAU	F	3	4	107	41	100	42	226	301	2.252	0.685
8	Bruno	ROT	F	2	4	140	63	104	39	200	305	1.378	0.085
9	Major	ALS	M	24	7	126	69	119	70	233	337	1.571	0.355
10	Brownie	ALS	F	3	8	111	47	125	55	245	333	1.209	0.407
11	Angel	CAU	F	3	7	119	38	112	41	223	314	1.341	0.114
12	Cooky	CRS	F	5	11	116	61	116	52	218	303	1.799	0.136
13	Oscar	BBL	M	3	12	167	68	107	56	216	360	1.204	0.15
14	Smart	BBL	M	7	20	125	50	94	50	200	288	1.993	0.115
15	Craft	CAU	F	9	18	144	46	101	51	207	320	1.378	0.155
16	Doffy	CRS	F	9	17	198	64	95	76	194	352	2.219	0.384
17	Puppy C	ROT	F	4	11	156	34	91	40	217	349	0.88	0.099
18	Bobby	CRS	M	48	18	124	37	113	37	194	278	2.407	-0.312
19	Billy	ALS	M	3	9	128	34	95	42	213	311	1.292	0.065
20	Squizzy	BBL	F	9	14	121	49	116	65	232	329	1.66	0.243
21	PuppyA	CRS	F	2	5	146	45	135	56	232	361	0.032	0.267
22	Terry	ALS	M	3	9	137	61	110	50	215	324	1.354	0.116
23	Alloy	ALS	M	10	12	114	53	119	70	205	282	0.704	-0.022
24	Mitchell	LHA	M	6	6	154	40	83	37	184	292	2.892	0.347
25	Berry	ALS	M	7	20	91	38	90	48	233	286	1.513	-0.1
26	Beliza	CAU	F	6	20	160	71	128	72	226	369	1.926	0.149
27	Fancy	ROT	M	3	7	170	57	107	50	188	316	1.408	0.236
28	Henry	BBL	M	18	19	103	217	240	43	171	224	1.501	-0.164
29	Argon	ROT	M	7	32	95	45	119	65	209	262	2.781	0.3
30	Puppy Xb parvo	CRS	M	2	7	120	34	89	54	200	282	2.291	-0.039
31	Puppy Xa parvo	CRS	M	2	7	104	41	121	44	244	321	0.031	0.257
32	Jesssica	BBL	F	7	16	200	43	71	65	167	304	1.354	0
33	Fortune	BBL	F	3	25	143	40	78	47	194	299	1.881	0.213
34	Douglas	LOC	M	6	11	130	33	103	40	187	275	1.529	0.198
35	Ruby	CHO	F	4	16	105	55	168	76	226	298	0.048	-0.026
36	Lizzy	MAS	F	8	15	136	42	82	41	194	292	1.271	0.13
37	Eric	CAU	M	5	20	126	51	105	78	170	246	2.229	-0.27
38	Empress	ROT	F	2	4	168	36	98	40	200	334	1.004	0.458
39	Scoopy	ALS	F	9	25	98	41	151	50	242	309	1.93	-0.31
40	Snow	ALS	M	7	12	257	48	73	55	127	262	0.439	-0.217

Appendix 10: ECG tracings from CPV-positive dogs studied



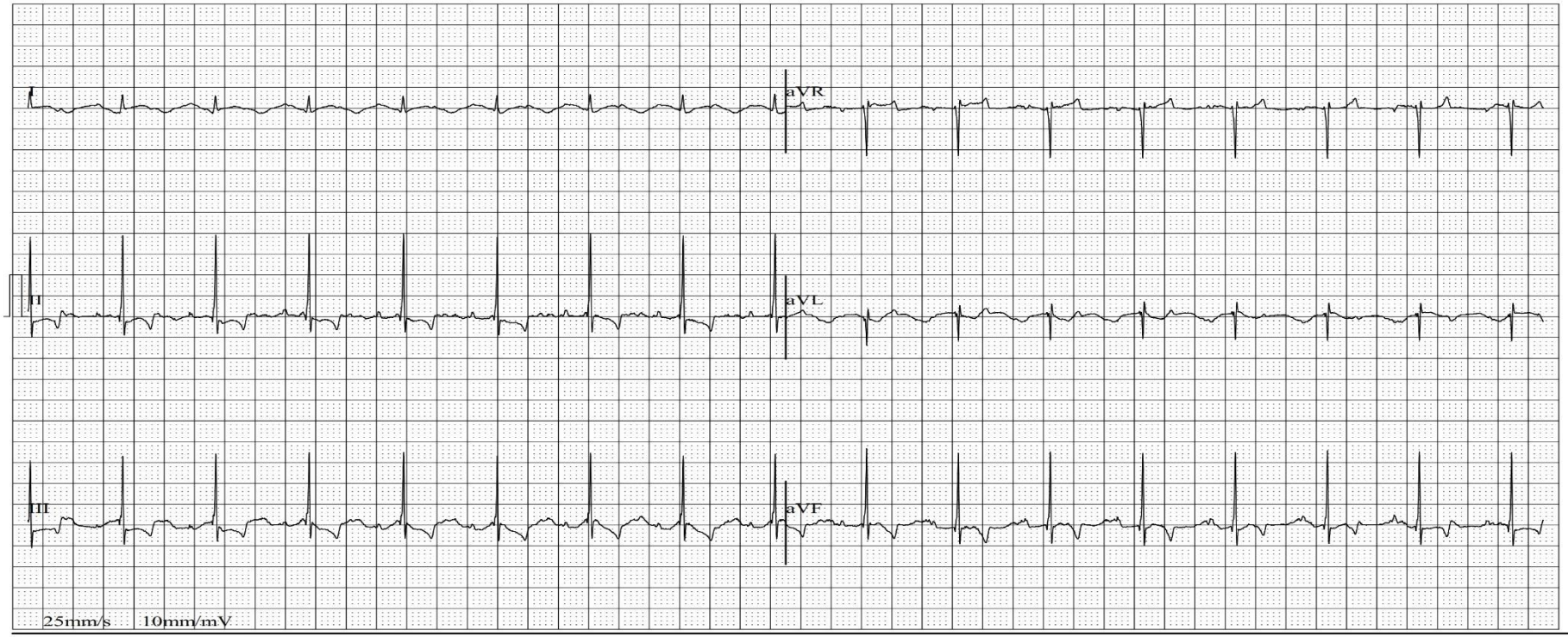
6 lead ECG Report

ID:201701110021 Name:scoopy Sex:Female Age:9Month Owner:Dr Oluwaseun Esan

HR : 98 bpm
P : 47 ms
PR : 155 ms
QRS : 63 ms
QT/QTc : 282/360 ms
P/QRS/T : 109/79/251 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/01/11 06:32 Print:2019/07/17 21:19 VET PC ECG2.0 SEMIP1.5

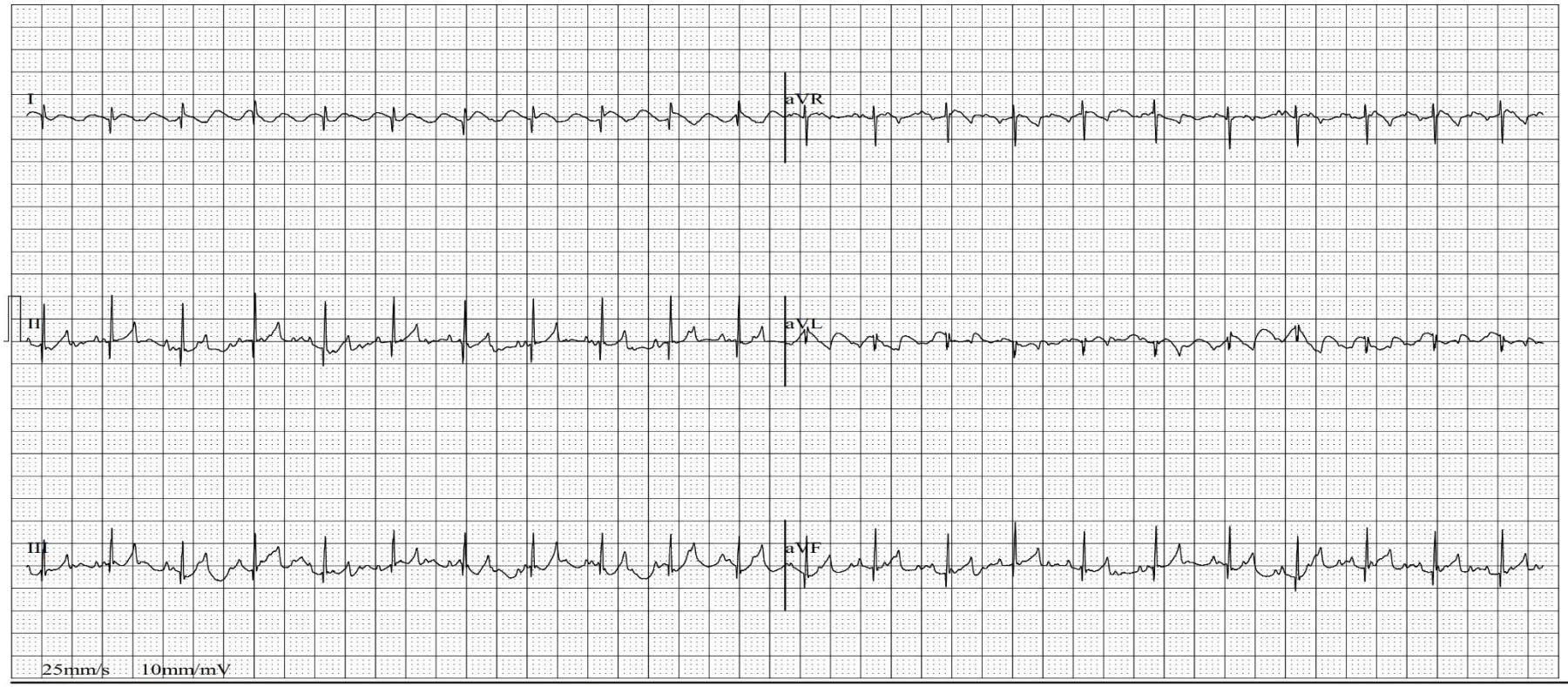
6 lead ECG Report

ID:201701170000 Name:Empress parvo enteritis Sex:Female Age:9Month Owner:Mr Richard

HR : 168 bpm
P : 65 ms
PR : 100 ms
QRS : 63 ms
QT/QTc : 211/353 ms
P/QRS/T : -90/87/107 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/01/17 06:21 Print:2019/07/17 21:18 VET PC ECG2.0 SEMIP1.5

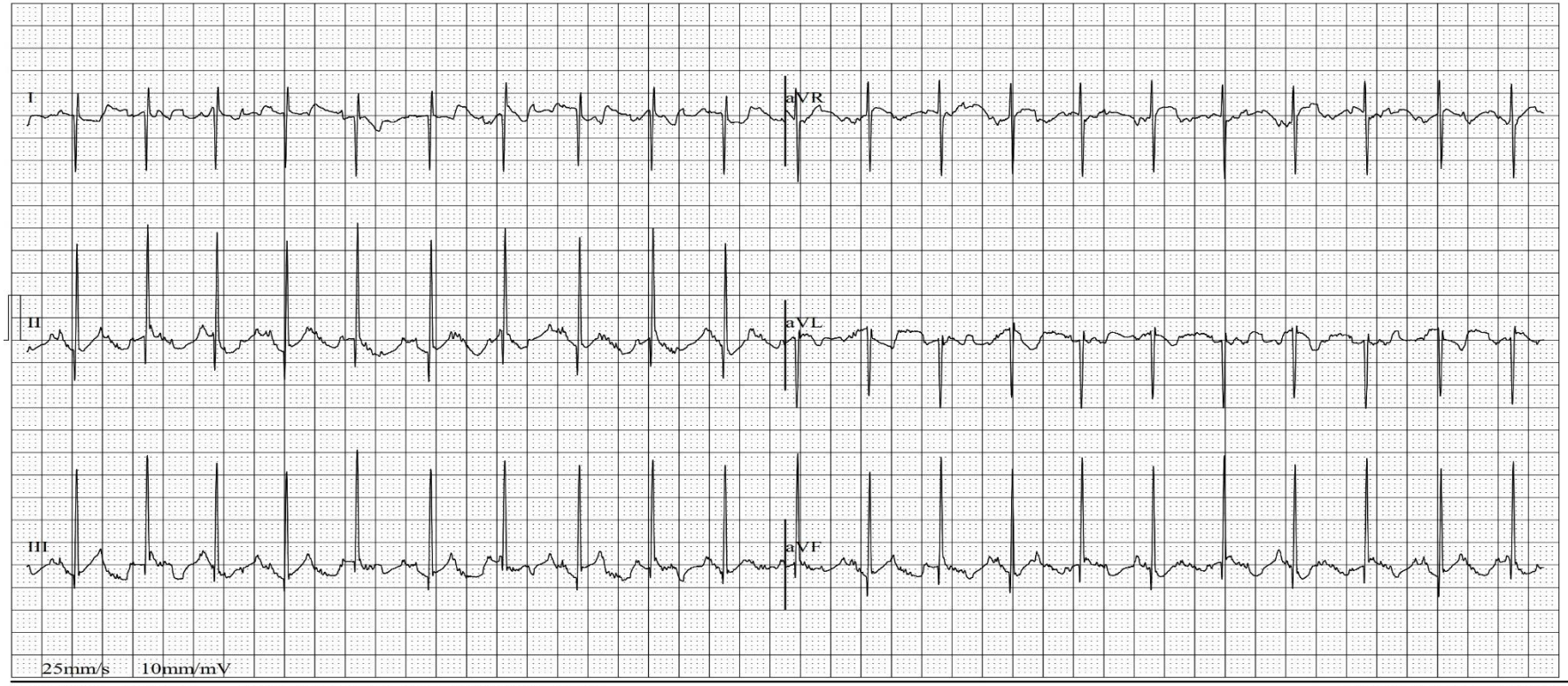
6 lead ECG Report

ID:201701170001 Name:Eric parvo enteritis Sex:Male Age:5Month Owner:Mr Onyekachi

HR : 126 bpm
P : 78 ms
PR : 114 ms
QRS : 61 ms
QT/QTc : 211/305 ms
P/QRS/T : 90/100/161 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejum



0.67-100Hz AC50 Exam:2017/01/17 06:37 Print:2019/07/17 21:17 VET PC ECG2.0 SEMIP1.5

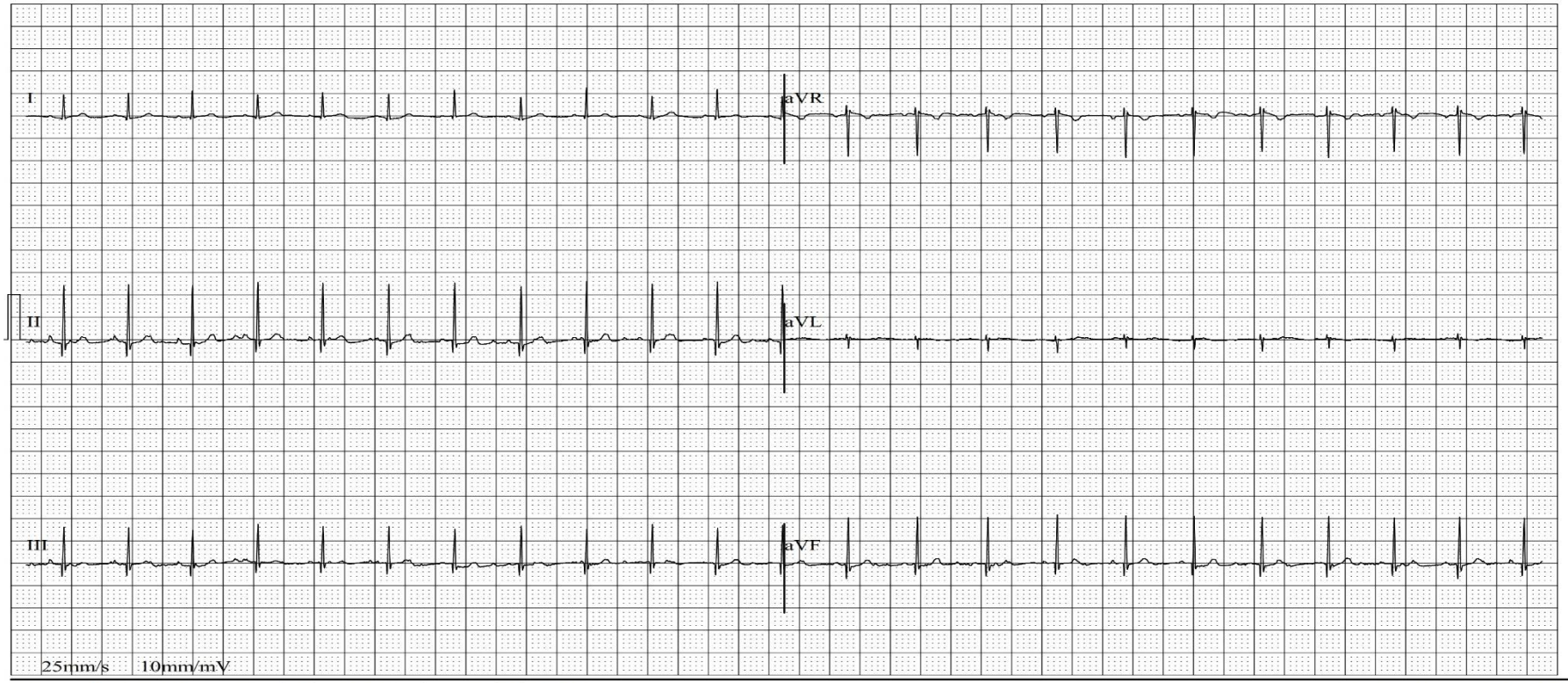
6 lead ECG Report

ID:201701230000 Name:Lizzy parvo 1st day Sex:Female Age:9Week Owner:Mr Adeyemi

HR : 136 bpm
P : 58 ms
PR : 89 ms
QRS : 41 ms
QT/QTc : 199/299 ms
P/QRS/T : 47/62/8 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejum



0.67-100Hz AC50 Exam:2017/01/23 08:17 Print:2019/07/17 21:16 VET PC ECG2.0 SEMIP1.5

