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Review Article

Molecular Epidemiology of Canine Parvovirus in Nigeria

Adeyemo A.A.[1], Aiki-Raji C.O.[1], Akinniyi O.O.[2] and *Fagbohun O.A[1]

Departments of [1]Veterinary Microbiology and [2]Veterinary Medicine,
Faculty of Veterinary Medicine, University of Ibadan. Ibadan, Nigeria

ABSTRACT

The emergence of canine parvovirus (CPV) in 1978, probably as a result of the cross-species incursion of feline panleukopenia virus, resulted in the current pandemic of canine parvoviral enteritis. It has been 40 years since the virus was first identified in Nigeria and it has been afflicting dog population in the country unabatedly. As such, in this review, CPV molecular epidemiology in Nigeria entailing its prevalence, occurrence of subtypes, co-infection and genetic evolution are analysed. All the three subtypes of the virus have been identified in the country with CPV-2a subtype being preponderant. However, in recent years there has been an upsurge in the number of CPV-2c and it is often associated with bloody diarrhoea even in vaccinated puppies. Therefore, there is need for proper assessment of the molecular epidemiology of the virus for proper institution of effective control policies to eradicate this pathogen.

Keywords: *Canine Parvovirus; Dogs; VP2 gene; Epidemiology; Nigeria*.

**Author for correspondence: Email: oa.fagbohun@ui.edu.ng; Tel: +234-8107128206*

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INTRODUCTION

Dogs are the cardinal companion animals in Nigeria, aside being pets in some urban areas; some are used as guard dogs whereas in rural areas they may be used for hunting. A very important infectious disease which poses a high risk to the survival of dogs in Nigeria is canine parvoviral enteritis. The disease is acute and often fatal and it is characterized by a sudden onset of vomiting, diarrhoea (often haemorrhagic), fever, anorexia, progressive dehydration, depression and sometimes myocarditis (Goetschius *et al*., 2021). The disease is cosmopolitan in distribution and although all ages of dogs are susceptible, puppies are more susceptible and show higher mortality figures. Puppies may also present with myocarditis without showing signs of gastroenteritis (Goddard and Leisewitz, 2010).

The aetiological agent of canine parvoviral enteritis is Canine parvovirus (CPV), also known as Carnivore protoparvovirus 1. It is a 27 nm in diameter, resistant, non-enveloped virus which belongs to the family Parvoviridae, subfamily Parvovirinae, and genus Protoparvovirus (Cotmore *et al*., 2014; Cotmore *et al*., 2019). It is a DNA virus with a single-stranded genome of about 5200 nucleotides, with a pair of open reading frames (ORF), encoding non-structural proteins, NS1 and NS2, and two structural proteins, VP1 and VP2 by alternative mRNA splicing (Decaro and Buonavoglia, 2012; Miranda and Thompson, 2016; Mietzsch *et al*., 2019). The nucleocapsid has a T=1 icosahedral symmetry with the capsid being made of 60 copies of three different proteins (VP1, VP2, and VP3). VP1 contains the full-length VP2 sequence with an extra N-terminal domain and makes up approximately 10% of the capsid protein. VP2 is the most abundant of the capsid proteins, making up about 90%. VP2 is also the major determinant of the virus' host range and its manner of interaction with host cells; VP3 is cleaved from VP2 by host proteases (Tsao *et al*., 1991; Decaro and Buonavoglia, 2012). The 3-dimensional structure of the CPV virion reveals a long protrusion on the 3-fold axes of the icosahedral formation, a deep depression (canyon) which encircles the five cylindrical structures at the 5-fold axes, and a depression (dimple) at the 2-fold axes (Tsao *et al*., 1991). The virus replicates in rapidly dividing cells and thus its tropism for foetuses, bone marrow, lymphoid tissues and intestinal tissues (Goddard and Leisewitz, 2010).

The emergence of the virus in 1978 is the result of mutations K80R, K93N, V103A, D323N, D375N, N564S and A568G in the feline panleukopenia virus (FPV), which led to

alterations in its receptor and antibody binding sites and a resultant change in host specificity (Parrish *et al*., 1985; Parrish *et al*., 1988; Parrish *et al*., 1991; Hueffer *et al*., 2002; Parrish and Kawaoka, 2005). Other carnivore parvoviruses such as raccoon parvovirus (RPV), blue-fox parvovirus (BFPV) and mink enteritis virus (MEK) are described as FPV-like viruses, may present a virus intermediate between FPV and CPV (Truyen *et al*., 1995; Steinel *et al*., 2001). Since then, CPV has been evolving and even faster than FPV with the substitution rate of 1.7 × 10−4 compared with FPV 9.4 × 10−5 substitutions per site per year (Shackelton *et al*., 2005; Sun *et al*., 2019). Despite this substitution rate, all CPV subtypes have a nucleotide sequence homology of ~99%; as such, the virus has been described as a successive pathogen (Voorhees *et al*., 2019). CPV was originally identified as canine parvovirus type 2 (CPV-2) to differentiate it from the Canine Minute Virus, which was known as Canine parvovirus-1 (CPV-1). The canine minute virus is now designated Carnivore bocaparvovirus 1 instead of CPV-1. Although not antigenically or genetically related to CPV-2, Canine minute virus – which initially was assumed to be non-pathogenic – has been shown to cause neonatal mortality in puppies (Appel *et al*., 1979; Parrish *et al*., 1985; Parrish *et al*., 1988; Parrish *et al*., 1991; Pratelli *et al*., 1999; Tattersall *et al*., 2005).

Five non-synonymous point mutations M87L, I101T, A300G, D305Y, N375D and V55I in the capsid protein, VP2 gene of wild-type CPV resulted in a new subtype of canine parvovirus, which was identified in 1980, and was named CPV-2a. Within a year of its emergence, CPV-2a replaced wild-type CPV as the dominant aetiology of canine parvovirus enteritis worldwide (Parrish *et al*., 1985; Parrish *et al*., 1988; Parrish *et al*., 1991). Further mutations led to the formation of another subtype with N426D substitution on the VP2 capsid protein and was subsequently named CPV-2b (Parrish *et al*., 1991). In 1990, Variants of CPV 2a and CPV 2b with S297A mutation, were recognized and designated as New CPV 2a, and New CPV 2b; they have been reported in various parts of the world, and are progressively replacing the circulating old CPV 2a and CPV 2b variants (Hoelzer and Parrish, 2010; Stucker *et al*., 2012). CPV-2c was first discovered in Italy in the year 2000. It has a D426E substitution on the VP2 capsid protein (Buonavoglia *et al*., 2001) (Fig. 1).

Fig. 1:
A schematic diagram detailing canine parvovirus evolution.