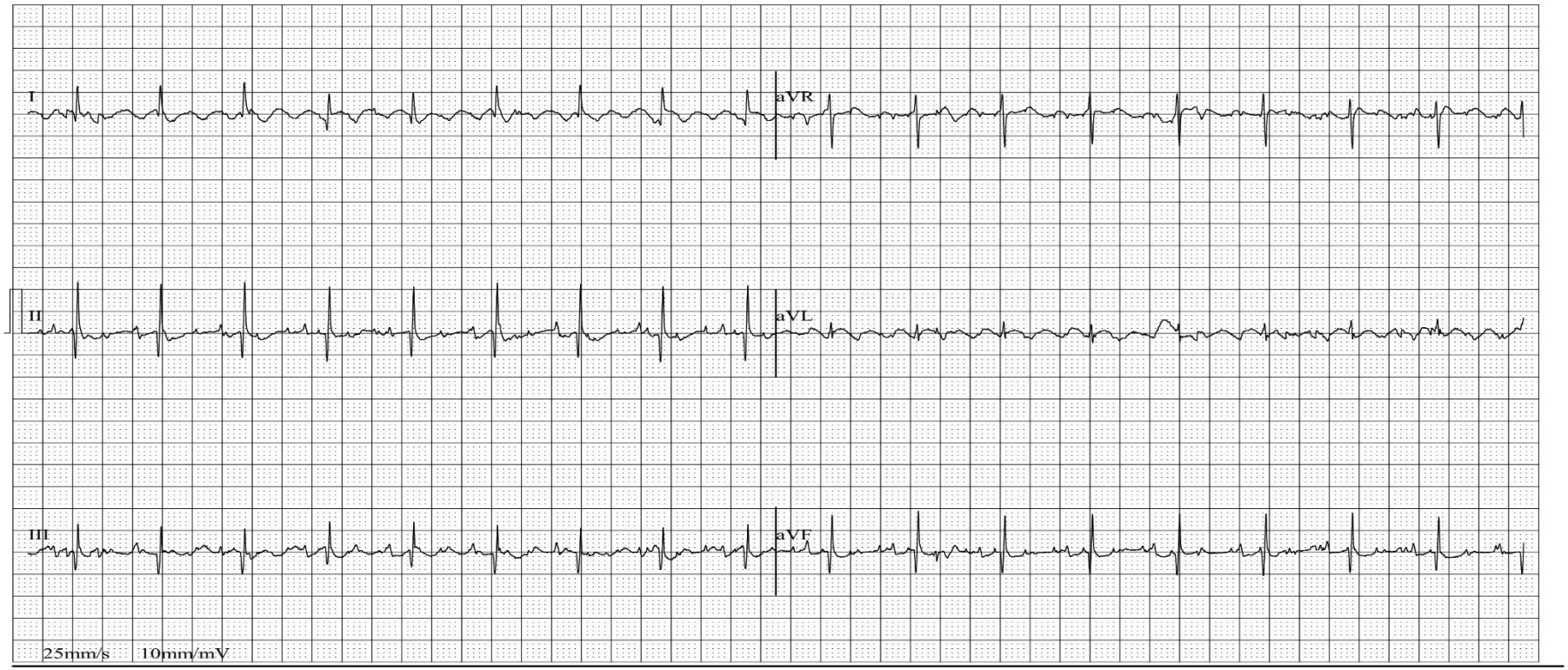
6 lead ECG Report

ID:201701230001 Name:Ruby parvo 1st day Sex:Female Age:5Month Owner:Mr Deji

HR : 105 bpm
P : 127 ms
PR : 151 ms
QRS : 70 ms
QT/QTc : 277/366 ms
P/QRS/T : -7/33/238 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/01/23 08:34 Print:2019/07/17 21:16 VET PC ECG2.0 SEMIP1.5

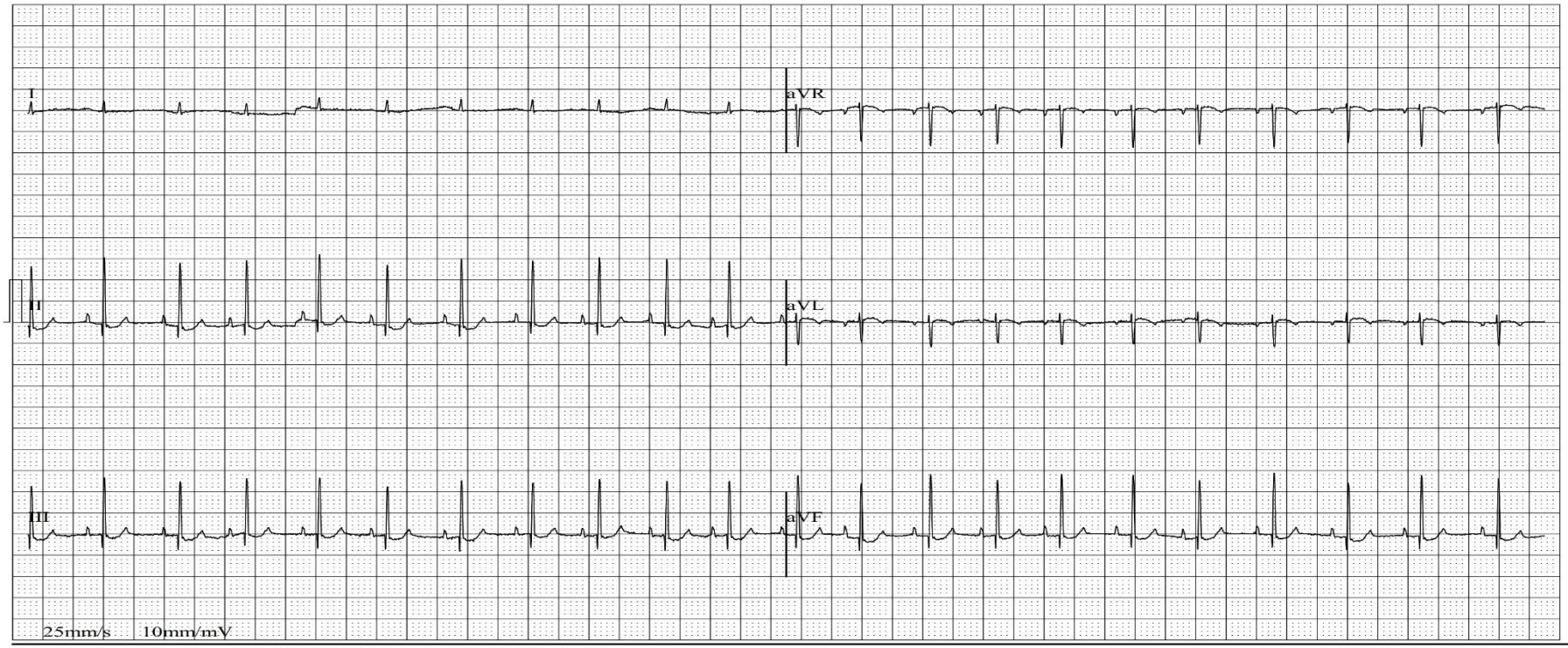
6 lead ECG Report

ID:201701250000 Name:DOUGLAS Sex:Male Age:3Month Owner:Pastor Aderibigbe

HR : 130 bpm
P : 49 ms
PR : 114 ms
QRS : 74 ms
QT/QTc : 234/344 ms
P/QRS/T : 90/83/90 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/01/25 03:36 Print:2019/07/17 21:15 VET PC ECG2.0 SEMIP1.5

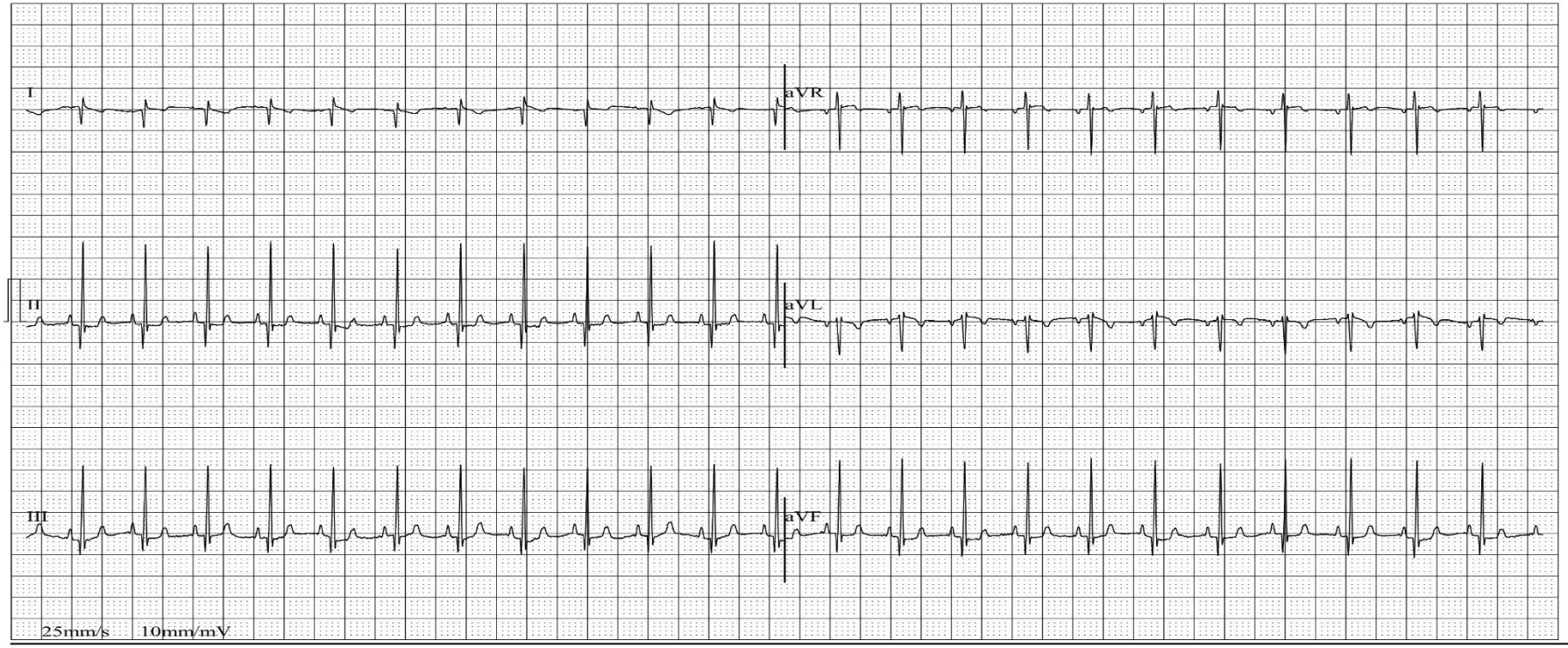
6 lead ECG Report

ID:201701250001 Name:fortune Sex:Male Age:10Month Owner:Wale Omole

HR : 143 bpm
P : 51 ms
PR : 81 ms
QRS : 67 ms
QT/QTc : 214/330 ms
P/QRS/T : 90/95/100 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/01/25 03:47 Print:2019/07/17 21:14 VET PC ECG2.0 SEMIP1.5

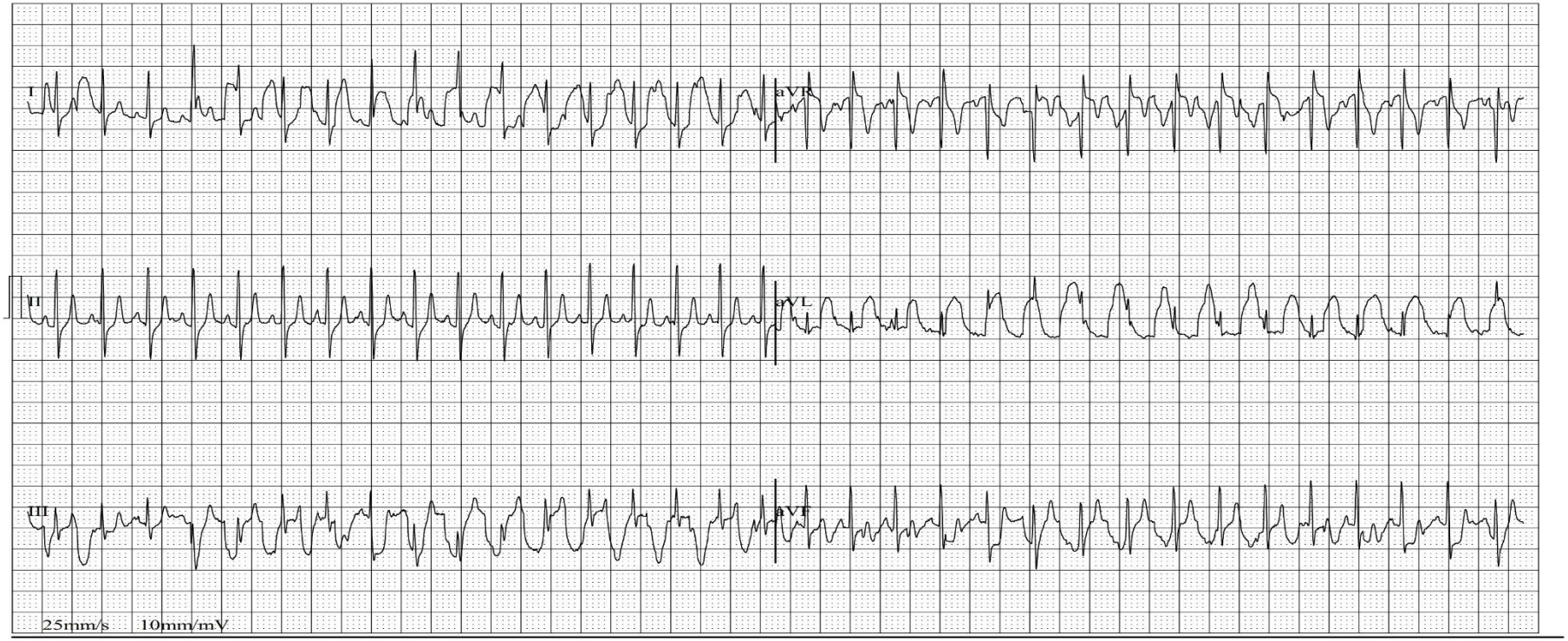
6 lead ECG Report

ID:201701260000 Name:Jessica Sex:Female Age:7Month Owner:Wale Omole

HR : 200 bpm
P : 81 ms
PR : 109 ms
QRS : 150 ms
QT/QTc : 217/396 ms
P/QRS/T : 21/98/50 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/01/26 07:39 Print:2019/07/17 21:14

VET PC ECG2.0 SEMIP1.5

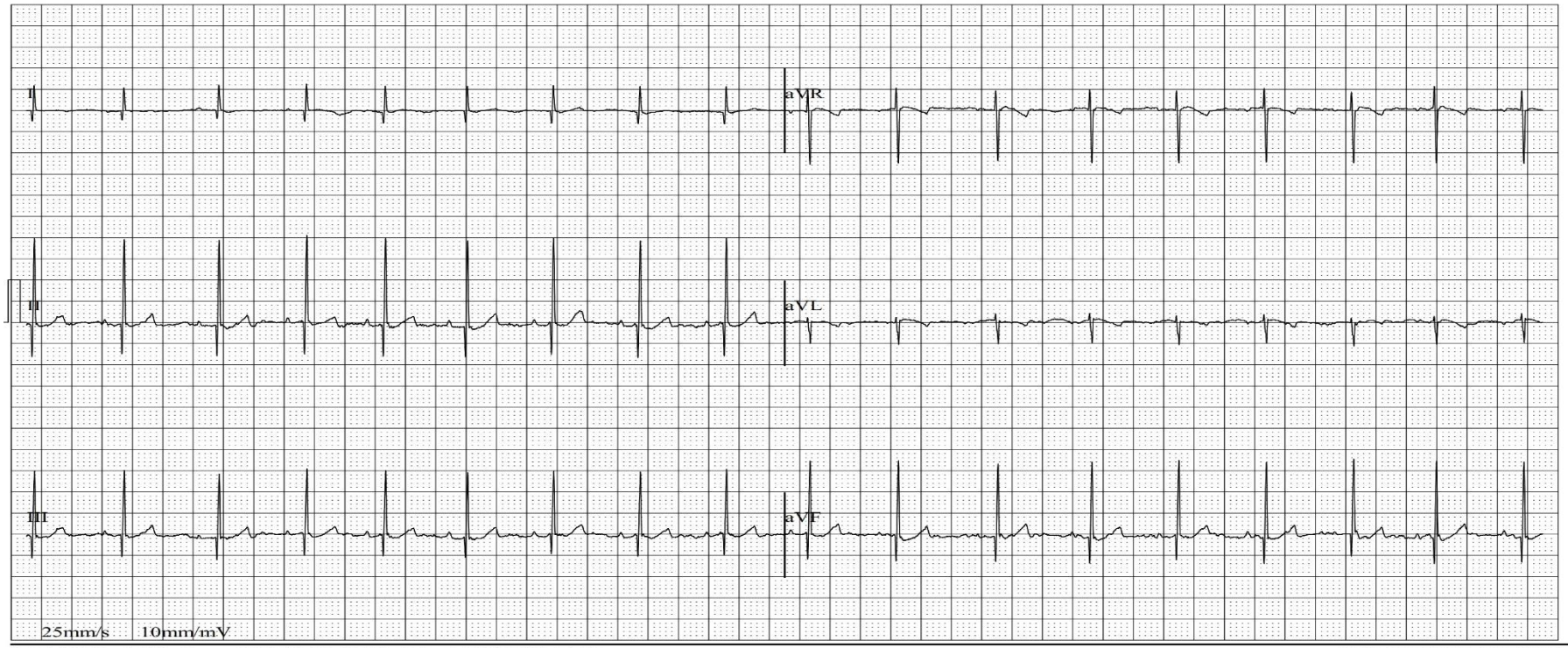
6 lead ECG Report

ID:201701300000 Name:puppy A parvo Sex:Female Age:10Month Owner:Mr Emmanuel

HR : 104 bpm
P : 52 ms
PR : 121 ms
QRS : 56 ms
QT/QTc : 249/327 ms
P/QRS/T : 90/80/90 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