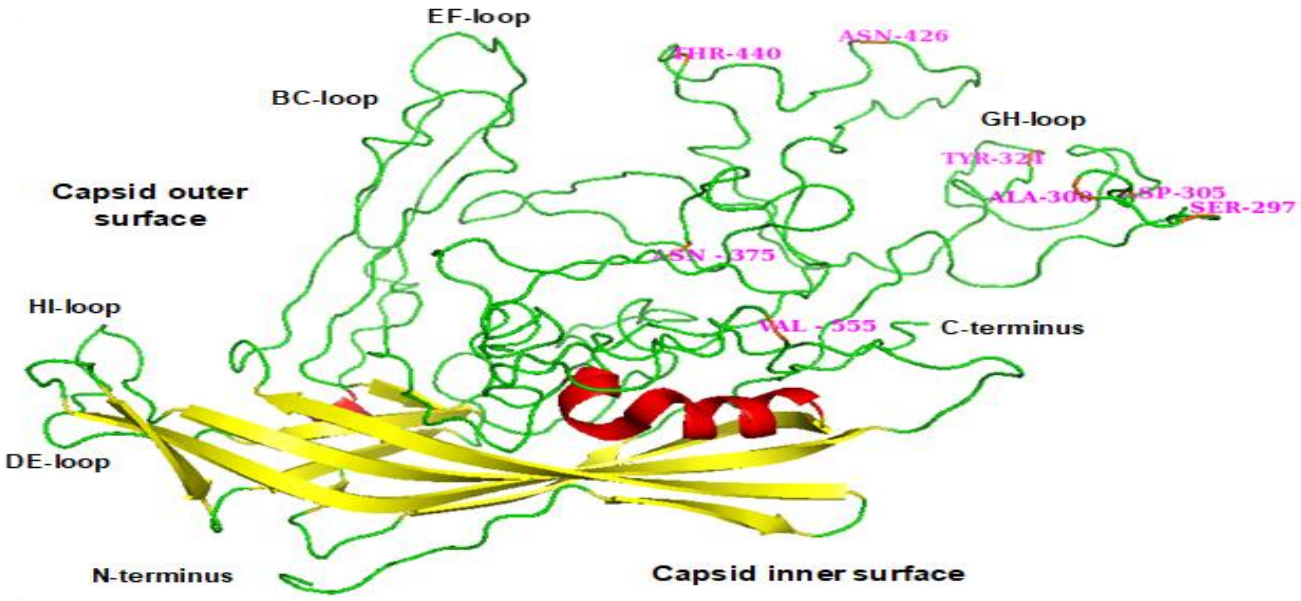

Figure 2:
A cartoon rendering of the dimensional structure of a CPV-2a VP2 protein (PDB-ID: 2CAS). The beta sheets, α-helix strand and interconnecting surface loops are depicted in yellow, red and green, respectively. Residues labelled in magenta are the regions prone to mutations. This image was manipulated in PyMOL.

The polypeptide chain of the VP2 protein resides as an antiparallel arrangement of 8 β strands named βB, βC, βD, βE, βF, βG, βH, and βI. These are arranged into 2 β sheets BIDG and CHEF with strands βB, βC, βD, and βE bonded by hydrogen bond to strands βI, βH, βG, and βF respectively to form a β barrel. There are 2 α helixes between strands βC and βD, and βE and βF. the other β strands are connected by loops

There is a prominence, in the region of the 3-fold axis, known as the 3-fold spike, and another cylindrical structure around the 5-fold axis. There is a depression between the 3-fold spikes in the region of the 2-fold axis; this depression is smaller than another depression, the canyon, in the region of the 5-fold axis, which encircles the cylindrical structure. These two depressions are separated by a slightly raised wall known as the 2-/5-fold wall (Tsao *et al*., 1991; Agbandje and Kleinschmidt, 2011; Mietzsch *et al*., 2019).

Langeveld *et al* (1993) mapped 10 antigenic sites on the VP2 capsid protein and their respective locations on the surface of the CPV capsid. Amino acid residues at positions 91, 92, 93, 95 on the 3-fold spike region; 292-297 on part of the canyon ridge; 297-303, 305, 307-309 on the wall of the dimple (2-fold depression); 507-509 on the ridge of the canyon; 550-552, 554-557, 573-575, 594 in the dimple region of the 2-fold axis (although unable to bind antibodies).

Strassheim *et al* (1994) was able to narrow down on residues 426, 222, 224, 93, 299, 300, and 302 as being antigenic. They also found changes A300D and I301V in an antigenically variant mutant CPV (by extended passage) which was unable to grow in canine cells or dogs. Furthermore, residues 300 and 305 together were determinants for an epitope found in CPV-2a and 2b, although 305 alone did not give the epitope. Changes in residues 375 and 103 of VP2 did not affect antigenicity as they are not exposed on the capsid surface (Chang *et al*., 1992).

Residues 93, 299, 301 have been shown to form the transferrin footprint in the 2-/5-fold wall (a raised region on the capsid surface that separates the 2-fold and 5-fold depressions) (Hueffer *et al*., 2003; Hafenstein *et al*., 2007). The 2-fold depression is the site for glycan receptor interaction; the 2-/5-fold wall is involved in receptor and antibody interaction (Agbandje and Kleinschmidt, 2011).