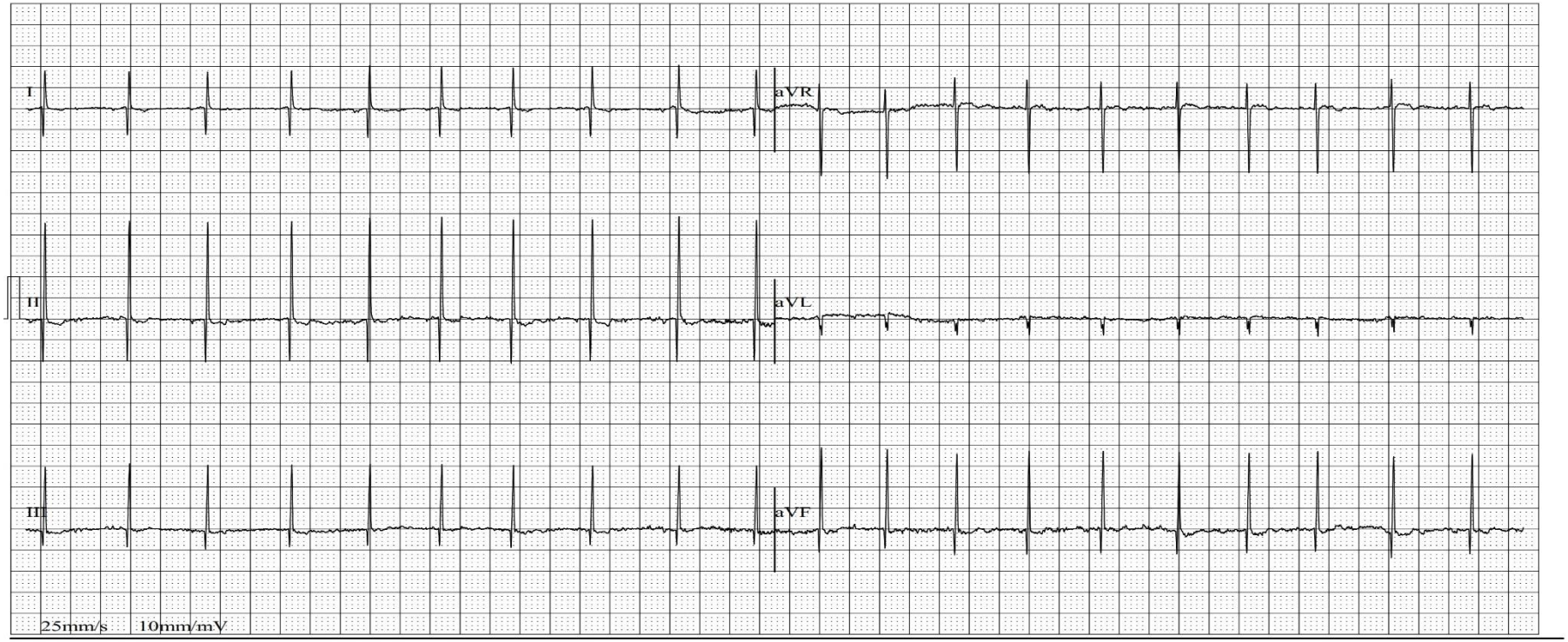
0.67-100Hz AC50 Exam:2017/01/30 06:33 Print:2019/07/17 21:13 VET PC ECG2.0 SEMIP1.5

6 lead ECG Report

ID:201701300001 Name:puppy B parvo Sex:Female Age:3Month Owner:Mr Emmanuel

HR : 120 bpm Diagnose:
P : 66 ms
PR : 94 ms
QRS : 66 ms
QT/QTc : 196/277 ms
P/QRS/T : 0/74/249 deg.

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/01/30 06:39 Print:2019/07/17 21:05 VET PC ECG2.0 SEMIP1.5

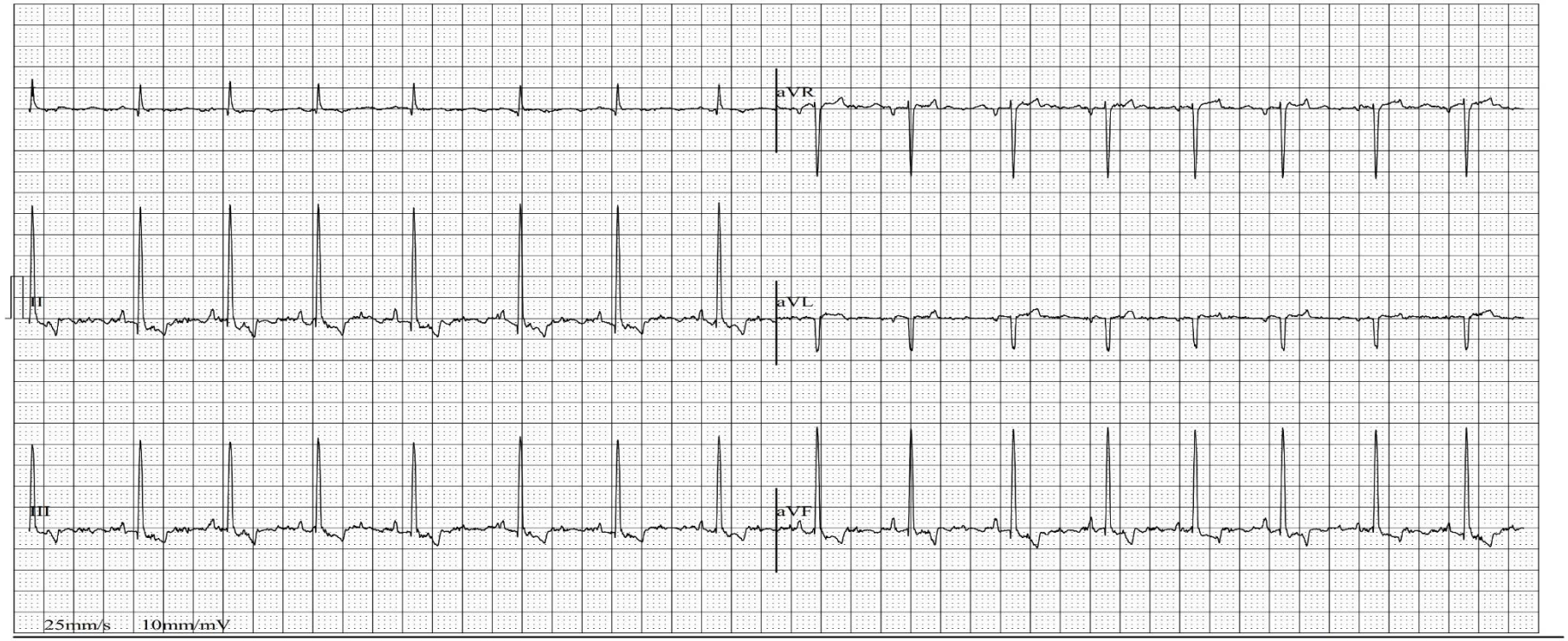
6 lead ECG Report

ID:201701300002 Name:Argon parvo Sex:Male Age:7Month Owner:Mr john

HR : 95 bpm
P : 55 ms
PR : 115 ms
QRS : 83 ms
QT/QTc : 252/317 ms
P/QRS/T : 82/83/260 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/01/30 07:05 Print:2019/07/17 21:04 VET PC ECG2.0 SEMIP1.5

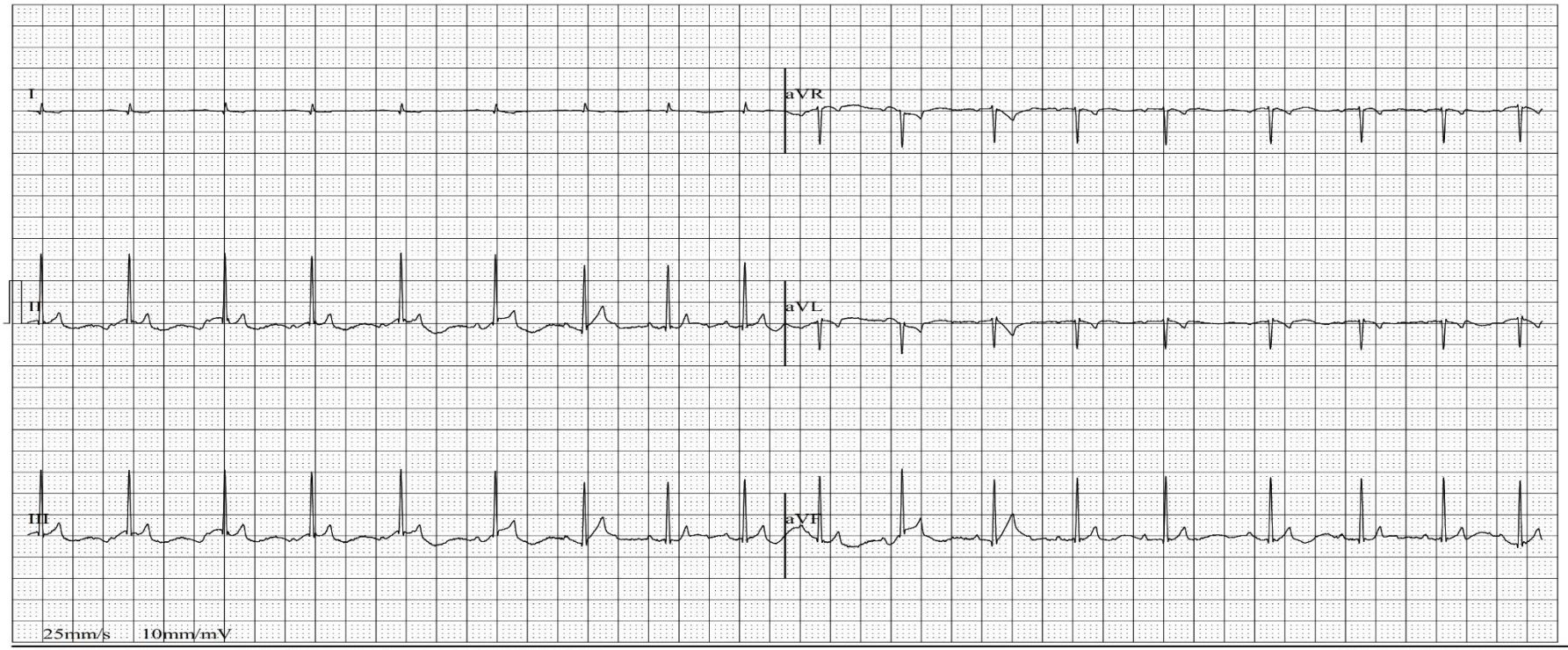
6 lead ECG Report

ID:201702030002 Name:HENRY Sex:Male Age:18Month Owner:Mr. Oyediran

HR : 103 bpm
P : 147 ms
PR : 190 ms
QRS : 80 ms
QT/QTc : 319/417 ms
P/QRS/T : 30/83/269 deg.

Diagnose:

Technician:Dr Victor Oriaku
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/02/03 02:45 Print:2019/07/17 21:03

VET PC ECG2.0 SEMIP1.5

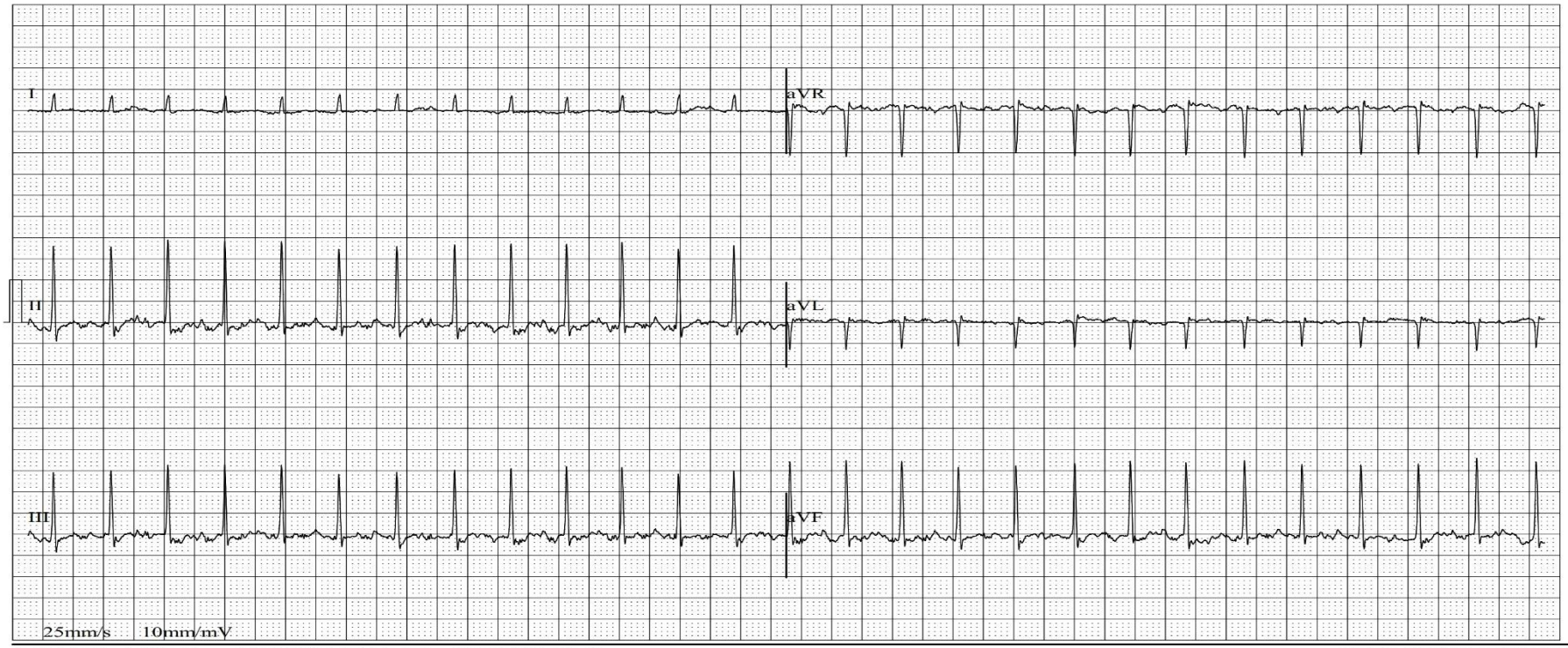
6 lead ECG Report

ID:201702130000 Name:Beliza Sex:Female Age:6Month Owner:MR OYERINDE

HR : 160 bpm
P : 85 ms
PR : 140 ms
QRS : 65 ms
QT/QTc : 275/449 ms
P/QRS/T : 0/78/269 deg.

Diagnose:

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/02/13 06:14 Print:2019/07/17 21:02 VET PC ECG2.0 SEMIP1.5

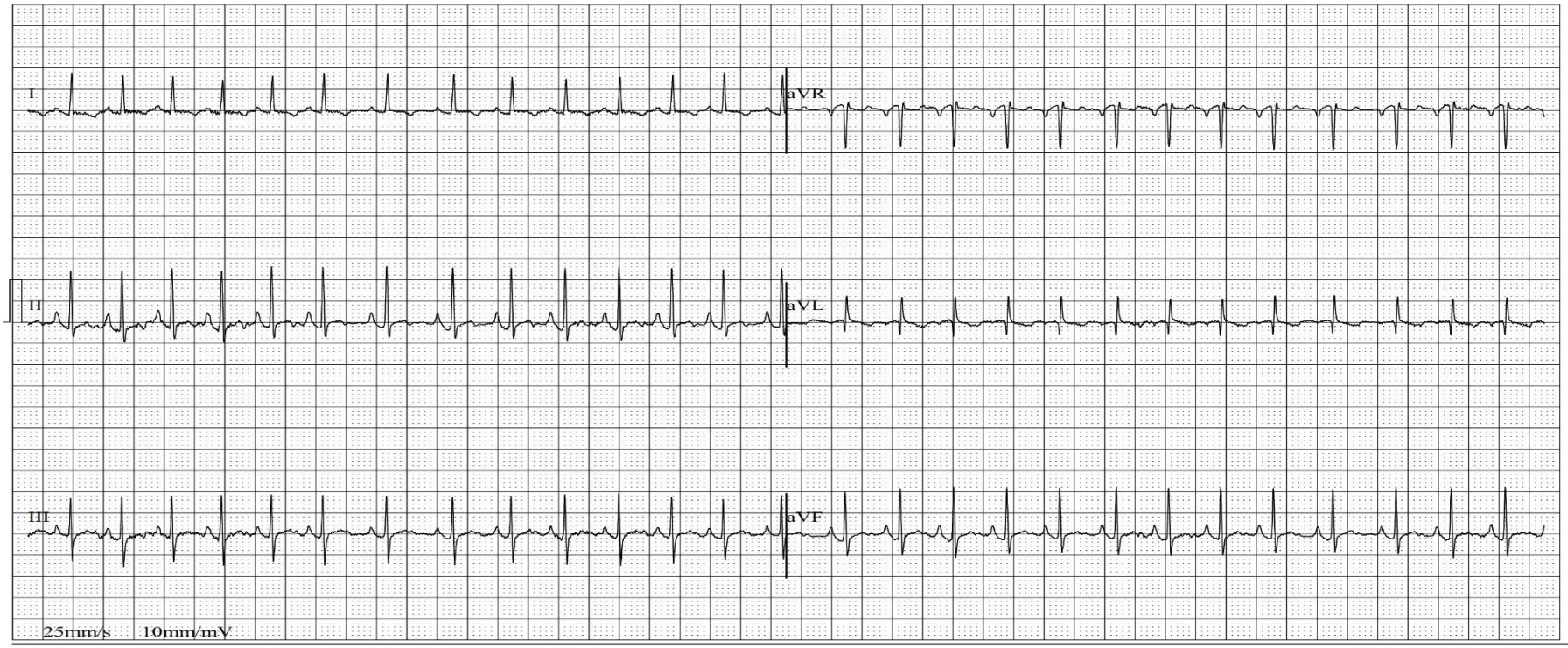
6 lead ECG Report

ID:201702170000 Name:Fancy Sex:Male Age:3Month Owner:MR Ademikanra

HR : 170 bpm
P : 71 ms
PR : 102 ms
QRS : 53 ms
QT/QTc : 292/491 ms
P/QRS/T : 70/40/74 deg.

Diagnose:

Technician:
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/02/17 02:19 Print:2019/07/17 21:01 VET PC ECG2.0 SEMIP1.5

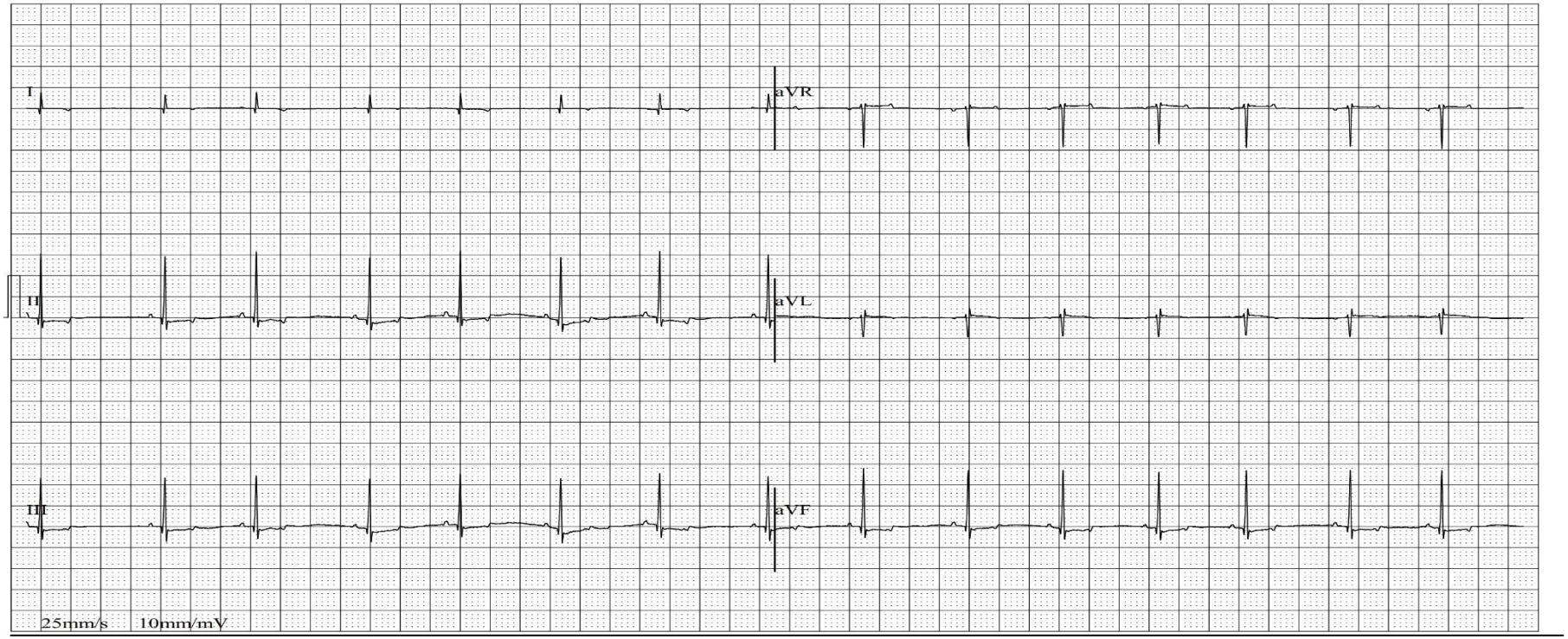
6 lead ECG Report

ID:201702170001 Name:Berry (parvo) Sex:Male Age:7Month Owner:Mrs Abiodun

HR : 91 bpm
P : 49 ms
PR : 96 ms
QRS : 55 ms
QT/QTc : 231/284 ms
P/QRS/T : 90/79/255 deg.

Diagnose:

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/02/17 02:30 Print:2019/07/17 21:00 VET PC ECG2.0 SEMIP1.5

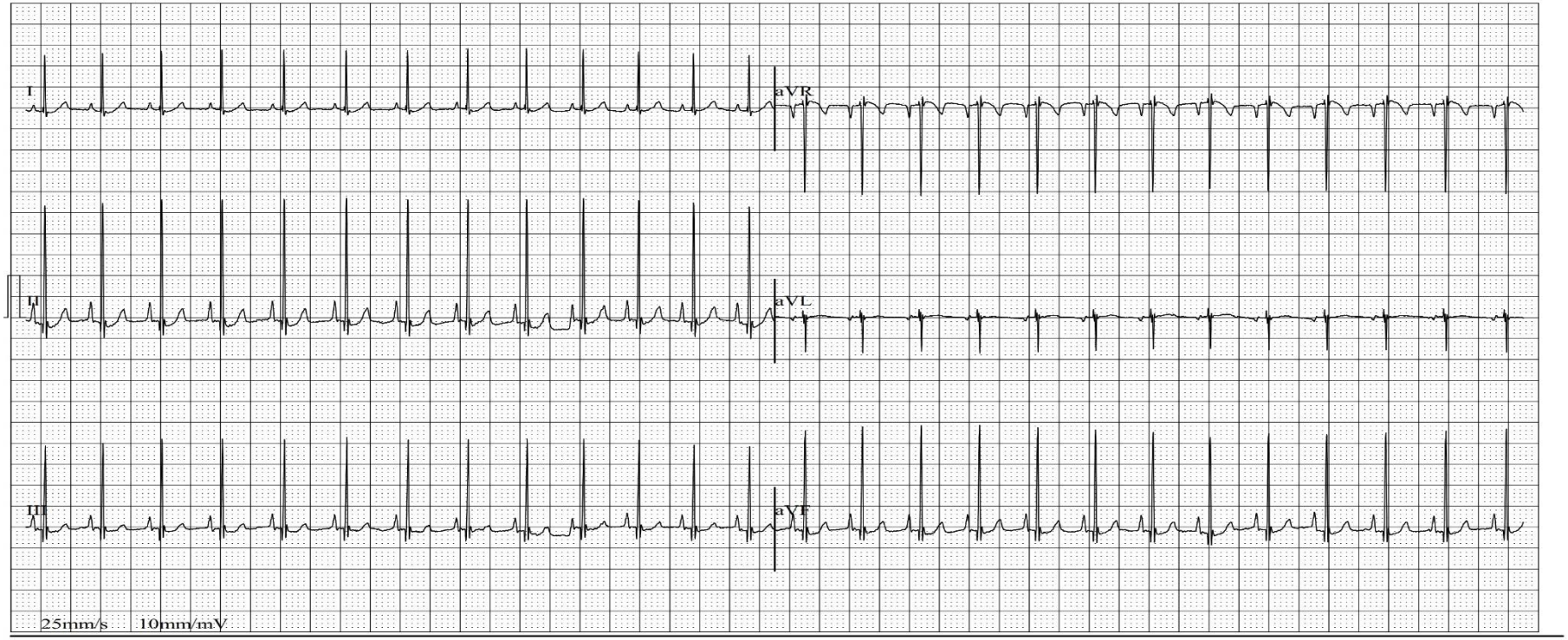
6 lead ECG Report

ID:201702170003 Name:mitchell (parvo) Sex:Male Age:5Month Owner:Prof Oyeyemi

HR : 154 bpm
P : 47 ms
PR : 85 ms
QRS : 35 ms
QT/QTc : 251/402 ms
P/QRS/T : 71/69/71 deg.

Diagnose:

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



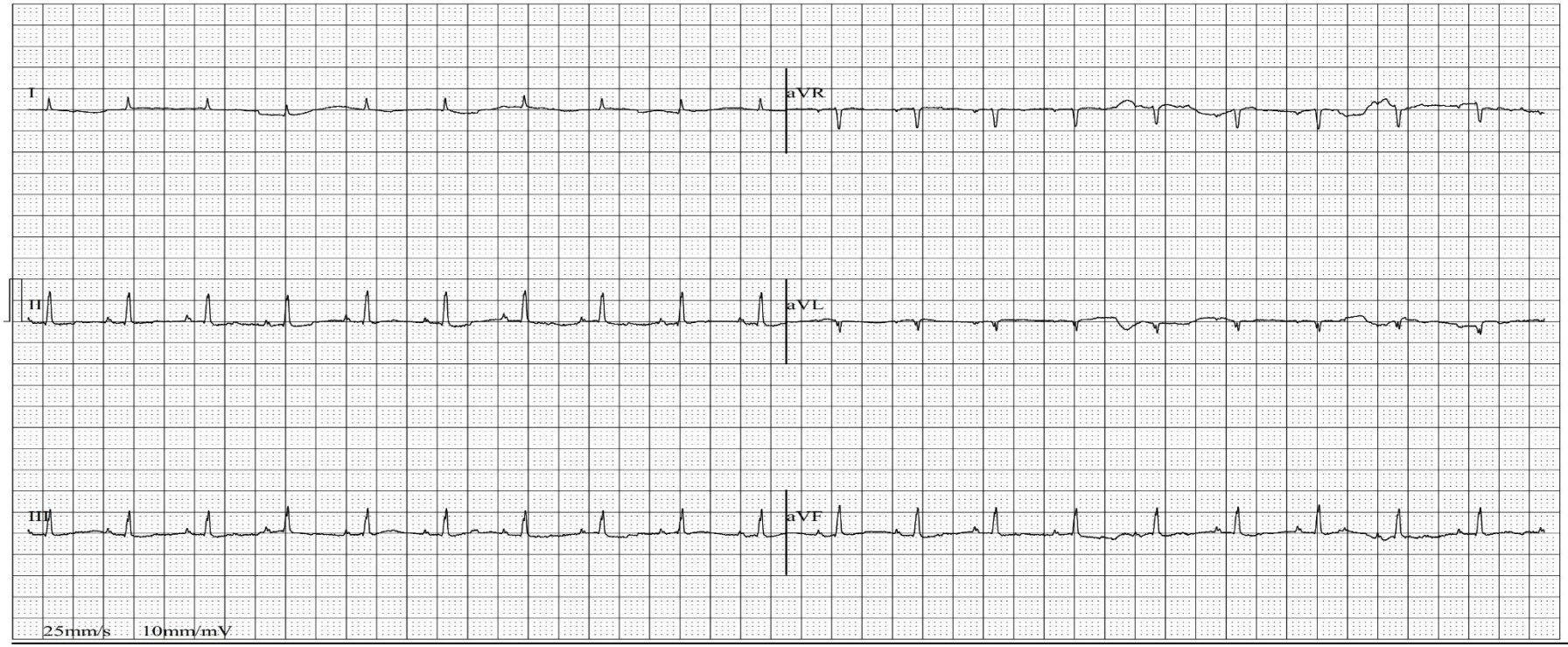
0.67-100Hz AC50 Exam:2017/02/17 02:42 Print:2019/07/17 21:00 VET PC ECG2.0 SEMIP1.5

6 lead ECG Report

ID:201702200001 Name:ALLOY (parvo) Sex:Male Age:8Month Owner:Mrs ABIOLA

HR : 114 bpm Diagnose:
P : 62 ms
PR : 125 ms
QRS : 77 ms
QT/QTc : 213/293 ms
P/QRS/T : 90/76/-90 deg.

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/02/20 03:21 Print:2019/07/17 20:59 VET PC ECG2.0 SEMIP1.5

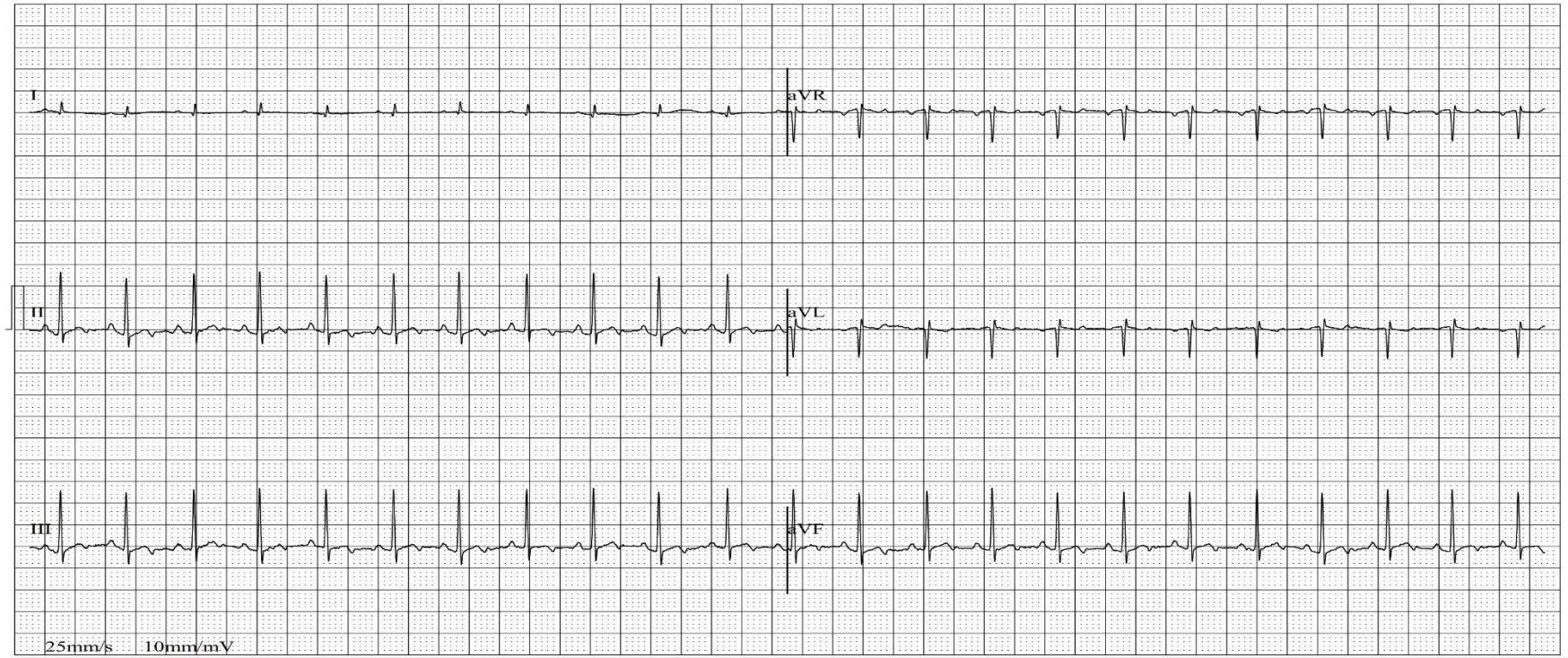
6 lead ECG Report

ID:201702200002 Name:TERRY (parvo) Sex:Male Age:4Month Owner:Mr FATOKUN

HR : 137 bpm
P : 67 ms
PR : 116 ms
QRS : 52 ms
QT/QTc : 243/367 ms
P/QRS/T : 81/85/90 deg.

Diagnose:

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/02/20 03:36 Print:2019/07/17 20:58

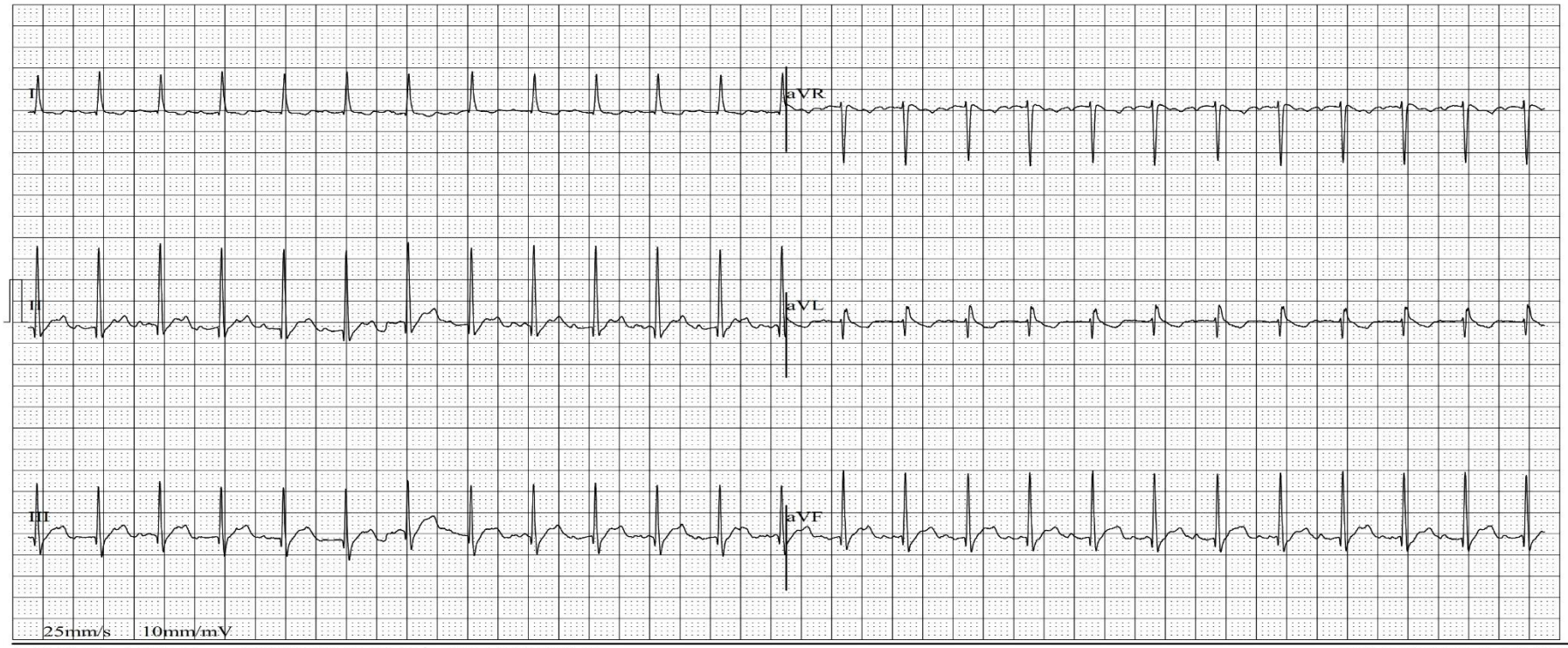
VET PC ECG2.0 SEMIP1.5

6 lead ECG Report

ID:201702220000 Name:PUPPYA (parvo) Sex:Female Age:2Month Owner:Mr Gafar

HR : 146 bpm Diagnose:
P : 66 ms
PR : 138 ms
QRS : 81 ms
QT/QTc : 250/389 ms
P/QRS/T : 47/45/94 deg.