Two amino-acid residues of major importance in the successful host shift of CPV was at positions 93 and 323 of VP2, which gave it the ability to bind canine transferrin receptor (Parrish and Kawaoka, 2005). Other residues of importance in this host shift include 80, 564, 568 which are found in the shoulder region of the 3-fold spike (Truyen *et al*., 1994; Parrish and Kawaoka, 2005). Also, CPV-2a which, unlike wild-type CPV, is capable of infecting cats has residue changes at positions 87, 300 and 305, which are also found in the shoulder region of the 3-fold spike (Parrish *et al*., 1985; Parrish *et al*., 1988; Truyen *et al*., 1996; Parrish *et al*., 1991). It has also been found that residue changes G299E or A300D, also found in the shoulder region of the 3-fold spike, prevents CPV from binding to canine transferrin receptor (Llamas-Saiz *et al*., 1996; Lukashov and Goudsmit, 2001). Amino acid residues at positions 375, 377, 562, 564 and 568 are important which form the surface of the viral capsid. The largest of these loops, the GH loop, is located between the βG and βH strands, and forms the 3-fold spike (Fig. 2). The DE loop between the βD and βE strands, together with 5 of the β strands forms part of the cylindrical structure around the 5-fold spike. The β barrel is internally situated within the capsid structure (Tsao *et al*., 1991; Xie and Chapman, 1996; Mietzsch *et al*., 2019). for its ability to hemagglutinate (Parrish *et al*., 1988). Based on their position within the capsid structure, residue positions 75-108, 211-248, 273-461 make up the 3-fold spike; 154-167 make up the 5-fold cylindrical structures; 539-584 make up the 2-fold depressions; 38-74, 109-153, 168-210, 244-272, 462-538 make up the β barrel which is normally occluded except at the canyon (Tsao *et al*., 1991).

Canine parvovirus in Nigeria: The first report of canine parvovirus in Nigeria can be traced back to the 1980s. Serological identification of the virus in Nigeria was reported in 1981 (Ezeokoli *et al*., 1985; Kamalu, 1985) while its first molecular identification was done by Chollom *et al* (2013). Studies have been carried out in parts of the country to detail the epidemiology of canine parvovirus enteritis and a reasonable number of these studies have been carried out to better characterize the subtypes of the virus circulating in the country. Since its emergence, CPV has shown its immense ability to adapt in the face of host immunity and has remained endemic in dog populations worldwide, a testament to its relatively high substitution rate which is more like an RNA-virus than a DNA-virus.

Epidemiology

Incidence, prevalence and mortality rate: The incidence and prevalence of canine parvovirus in Nigeria vary with different locations, the prevalence appears to range from 6.4% to 61%. The lowest prevalence recorded was 6.4% in Ilorin, Kwara State (Daodu and Ajiboye, 2018); the highest, 61%, was recorded for Ibadan, Oyo state in South-Western Nigeria (Adejumobi *et al*., 2017). These prevalence figures were obtained from retrospective studies, which only gives figures based on records. There are no annual prevalence reports by individual clinics or state veterinary services to provide a clearer period prevalence. The case mortality rate reported was between 72% to 80.6% (Ukwueze *et al*., 2018; Ogbu *et al*., 2020).

Age distribution: The highest prevalence of canine parvovirus enteritis was found in dogs aged 0-7 months old (Fig. 2). For this age range, the prevalence was between 28.75% and 80% (Adejumobi *et al*., 2017; Ogbu *et al*., 2020). This is similar to reports from other parts of the world (Goddard and Leisewitz, 2010; Pinto *et al*., 2010). Older dogs (i.e., 12 months and above) were least represented in these studies since they are most likely to be exposed to the virus either by vaccination or by infection and recovery and have developed immunity (Plate 1).