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/02/22 03:30 Print:2019/07/17 20:58 VET PC ECG2.0 SEMIP1.5

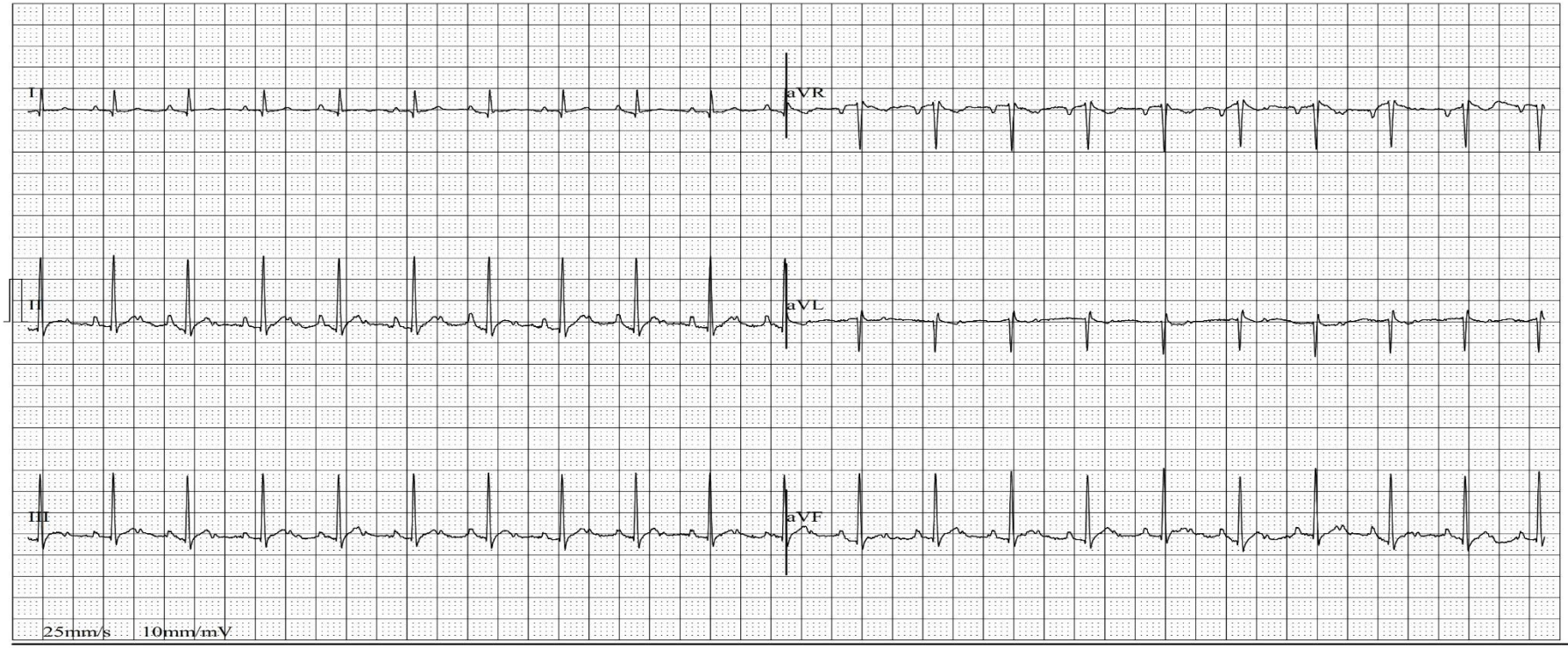
6 lead ECG Report

ID:201702220001 Name:Squizy (parvo) Sex:Female Age:9Month Owner:Dr Raji

HR : 121 bpm
P : 65 ms
PR : 125 ms
QRS : 59 ms
QT/QTc : 240/340 ms
P/QRS/T : 64/78/74 deg.

Diagnose:

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/02/22 03:43 Print:2019/07/17 20:57 VET PC ECG2.0 SEMIP1.5

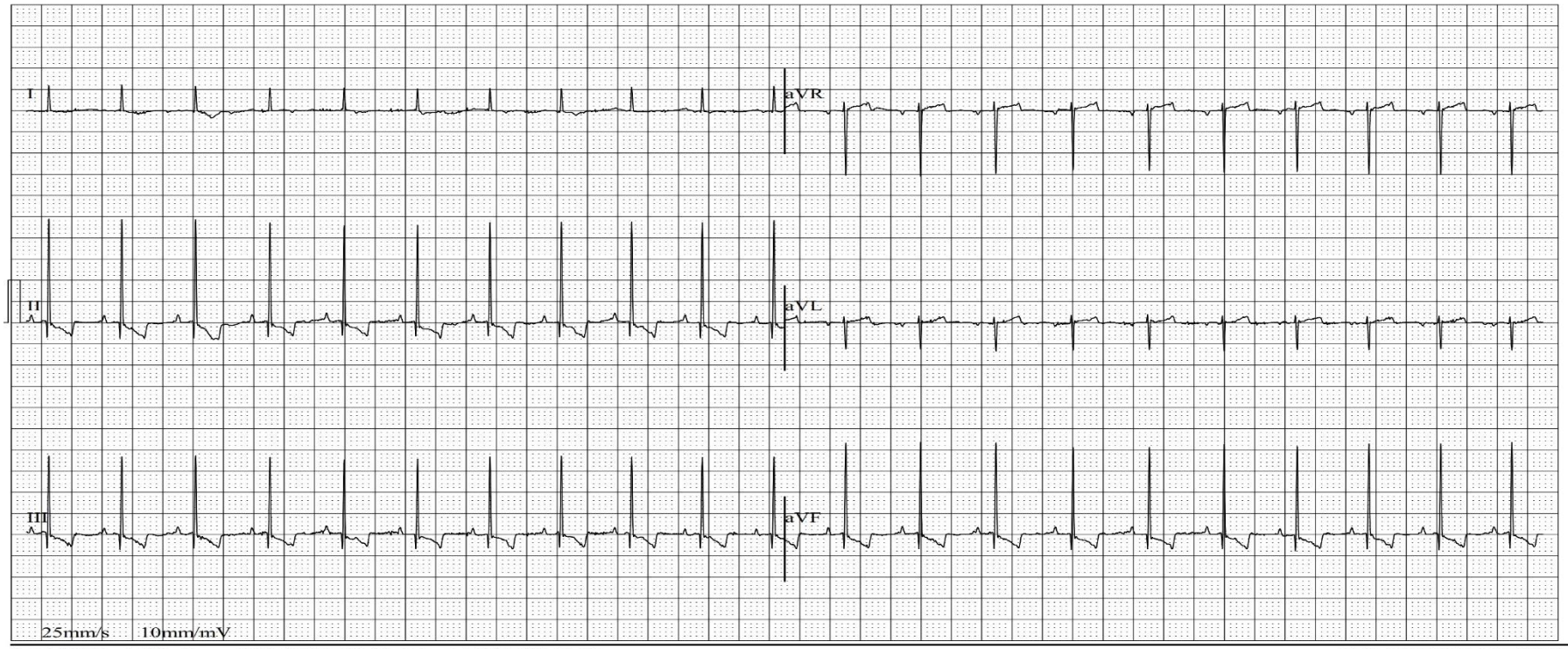
6 lead ECG Report

ID:201703020001 Name:bobby Sex:Male Age:2Year Owner:Miss Folayemi

HR : 124 bpm
P : 50 ms
PR : 119 ms
QRS : 57 ms
QT/QTc : 199/286 ms
P/QRS/T : 75/74/264 deg.

Diagnose:

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/03/02 04:47 Print:2019/07/17 20:56

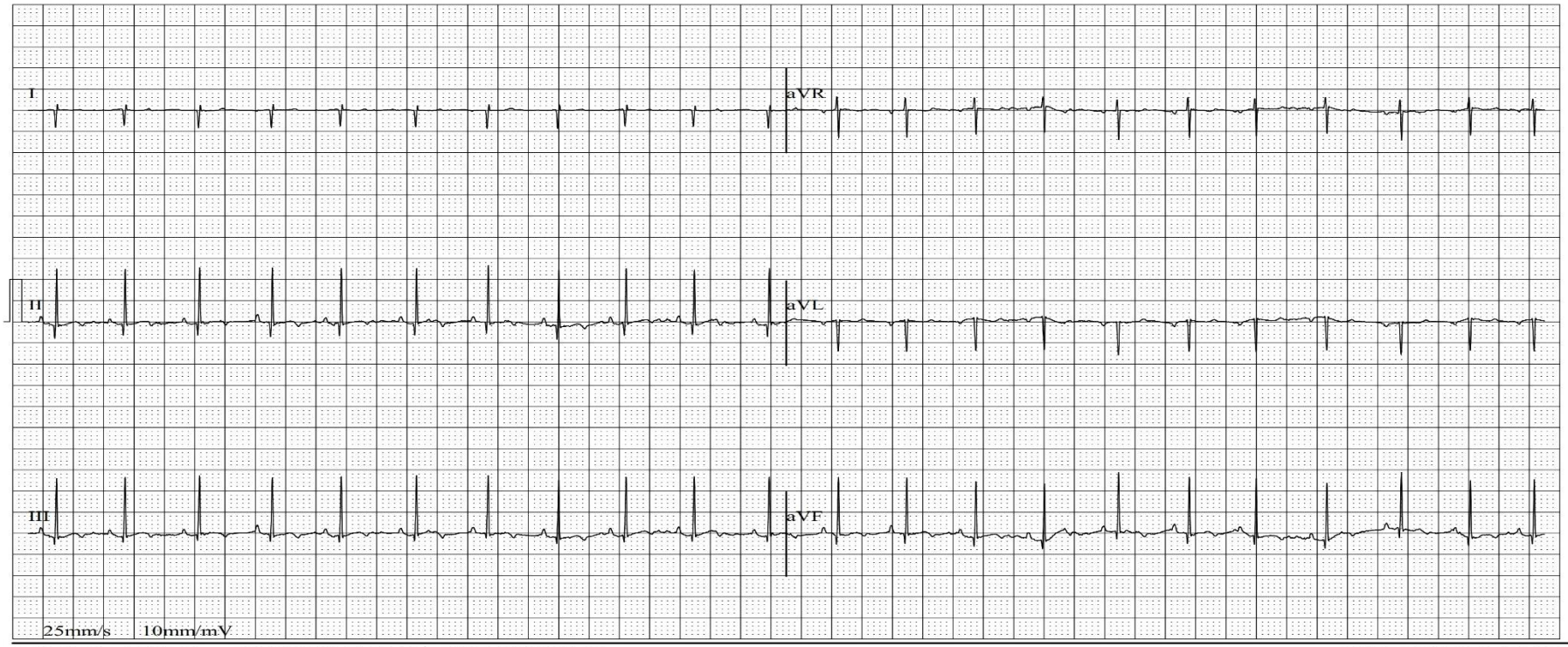
VET PC ECG2.0 SEMIP1.5

6 lead ECG Report

ID:201703020000 Name:billy Sex:Male Age:3Month Owner:Mr Chigbundu

HR : 128 bpm Diagnose:
P : 61 ms
PR : 106 ms
QRS : 52 ms
QT/QTc : 199/290 ms
P/QRS/T : 90/104/90 deg.

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/03/02 04:14 Print:2019/07/17 20:56 VET PC ECG2.0 SEMIP1.5

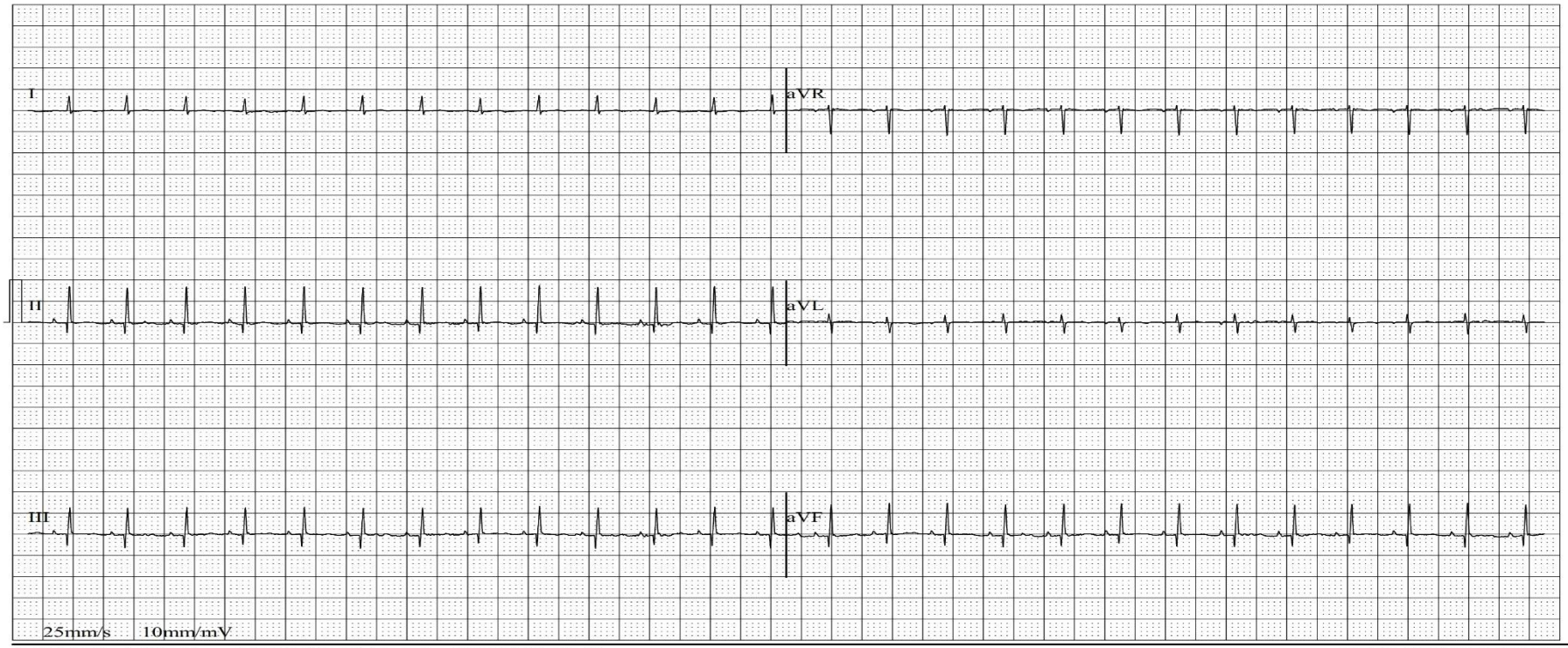
6 lead ECG Report

ID:201704030000 Name:John's pet no name) Sex:Female Age:3Month Owner:Mr. John Oluseyi

HR : 156 bpm
P : 48 ms
PR : 96 ms
QRS : 62 ms
QT/QTc : 314/506 ms
P/QRS/T : 90/73/90 deg.

Diagnose:

Technician:Mr George Ikokoh
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2017/04/03 03:00 Print:2019/07/17 20:55 VET PC ECG2.0 SEMIP1.5

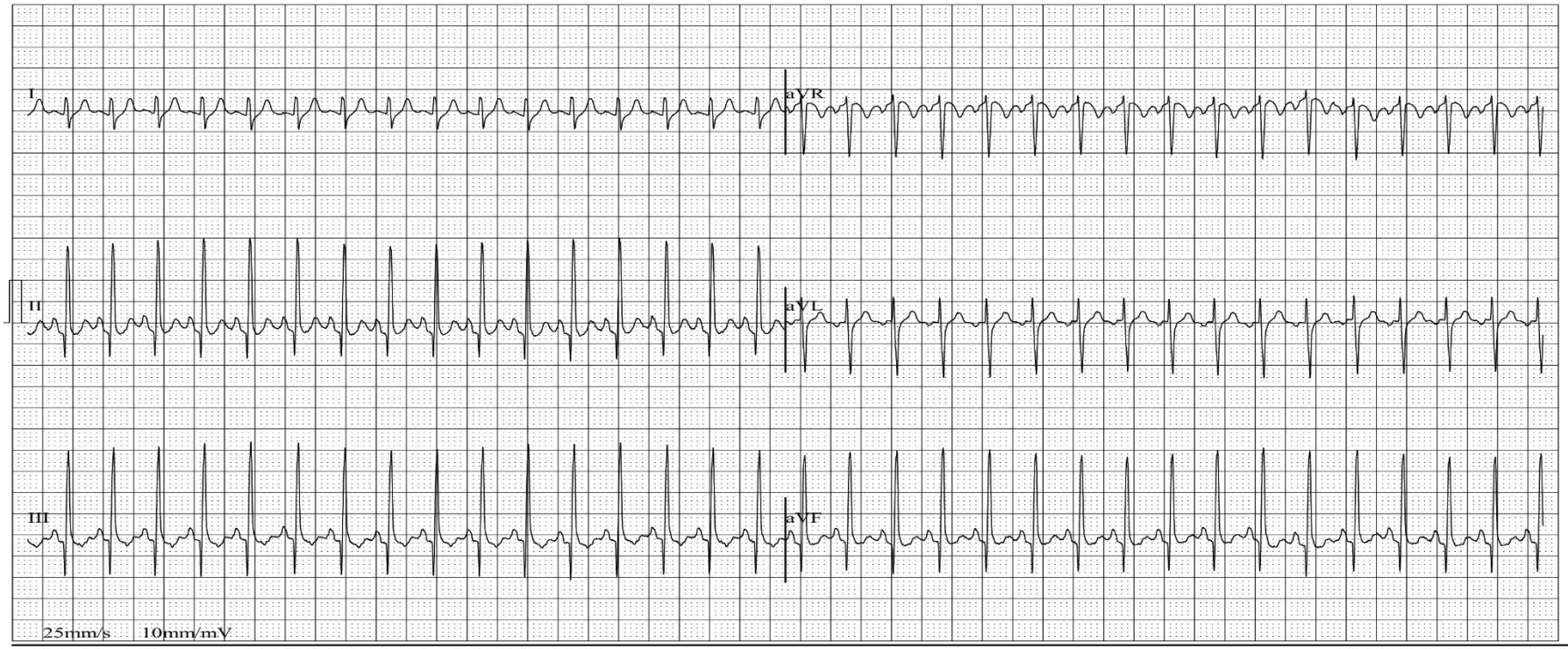
6 lead ECG Report

ID:201901240001 Name:doffy parvo Sex:Male Age:9Month Owner:Mr Ayeleru

HR : 198 bpm
P : 65 ms
PR : 87 ms
QRS : 81 ms
QT/QTc : 247/448 ms
P/QRS/T : 78/87/65 deg.