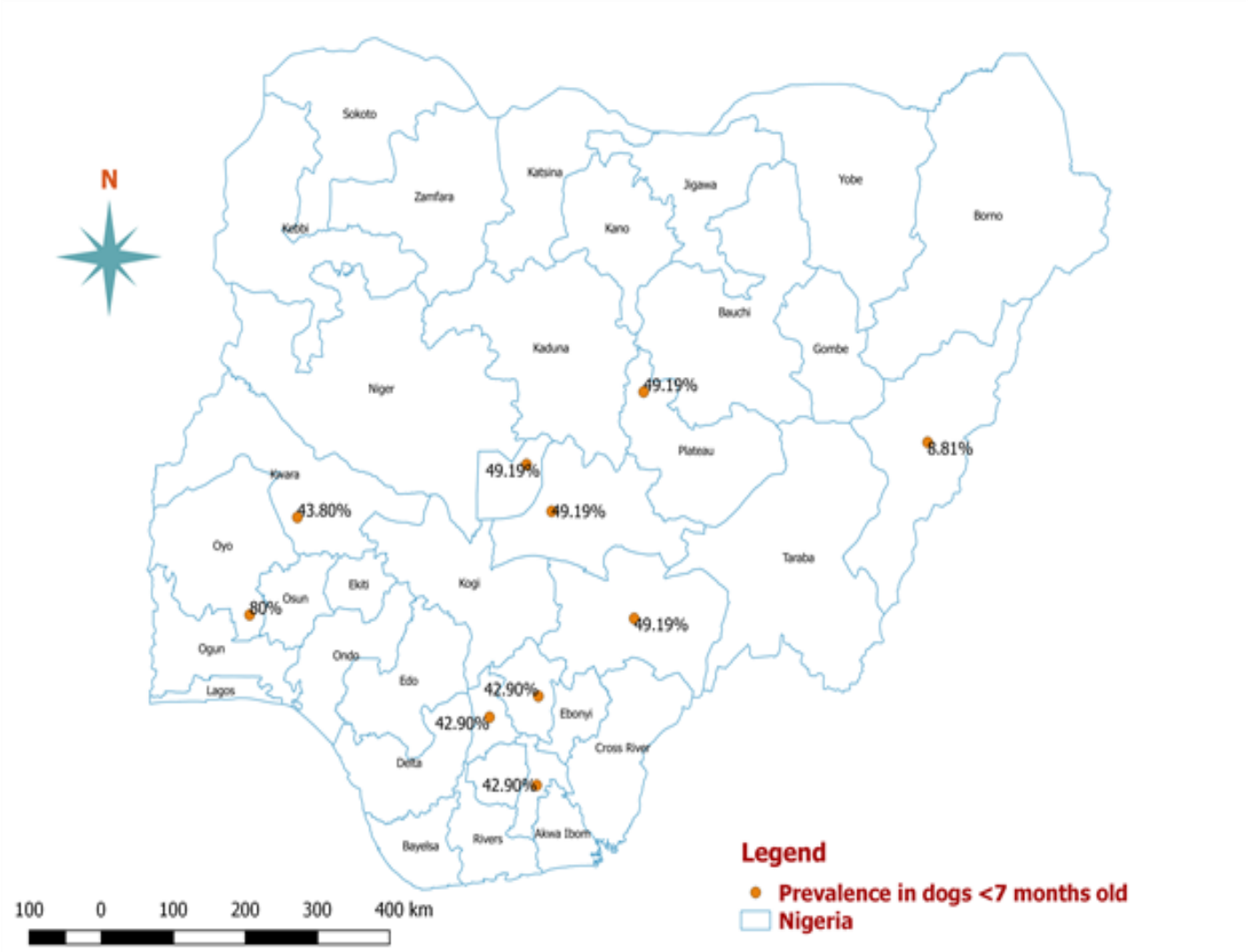

Plate 1:
Prevalence for dogs <7 months old in some states, based on available data.

Sex and breed distribution

The distribution of cases of parvovirus enteritis based on the sex of the dog was largely insignificant, although some studies showed a higher preponderance of cases in male dogs (Shima *et al*., 2015; Tion *et al*., 2018). This was suggested to be due to the use of male dogs for a series of mating and due to the behaviour of sniffing the perineal region of potentially infected females. Conversely, Adejumobi *et al* (2017) reported a significant preponderance of female subjects with canine parvovirus enteritis. The dog breeds most represented are Rottweilers and German shepherds; the least represented appear to be the local breeds (mongrel) which have been shown to sometimes have antibodies against canine parvovirus without prior vaccination (Babalola *et al*., 2016; Ukwueze *et al*., 2018; Tion *et al*., 2019; Ogbu *et al*., 2020).

Seasonal prevalence: There is a pronounced seasonal influence on the incidence of canine parvovirus enteritis, although this varies slightly with the different locations in the country. The highest incidence is usually recorded in the dry months of the year (i.e., January, February, November, December) regardless of location (Shima *et al*., 2015; Adejumobi *et al*., 2017; Tion *et al*., 2018; Francis *et al*., 2019; Tion *et al*., 2019). Conversely, Shima *et al* (2015) and Tion *et al* (2018) also recorded a relative spike in occurrence during June (rainy season) and September (rainy season) respectively. Nevertheless, there is an all-year-round occurrence of canine parvovirus cases.

CPV subtypes present in Nigeria

At the emergence of the virus in Nigeria, the CPV-2a was only detected (Dogonyaro *et al*., 2013; Apaa *et al*., 2016) until Fagbohun and Omobowale (2018) reported the presence of all three subtypes. This was later confirmed by Ogbu *et al* (2019), Shima *et al* (2020), and Ukwueze *et al* (2020). Currently, the most frequently identified subtype is CPV-2c followed by CPV-2a (Ogbu *et al*., 2019; Shima *et al*., 2020; Ukwueze *et al*., 2020; Tion *et al*., 2021). Recent studies have highlighted CPV-2a variants with S292A mutations. These are designated as the "New CPV-2a," and is thought to have replaced the old CPV-2a variants in Nigeria (Ndiana *et al*., 2021; Tion *et al*., 2021). Furthermore, co-infection of CPV subtypes within the same animal, especially CPV-2a with CPV-2b and CPV-2a with CPV-2c has been reported (Fagbohun and Omobowale, 2018). This finding is corroborated by the reports of co-

infection of CPV-2a and CPV-2c (Battilani *et al*., 2007) and the co-existence of FPV and wild-type CPV in the same animal (Url *et al*., 2003). On phylogenetic analysis of the VP2 genes of Nigerian origin, the sequences clustered into their respective clades. Most of the sequences were CPV 2a (52%), while the rest were CPV-2c (47%) except for two CPV-2b sequences (a viral sequence and a vaccine sequence) (Fig. 3).

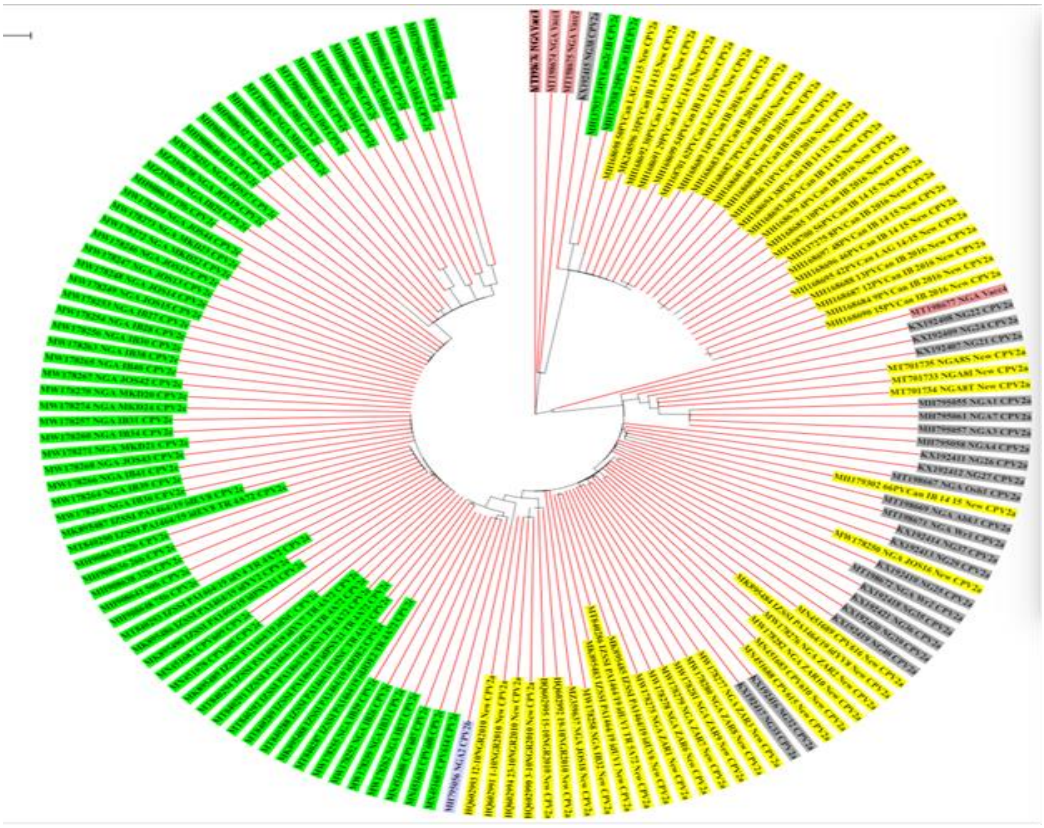

Fig. 3:
Phylogenetic analysis based on CPV VP2 gene sequences. A phylogenetic tree was constructed *via* multiple alignments of VP2 gene nucleotide sequences of Nigerian CPV isolates retrieved from the GenBank. Canine adenovirus-2 (CAV-2) nucleotide sequence was used as the outgroup. The tree was analysed by maximum likelihood method, with bootstrapping value set at 1000. CPV-2a, CPV-2b and CPV-2c clusters are labelled. Bar, 0.1 nucleotide substitutions per site.