Diagnose:

Technician:Dr Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/01/24 03:58 Print:2019/07/17 20:54 VET PC ECG2.0 SEMIP1.5

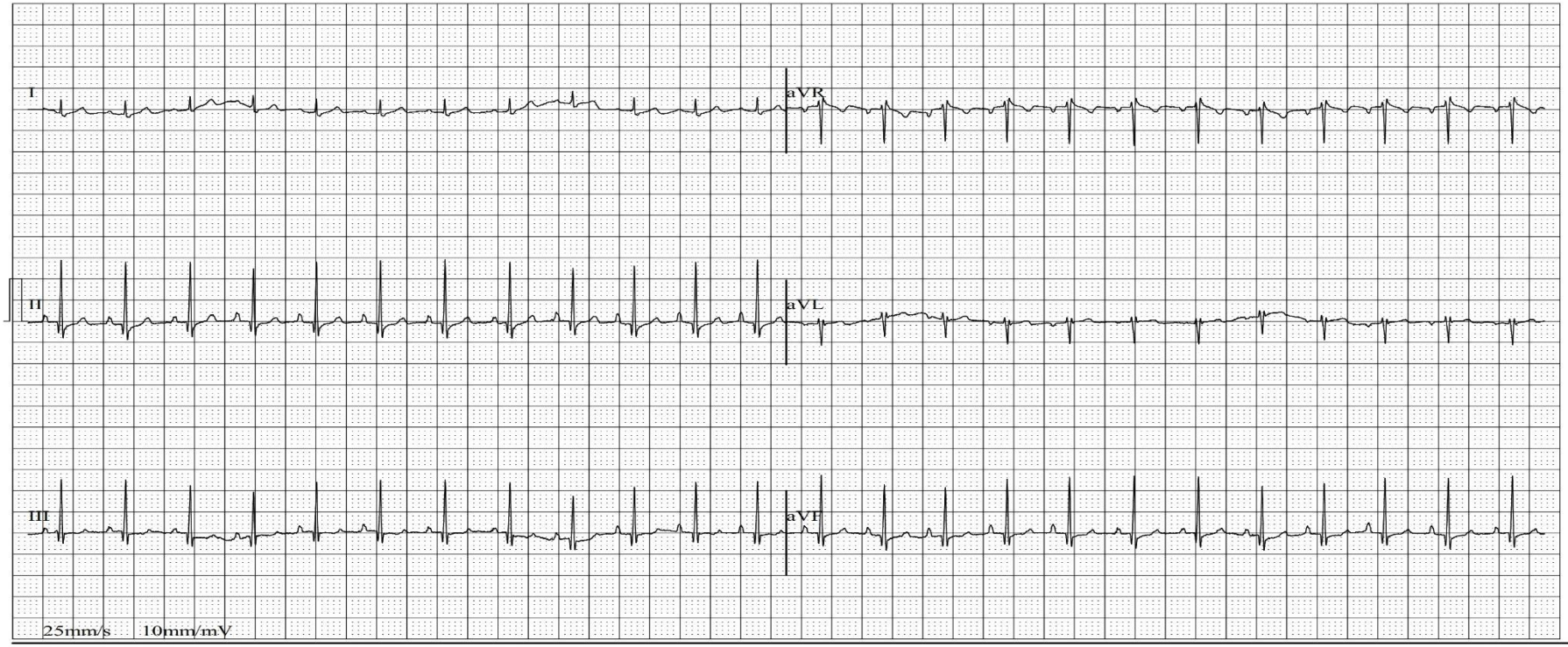
6 lead ECG Report

ID:201901250000 Name:craft parvo Sex:Female Age:10Month Owner:Mr Olaseinde

HR : 144 bpm
P : 55 ms
PR : 108 ms
QRS : 60 ms
QT/QTc : 283/438 ms
P/QRS/T : 58/87/50 deg.

Diagnose:

Technician:Dr Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/01/25 02:57 Print:2019/07/17 20:53

VET PC ECG2.0 SEMIP1.5

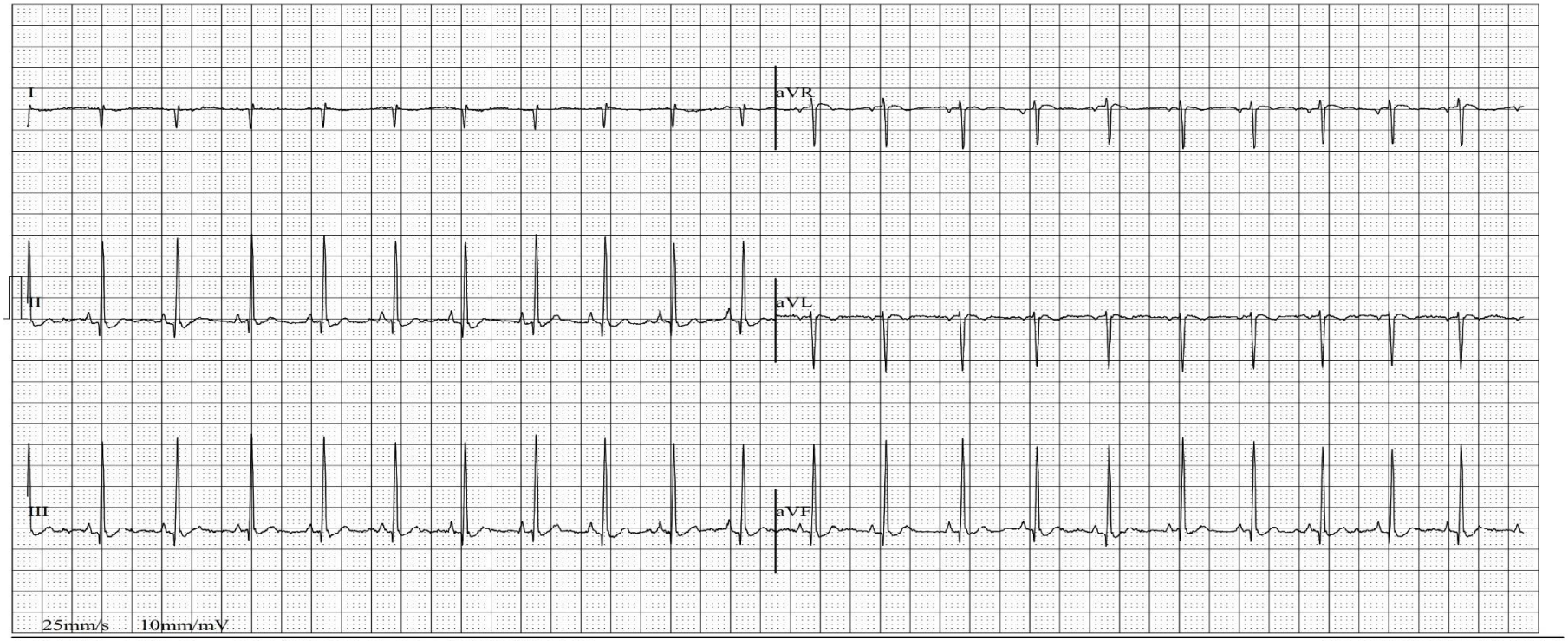
6 lead ECG Report

ID:201901250003 Name:SMART parvo Sex:Male Age:2Year Owner:Mr ADEKUNLE

HR : 125 bpm
P : 55 ms
PR : 94 ms
QRS : 68 ms
QT/QTc : 204/294 ms
P/QRS/T : 80/99/90 deg.

Diagnose:

Technician:Dr Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/01/25 03:10 Print:2019/07/17 20:53 VET PC ECG2.0 SEMIP1.5

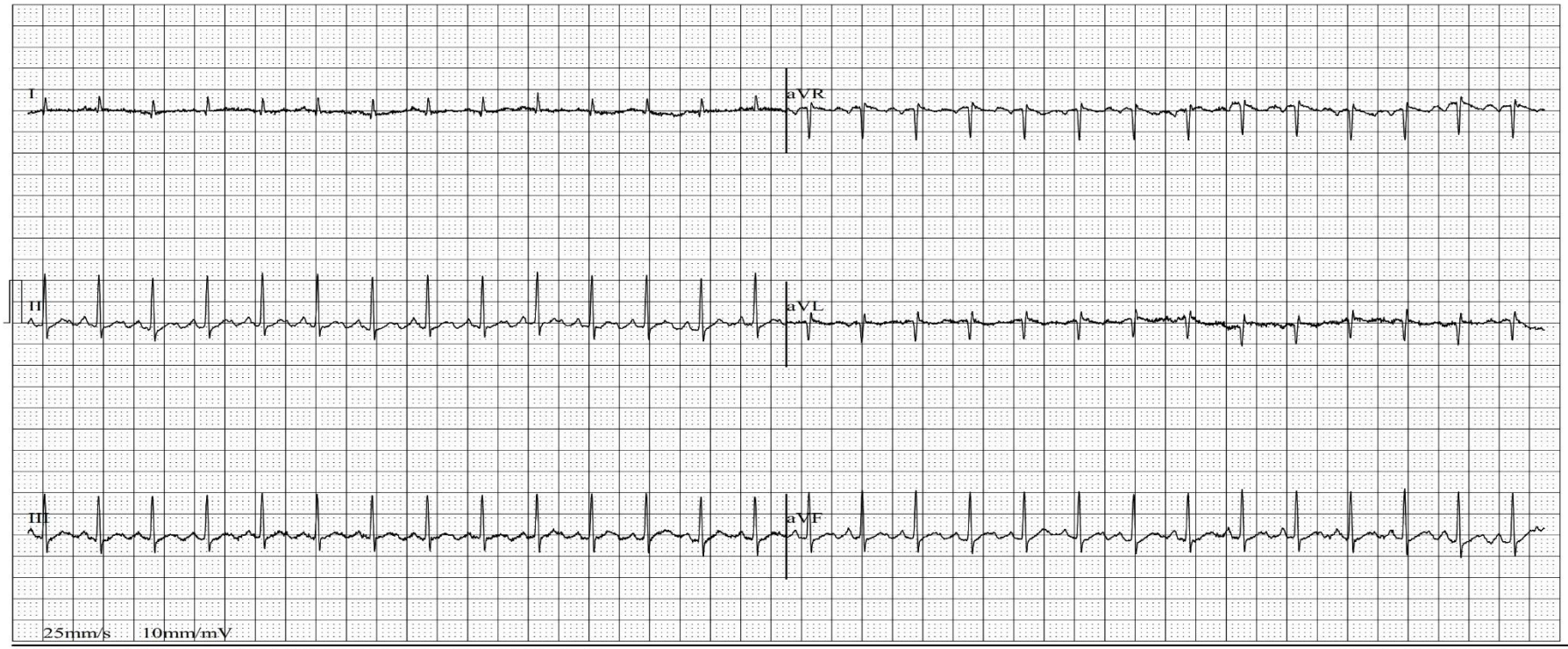
6 lead ECG Report

ID:201901280002 Name:Oscar parvo Sex:Male Age:2Year Owner:Mr GIBSON

HR : 167 bpm
P : 65 ms
PR : 104 ms
QRS : 62 ms
QT/QTc : 305/508 ms
P/QRS/T : 81/80/86 deg.

Diagnose:

Technician:Dr Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/01/28 09:32 Print:2019/07/17 20:52 VET PC ECG2.0 SEMIP1.5

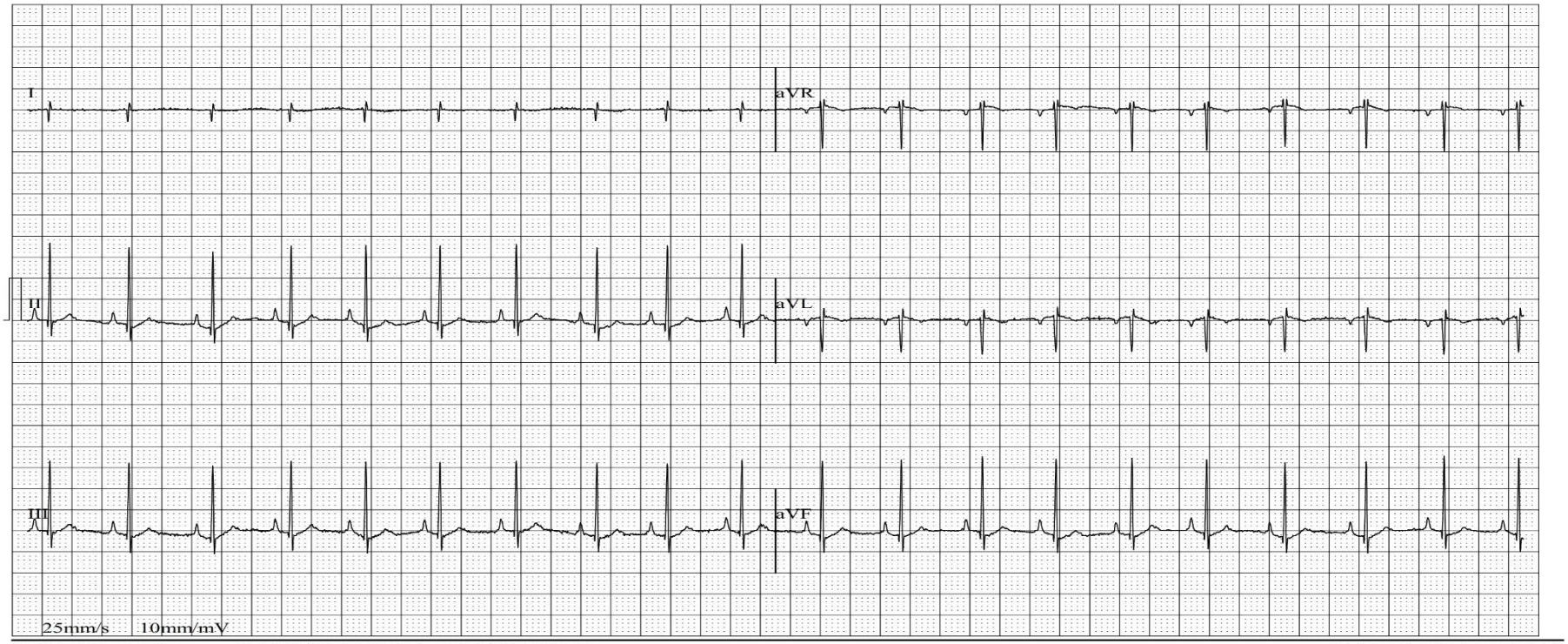
6 lead ECG Report

ID:201901290002 Name:Cooky parvo Sex:Female Age:5Month Owner:Prof. Aiyelero

HR : 116 bpm
P : 63 ms
PR : 112 ms
QRS : 60 ms
QT/QTc : 222/308 ms
P/QRS/T : 90/93/90 deg.

Diagnose:

Technician:Dr Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/01/29 05:51 Print:2019/07/17 20:52 VET PC ECG2.0 SEMIP1.5

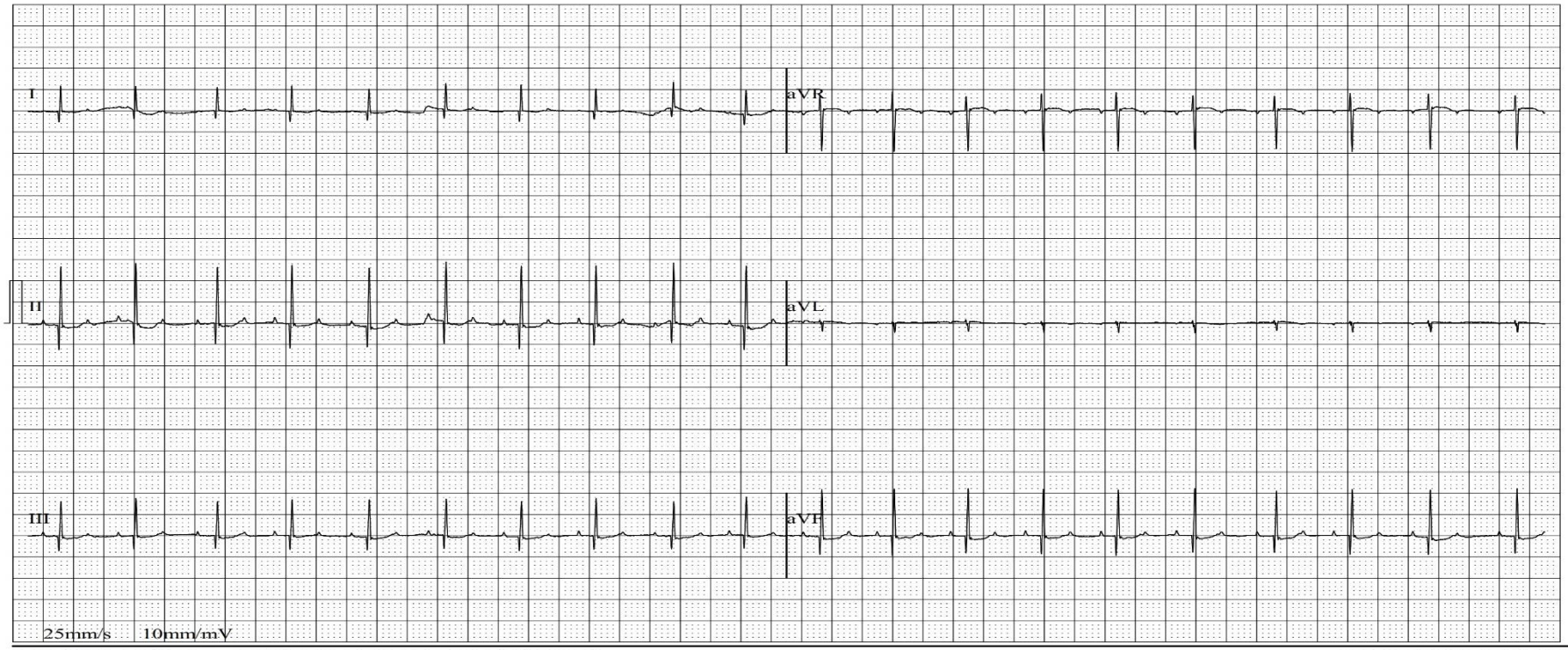
6 lead ECG Report

ID:201901300013 Name:angel boer boel parvo Sex:Female Age:3Month Owner:mr Dare

HR : 119 bpm
P : 52 ms
PR : 116 ms
QRS : 46 ms
QT/QTc : 249/350 ms
P/QRS/T : 76/72/95 deg.

Diagnose:

Technician:Dr Ronke
Physician:Drs Omobowale and Adejun



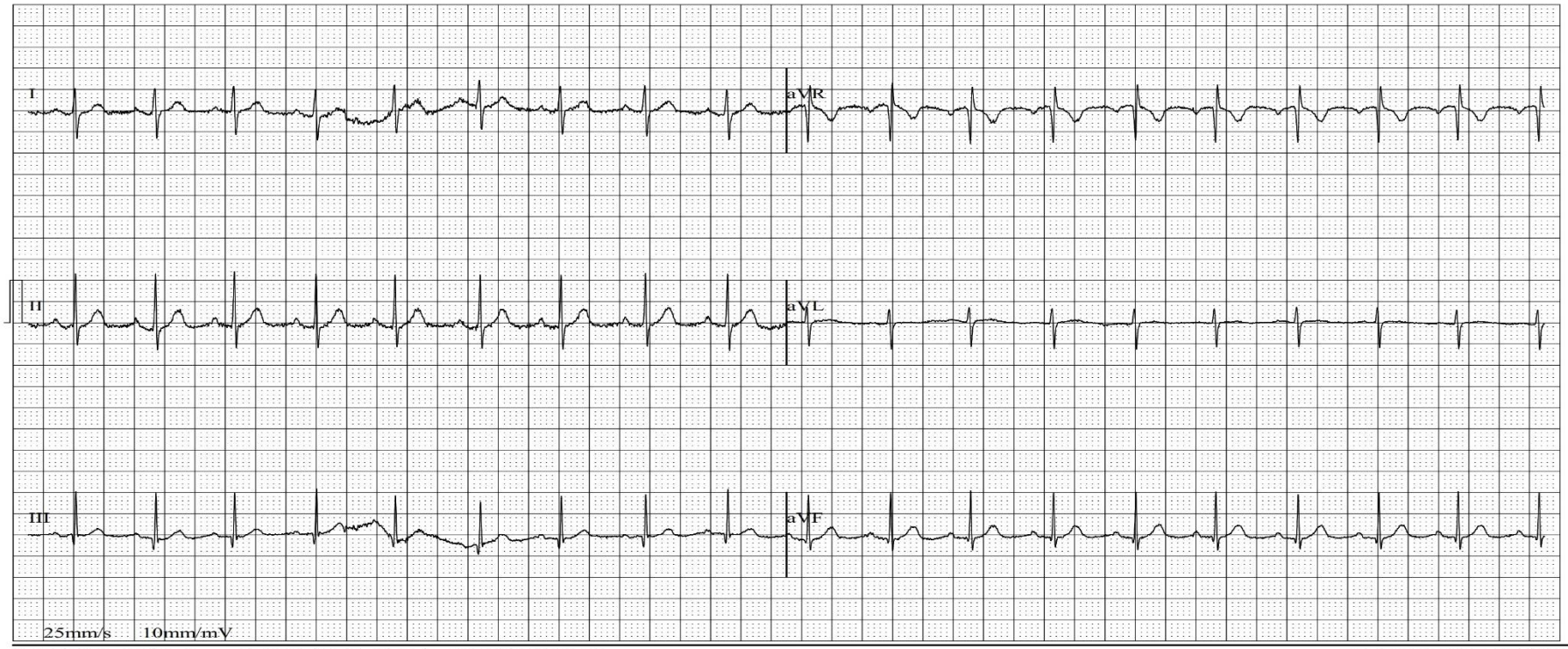
0.67-100Hz AC50 Exam:2019/01/30 04:46 Print:2019/07/17 20:51 VET PC ECG2.0 SEMIP1.5

6 lead ECG Report

ID:201902040005 Name:Brownby Parvo Sex:Female Age:3Month Owner:Mr Adeeko

HR : 111 bpm Diagnose:
P : 79 ms
PR : 134 ms
QRS : 53 ms
QT/QTc : 258/350 ms
P/QRS/T : 52/78/53 deg.

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/02/04 03:13 Print:2019/07/17 21:27 VET PC ECG2.0 SEMIP1.5

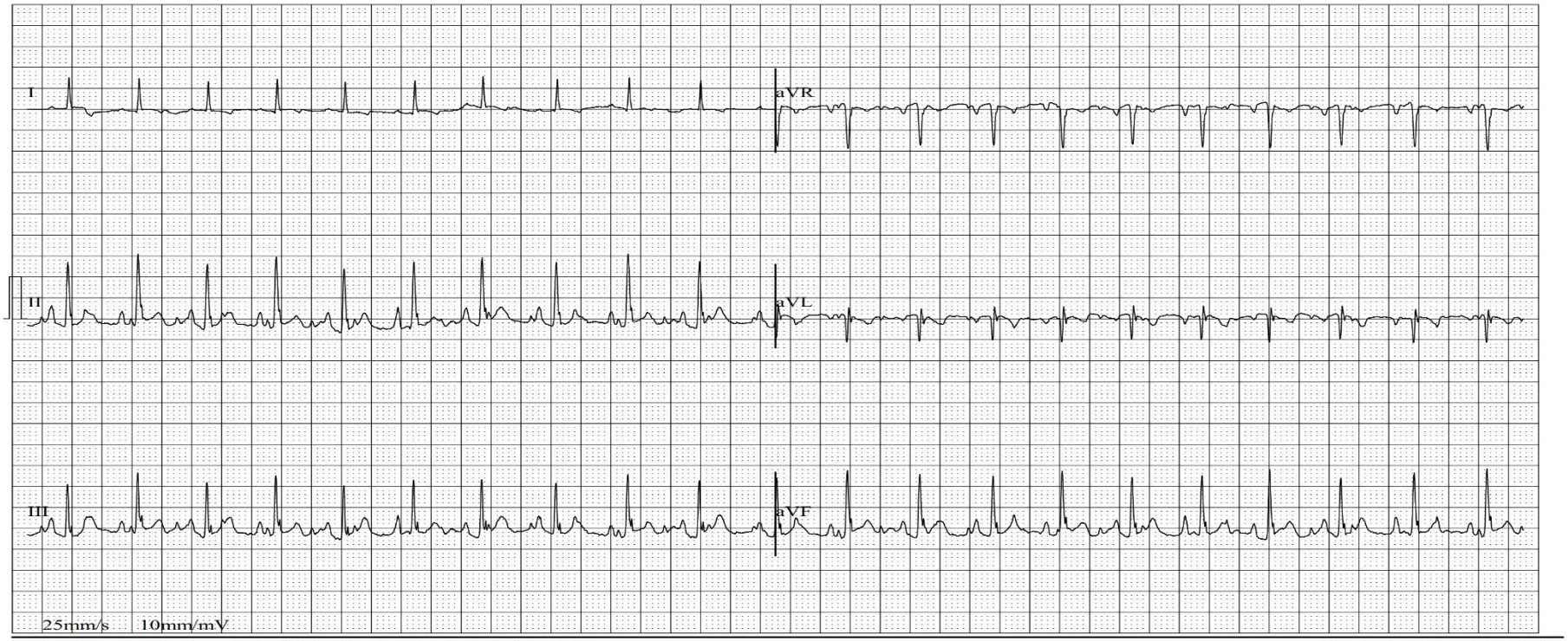
6 lead ECG Report

ID:201902070000 Name:major Sex:Male Age:1Year Owner:MR john

HR : 126 bpm
P : 63 ms
PR : 109 ms
QRS : 84 ms
QT/QTc : 238/344 ms
P/QRS/T : 85/74/96 deg.

Diagnose:

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/02/07 02:00 Print:2019/07/17 21:26

VET PC ECG2.0 SEMIP1.5

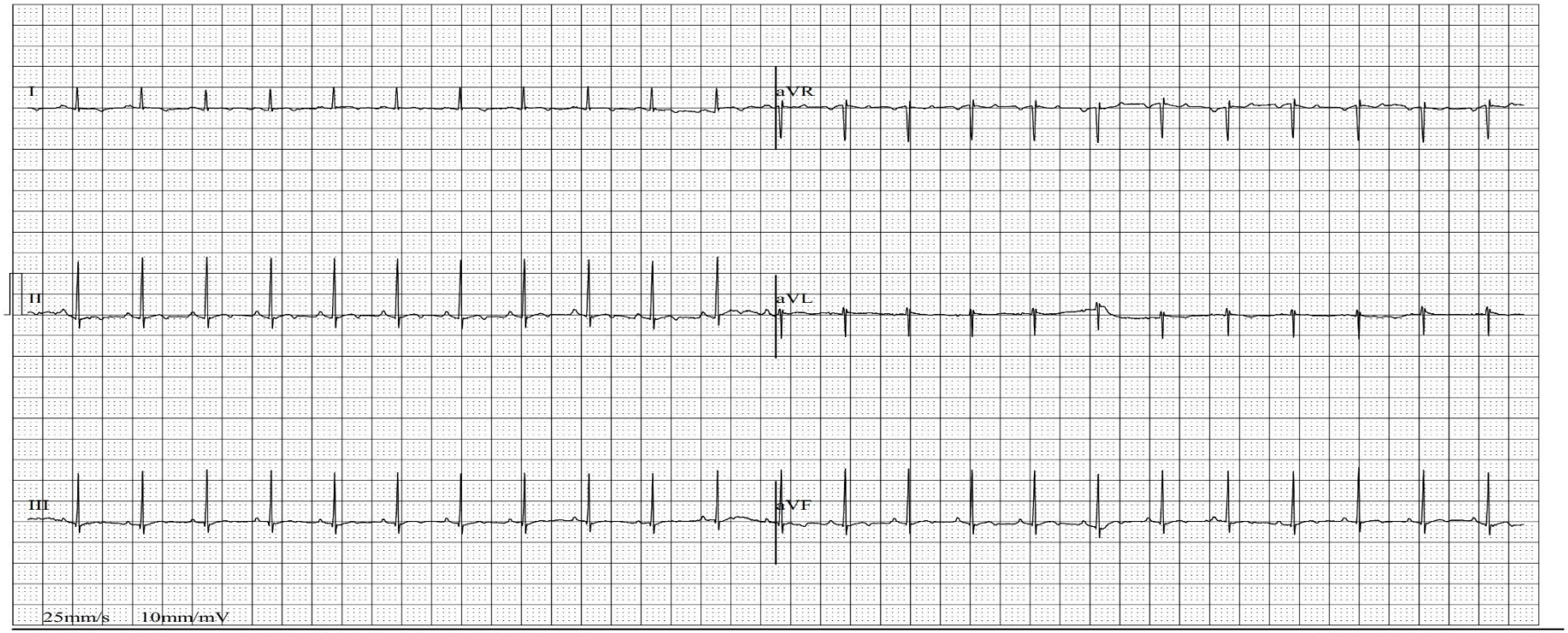
6 lead ECG Report

ID:201902100002 Name:BRUNO PARVO Sex:Male Age:3Month Owner:MRSDEBORA OLOFINTILA

HR : 140 bpm
P : 68 ms
PR : 108 ms
QRS : 47 ms
QT/QTc : 294/449 ms
P/QRS/T : 66/66/112 deg.

Diagnose:

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/02/10 07:15 Print:2019/07/17 21:25 VET PC ECG2.0 SEMIP1.5

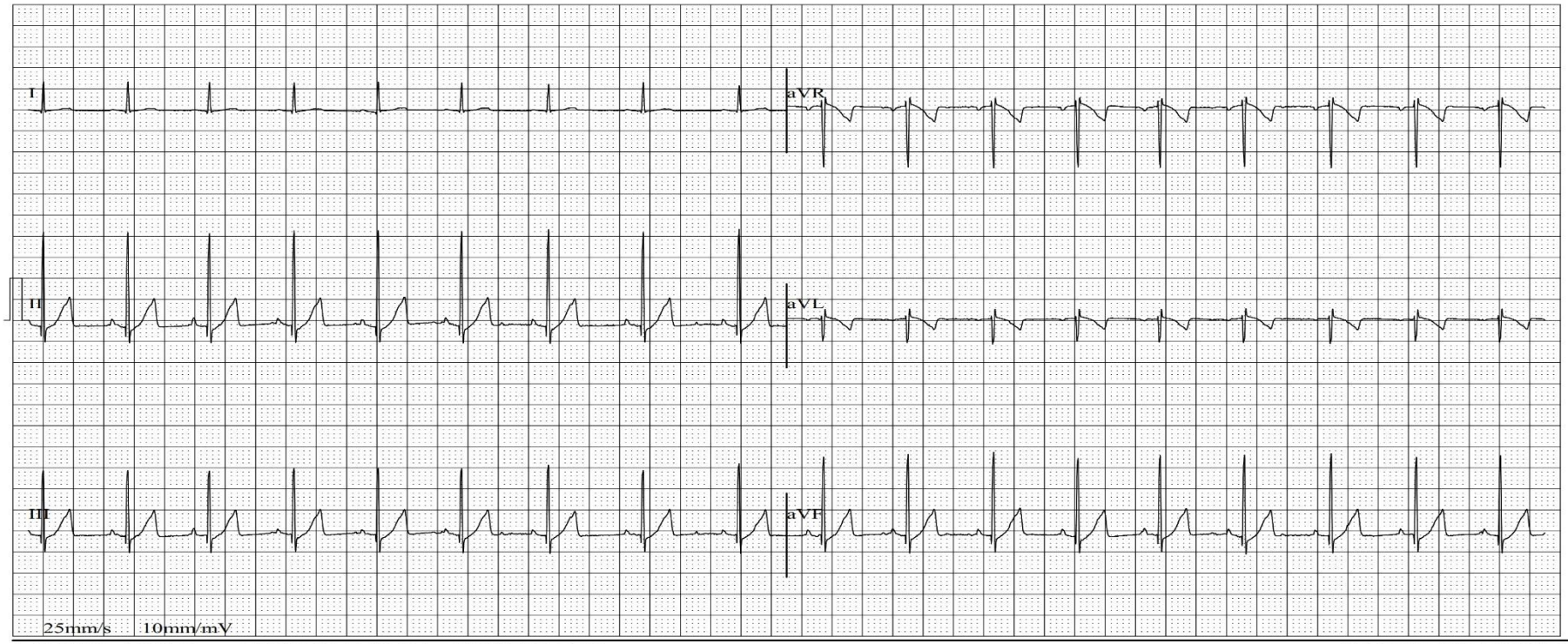
6 lead ECG Report

ID:201902190000 Name:Bella PARVO Sex:Female Age:9Week Owner:Mr Gbenga

HR : 107 bpm
P : 63 ms
PR : 110 ms
QRS : 47 ms
QT/QTc : 230/307 ms
P/QRS/T : 78/73/83 deg.

Diagnose:

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/02/19 03:52 Print:2019/07/17 21:25 VET PC ECG2.0 SEMIP1.5

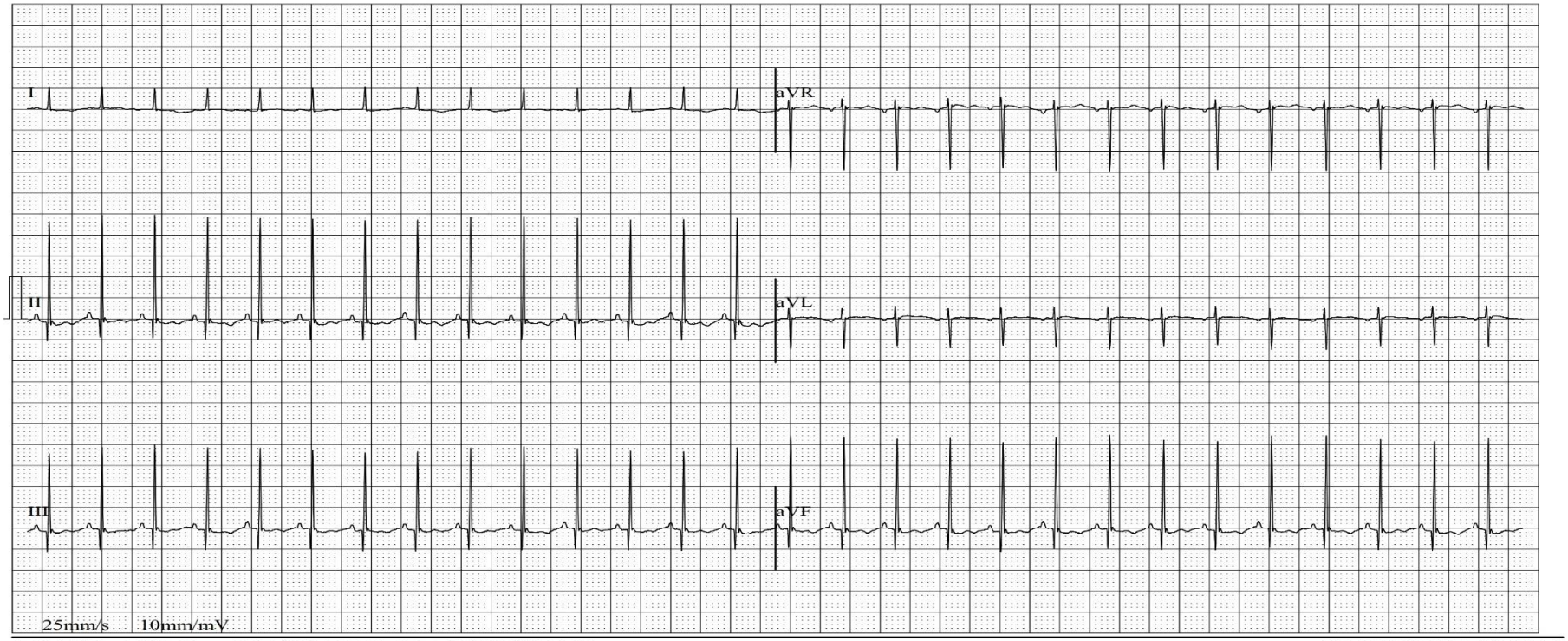
6 lead ECG Report

ID:201902190001 Name:SPARKLE PARVO Sex:Female Age:11Week Owner:DR BABALOLA

HR : 168 bpm
P : 63 ms
PR : 90 ms
QRS : 37 ms
QT/QTc : 303/507 ms
P/QRS/T : 90/76/90 deg.