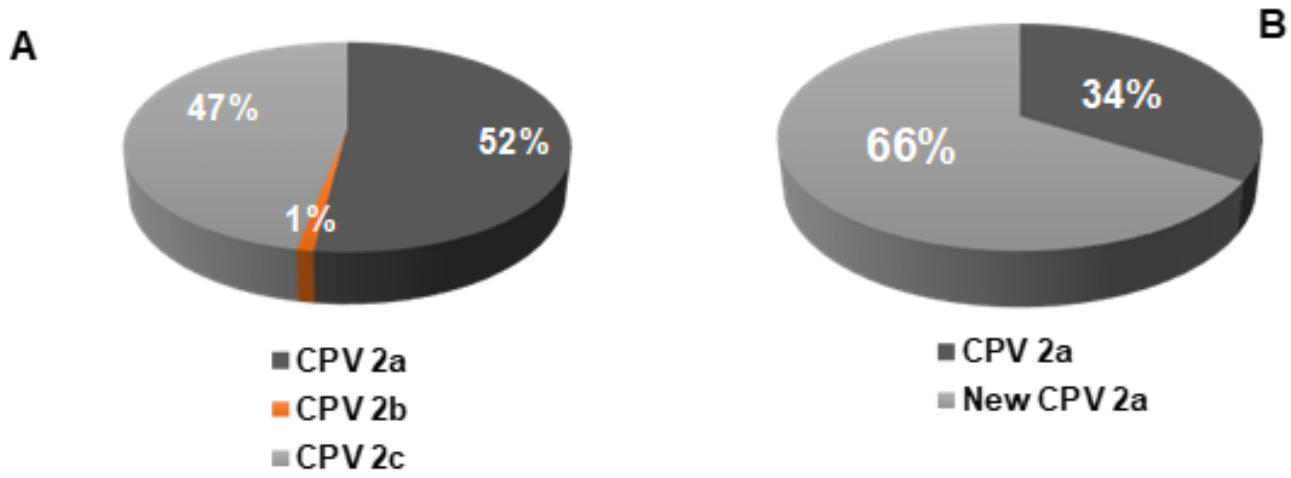

Fig. 4:
Proportion of the different CPV subtypes detected in Nigeria. (A) Proportion of three subtypes of CPV circulating in Nigeria. (B) Proportion of CPV-2a and New CPV2-a detected in Nigeria.

There was no wild-type CPV sequence based on the presence of the S297A mutation, 66% of the CPV 2a sequences were determined to be the New CPV 2a variant, the remaining 44% were partial sequences which did not include position 297 (Fig. 4).

Amino acid changes observed upon multiple sequence analysis of the translated sequence include A5G, L218F, F251S, F267Y, S297A, E298G, D305N, D305Y, Y324I, Q370R, D375N, N426D, N426E, T440A, and I447M. The S297A mutation appeared in all the sequences except a few short sequences which did not include this position. It can be assumed that practically all Nigerian CPV- 2a isolates, till date, have been the New CPV-2a. 93% of the sequences showed the Y324I amino acid change; 45% showed the Q370R amino acid change, whereas 46% showed the T440A change. Most of the amino acid changes clustered between positions 218 and 447 of the VP2 protein, and this includes positions 267 to 498 which forms the GH-loop of the VP2 protein, which is known to show the highest level of amino-acid variability (Chapman and Rossmann, 1993). This region forms part of the 3-fold spike region, a region which has been shown to have both antigenic function and also important for virus-host interactions (Tsao *et al*., 1991; Truyen *et al*., 1994; Lukashov and Goudsmit, 2001; Parrish and Kawaoka, 2005). The D305N change, which is located in the shoulder region of the 3-fold spike, although potentially important, but without a simultaneous change at position 300 may have no effect on antigenicity (Strassheim *et al*., 1994). The proximity to position 323, which plays a crucial role in transferrin receptor binding, gives significance to the Y324I change (Parrish and Kawaoka, 2005). Amino-acid change T440A and Q370R have been reported in other parts of the world (Battilani *et al*., 2002; Wang *et al*., 2015; Vannamahaxay *et al*., 2017; De Oliveira *et al*., 2019). Guo *et al* (2013) also reported amino acid change Q370R in a CPV-2a isolate from a Giant Panda in China. Amino-acid change T440A, F267Y and Y324I at the GH loop (Fig. 2), were suggested to be responsible for CPV immune evasion and resultant disease in dogs with complete vaccination records (De Oliveira *et al*., 2019). The I447M change which was first reported by Shima *et al* (2020) as being unique to Nigerian isolates has not been implicated as a significant antigenic site.

Vaccines and vaccination in Nigeria: There are often reports of vaccinated dogs succumbing to canine parvovirus infection: Daodu and Ajiboye (2018) and Tion *et al* (2018) reported a prevalence of 8.6% and 28.1%, respectively among vaccinated dogs. This has been reasoned to be due to vaccination failure which may be due to incomplete vaccinal regimen, interference by maternally derived antibody at the time of vaccination, the inability of some dogs to mount antibody response to the vaccine and poor-quality vaccine, and also as a result of vaccine failure due to poor vaccine immunogenicity (Shima *et al*., 2015; Babalola *et al*., 2016; Adejumobi *et al*., 2019). Similarly, Ukwueze *et al* (2018) was able to deduce from clinical records that there was a 48.7% prevalence among dogs with incomplete vaccinal records.

Although concerns have been raised about the possibility of reversion of vaccine-virus to virulence because on rare occasions, live attenuated vaccine virus has been detected in the faeces of vaccinated dogs (Decaro *et al*., 2007) since they can replicate in the vaccinated dog's intestine (Decaro *et al*., 2006; Riedl *et al*., 2016). Also, CPV with one or more mutation sites similar to vaccine subtypes has been isolated from vaccinated dogs with canine parvovirus enteritis in parts of China (Zhang *et al*., 2010). In a study carried out by Fagbohun and Omobowale (2018) in Nigeria, a similar concern was raised.

The polyvalent vaccine DHLPP (Distemper Hepatitis Leptospirosis Parvovirus Parainfluenza) against canine distemper, infectious canine hepatitis, canine leptospirosis, canine parvovirus and canine parainfluenza is in use in Nigeria. The canine parvovirus vaccine subtypes in this vaccine are either the wild-type CPV or CPV-2b (Fagbohun and Omobowale, 2018; Shima *et al*., 2020). Although several variables affect the outcome of vaccination, these vaccines are effective in stimulating sufficient antibody response in vaccinated dogs (Babalola *et al*., 2016; Adejumobi *et al*., 2019). There are reported cases of isolation of CPV-2c from dogs with complete vaccinal records but showing signs of canine parvovirus enteritis. This has raised questions as to the efficacy of these vaccines in protecting dogs against the CPV-2c subtype (Fagbohun and Omobowale, 2018; Shima *et al*., 2020; Ukwueze *et al*., 2020; Tion *et al*., 2021). This has also been observed in other parts of the world (Decaro *et al*., 2009; Chiang *et al*., 2016).

CONCLUSION

The journey of canine parvovirus from its early report as a local epidemic in the United States to its current pandemic status and its continued worldwide endemicity in canine populations are marked by various landmark genetic mutations all culminating in a better host-adapted virus. In this review, we have attempted a detailed summary of the origins, epidemiology and genetic dynamics of CPV in Nigeria. High prevalence and case-fatality figures, sometimes despite vaccination, raises concern as to the effectiveness of the current immunoprophylactic regimen against circulating subtypes of the virus. Socio-economic factors may play a role in the epidemiology of canine parvovirus enteritis in Nigeria. Studies on Carnivore protoparvovirus 1 in Nigeria has revolved around dogs; there is a paucity of data on other susceptible species such as domestic and feral cats as well as wildlife which is potentially important hosts (Calatayud *et al*., 2019). Therefore, studying the behaviour of the virus in other susceptible hosts in Nigeria may yet reveal more fascinating insight into the domestic and feral dynamics of canine parvovirus and other related parvoviruses both at the epidemiological level and the genetic level.

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