Diagnose:

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/02/19 03:58 Print:2019/07/17 21:24

VET PC ECG2.0 SEMIP1.5

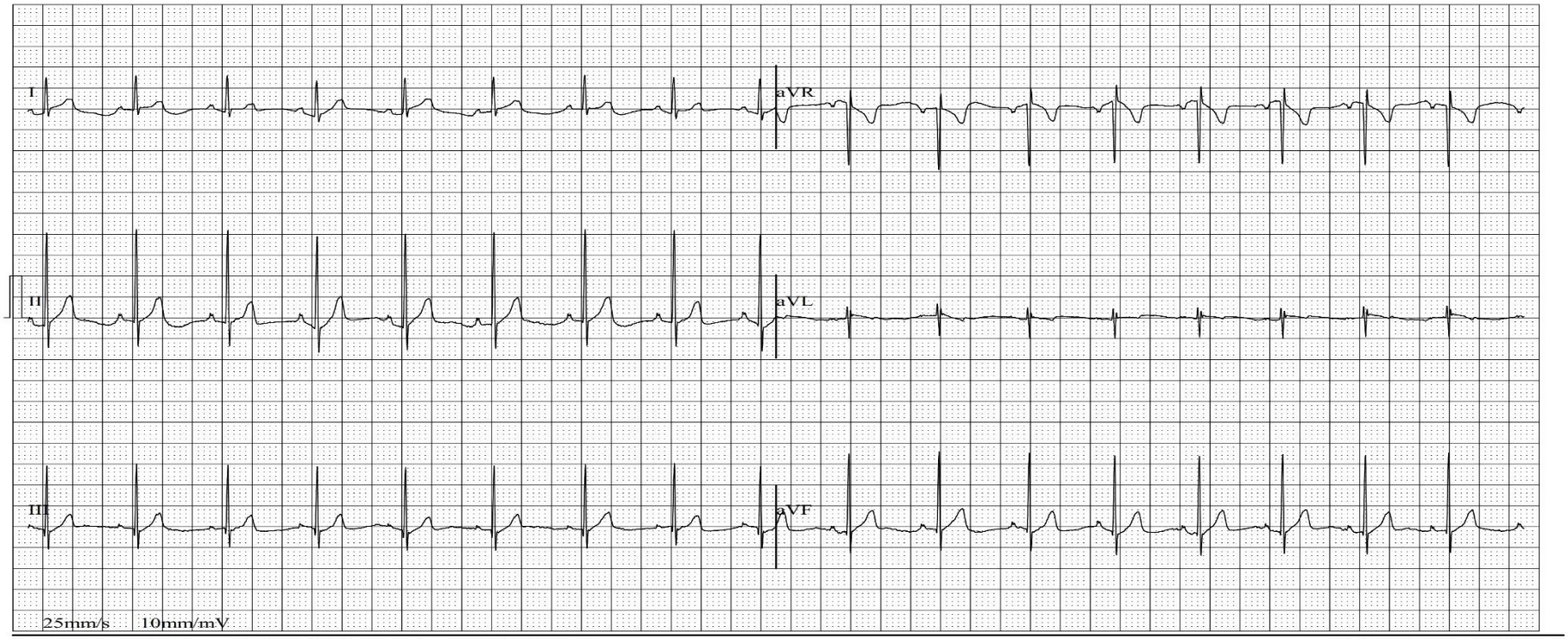
6 lead ECG Report

ID:201902190002 Name:KING PARVO Sex:Female Age:9Month Owner:DR BABALOLA

HR : 102 bpm
P : 69 ms
PR : 123 ms
QRS : 48 ms
QT/QTc : 231/301 ms
P/QRS/T : 51/59/57 deg.

Diagnose:

Technician: Ronke
Physician:Drs Omobowale and Adejum



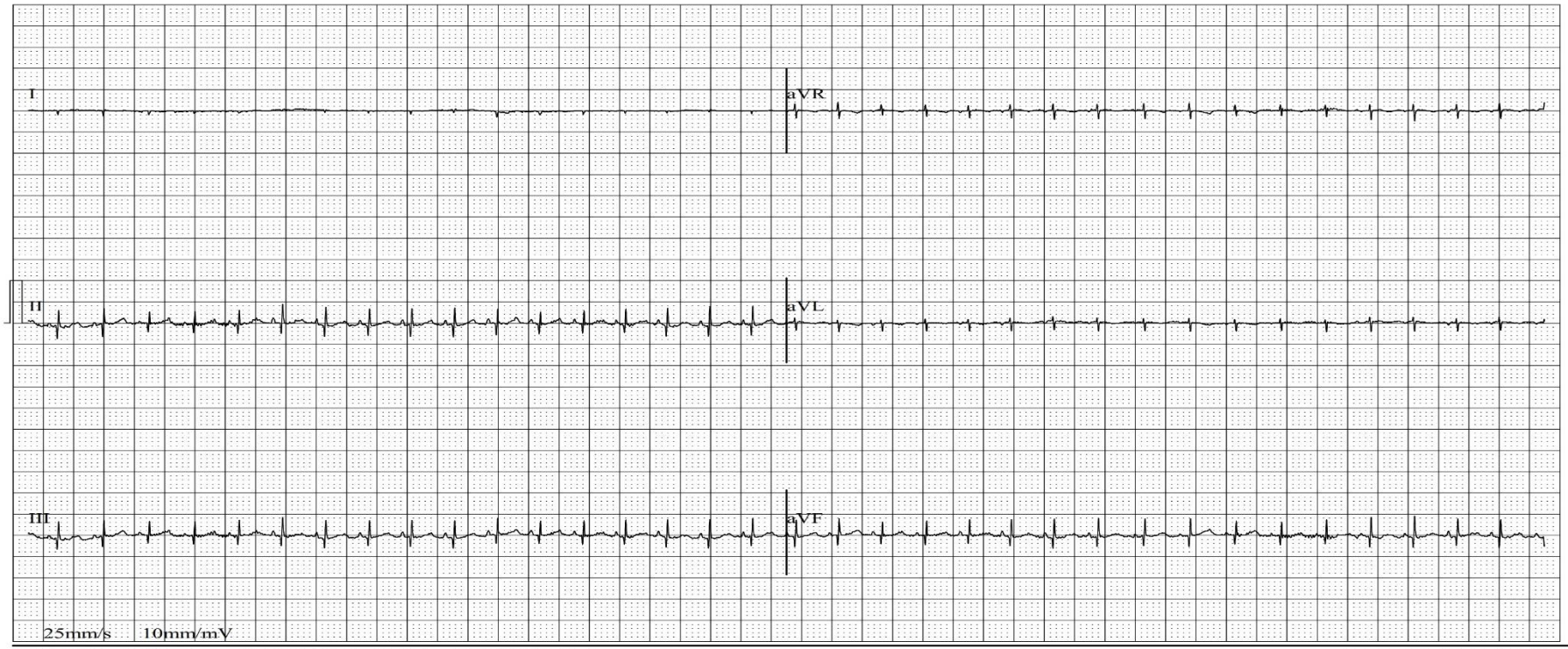
0.67-100Hz AC50 Exam:2019/02/19 04:07 Print:2019/07/17 21:24 VET PC ECG2.0 SEMIP1.5

6 lead ECG Report

ID:201902210002 Name:puppy B Sex:Female Age:5Week Owner:prof. Fadare

HR : 209 bpm Diagnose:
P : 0 ms
PR : 68 ms
QRS : 167 ms
QT/QTc : 182/339 ms
P/QRS/T : 90/90/90 deg.

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/02/21 02:41 Print:2019/07/17 21:22 VET PC ECG2.0 SEMIP1.5

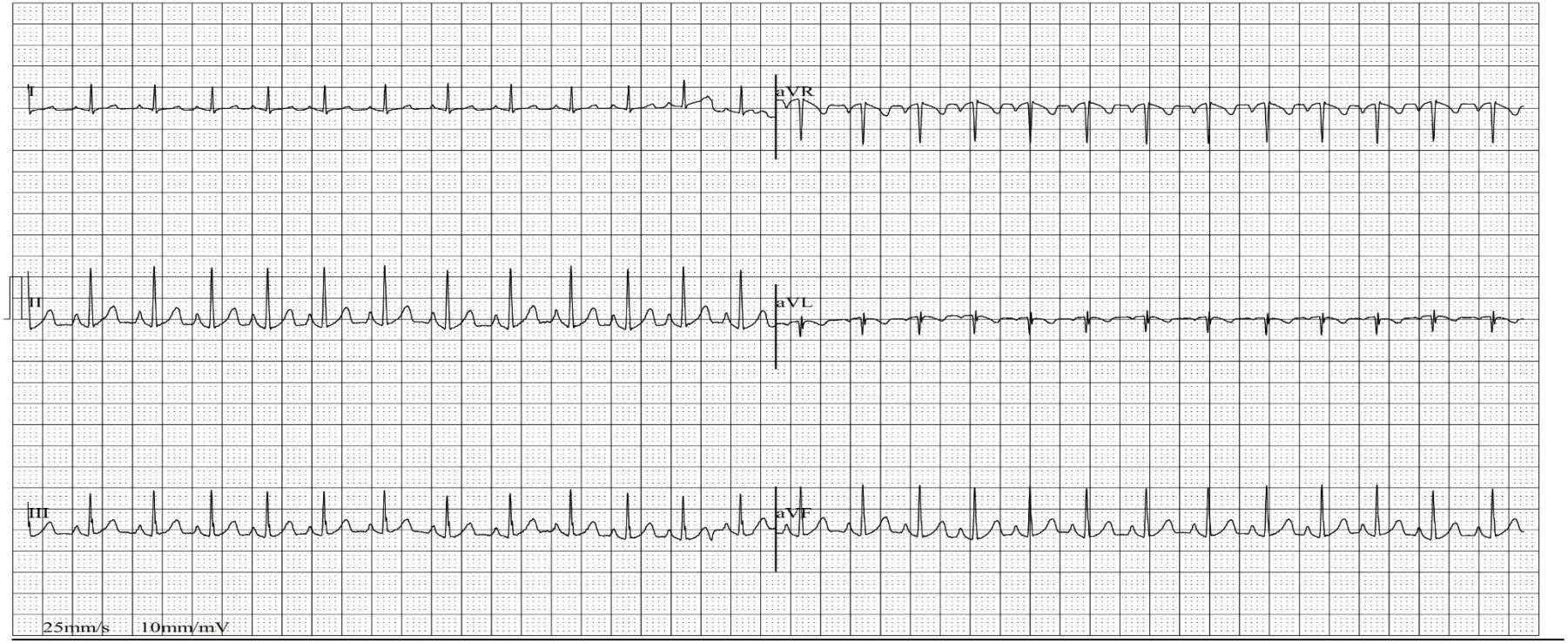
6 lead ECG Report

ID:201902250001 Name:Chika Sex:Male Age:8Week Owner:Mr Olorode

HR : 153 bpm
P : 68 ms
PR : 98 ms
QRS : 65 ms
QT/QTc : 231/368 ms
P/QRS/T : 75/76/79 deg.

Diagnose:

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/02/25 03:30 Print:2019/07/17 21:22

VET PC ECG2.0 SEMIP1.5

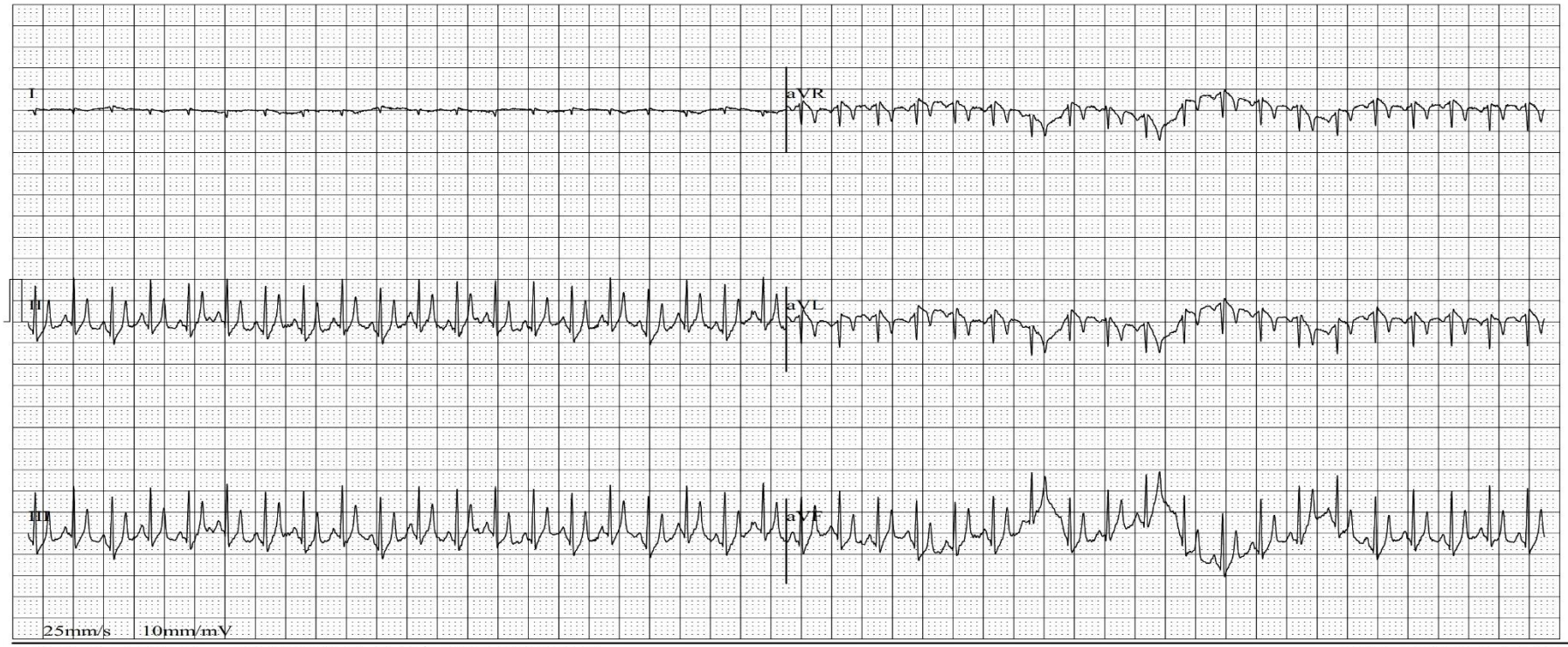
6 lead ECG Report

ID:201903150002 Name:Rocket parvo Sex:Male Age:6Year Owner:DR FADARE

HR : 238 bpm
P : 47 ms
PR : 92 ms
QRS : 156 ms
QT/QTc : 214/426 ms
P/QRS/T : 90/106/90 deg.

Diagnose:

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/03/15 04:40 Print:2019/07/17 21:21 VET PC ECG2.0 SEMIP1.5

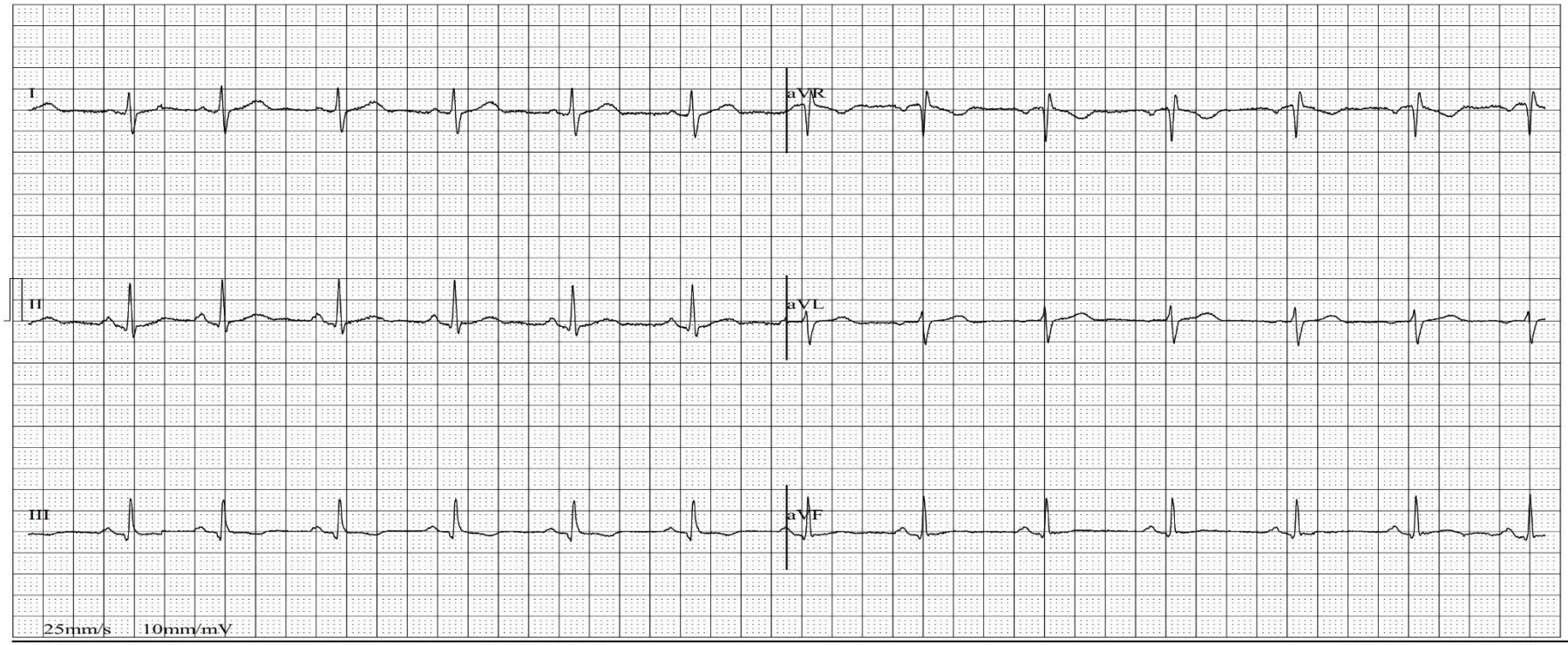
6 lead ECG Report

ID:201903150004 Name:WILLEA parvo Sex:Female Age:4Month Owner:MR AJUWON

HR : 76 bpm
P : 112 ms
PR : 159 ms
QRS : 90 ms
QT/QTc : 366/411 ms
P/QRS/T : 67/96/24 deg.

Diagnose:

Technician: Ronke
Physician:Drs Omobowale and Adejun



0.67-100Hz AC50 Exam:2019/07/06 10:21 Print:2019/07/17 21:20 VET PC ECG2.0 SEMIP1.5

Appendix 11: CPV sequences from vaccine and clinical cases analysed

>NGA_Abj1 (Accession #: MT198664)
TAACTTTGGTTATATAGGAGTTCAACAAGATAAAAGACGTGGTGTAACTCAAATGGGAAAC
ACAAACATTATTACTGAAGCTACTATTATGAGACCAGCTGAGGTTGGTTATAGTGCACCAT
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