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Short Paper

Genetic analysis and emergence of canine parvovirus type 2c in South Eastern Nigeria

Ukwueze, C. S.[1*]; Nwosuh, C. I.[2]; Obishakin, E. F.[3]; Anene, B. M.[4]; Ezeokonkwo, R. C.[5]; Owoludun, O. A.[3]; Chima, N. C.[3] and Luka, P. D.[3]

[1]Department of Veterinary Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria; [2]Research Directorate, National Veterinary Research Institute, Vom, Nigeria; [3]Biotechnology Division, National Veterinary Research Institute, Vom, Nigeria; [4]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria; [5]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria

[*]***Correspondence:*** *C. S. Ukwueze, Department of Veterinary Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Nigeria. E-mail: chynet2006@yahoo.com*

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Abstract

Background: Genetic analysis of canine parvovirus type 2 (CPV-2) variants circulating in South Eastern Nigeria was investigated. The original strain of CPV-2 emerged in 1978, mutated later to CPV-2a and has continued to be evolved. **Aims:** To genetically characterize CPV-2 strains detected in dogs in South Eastern Nigeria and to phylogenetically group the viruses with existing sequencing data. **Methods:** A total number of 82 rectal swabs were collected and stored in virus transport medium (VTM) from suspected cases of CPV-2 within the study area and were tested with polymerase chain reaction (PCR). **Results:** Seventy-nine samples (96.3%) were positive for CPV-2 and sequence analysis of partial *VP2* gene of 20 amplicons revealed circulation of CPV-2a (n=4) and CPV-2c (n=16) in the region. The obtained strains clustered together. However, the group was further divided into two clear clusters comprising of 2a and 2c strains. The vaccine strain and the CPV-2 reference strains from USA formed a monophyletic cluster. **Conclusion:** Canine parvovirus types 2a and 2c are co-circulating in South Eastern region of Nigeria and therefore, there is an urgent need for an improved vaccine to cover for the emerging strain (CPV-2c) in Nigeria.

Key words: Amino acids, Canine parvovirus-2c, Nigeria, Polymerase chain reaction, Sequence analysis

Introduction

Canine parvovirus has been a common and important cause of morbidity and mortality in young dogs, since its emergence in 1978. The disease is globally distributed and is endemic in most populations of domestic and wild canids (Parrish *et al*., 1988). Canine parvovirus type 2 (CPV-2) is a small (25 nm) non enveloped virus infecting domestic and wild carnivores. The virus consists of a spherical capsid, which is composed of three proteins and a single stranded DNA molecule (Muzyczka and Berns, 2001). Canine parvovirus type 2 belongs to the family parvoviridae and genus parvovirus, and is a variant adaptation of feline panleukopenia virus (FPLV) like parvovirus of the wild carnivores. The virus is also closely related to the mink enteritis virus (MEV), raccoon parvovirus (RPV) and blue fox parvovirus (BFPV) (Tattersall *et al*., 2005).

Control and prevention of CPV-2 remains a global challenge because it relies mainly on extensive vaccination, which has to be tailored to fit into geographical diversities. The vaccines currently used are based on the original antigenic type of CPV-2, and have been shown to protect dogs against infection with the new (CPV-2a/2b) antigenic types (Yule *et al*., 1997). However, certain vaccines based on FPLV have also been shown to protect cats (Chalmers *et al*., 1999). The ideal vaccines for CPV-2 should contain the latest antigenic types of the virus within a given geographical area, as this implies the most complete protection, provided that the new vaccines are as immunogenic as the old ones (Truyen, 2006).

The continued incidence of CPV-2 is partly due to the virus capability of evolving into more virulent and resistant subspecies with significant local gastrointestinal and systemic inflammatory reactions. New antigenic types including CPV-2a, CPV-2b, and CPV-2c have emerged from the original CPV-2, which have been detected in several parts of the world, even among fully vaccinated dogs (Buonavoglia *et al*., 2001). These mutations and antigenic variations of CPV-2 have made the control and eradication of parvoviral enteritis almost impossible, as seen in the continued emergence of new strains in several parts of the world including Africa (Dogonyaro *et al*., 2013). Canine parvovirus type 2 has been detected in some parts of Nigeria (Dogonyaro *et al*., 2013; Apaa *et al*., 2016), including South Eastern region, and constitutes a major source of morbidity and mortality

in young dogs less than six months of age (Pollock and Coyne, 1993). However, there is paucity of information on the strains of the virus circulating in the region. The vaccines used in the region are mainly imported from foreign countries and in most cases do not show full protection of dogs against other strains of CPV-2. Movement of dogs across the country especially in endemic areas, have been speculated to play a major role on the epidemiology of CPV-2 in Nigeria. Our study was therefore designed to genetically characterize CPV-2 strains in dogs within South Eastern rejoin of Nigeria and to compare with the vaccine used for possible control policy.

Materials and Methods

Study area and sample collection

A total of 82 rectal swabs were collected from three states of the South Eastern Nigeria (Abia, Anambra, and Enugu States) using randomly sampling method (the patient profile in this study is shown in Supplementary Table (ST1)). Sampling was carried out for ten months (June, 2016-March, 2017) from six major veterinary clinics and major kennels/breeders. The swabs were put into virus transport medium (VTM) (National Veterinary Research Institute, Vom, Nigeria) and stored at -20°C before use. DNA was extracted using the Quick-DNA™ Miniprep kit (Zymo Research, USA) following the manufacturer's instructions with some minor modifications. Lyophilized CPV-2 commercial vaccine Biocan®) (Bioveta, Czech Republic) was used as control. Five µL of the DNA template was amplified as described by Bounavoglia *et al*. (2001).

Ten µL of the polymerase chain reaction (PCR) product from each reaction were mixed with 1 µL of 6 × DNA loading dye (Thermo Scientific, USA) and separated along with a 100 bp DNA molecular weight marker (Gene Ruler, Thermo Scientific, USA) by electrophoresis at 80 V for 45 min in 1 × Tris-Boric acid-EDTA (TBE) buffer (10 × Tris-Boric acid-EDTA, BioRad, USA) (Fig. 1).

Out of the 82 faecal specimens collected, 79 samples (96.3%) were positive for CPV-2 and sequence analysis of partial *VP2* gene of 20 amplicons revealed circulation of CPV-2a (n=4) and CPV-2c (n=16) in the region which were not previously reported (Table 1).

Results

Amino acid sequence analysis

Our results revealed the presence of CPV-2a (n=4) and CPV-2c (n=16) among the samples (Fig. 1). However, CPV-2 and 2b were not detected. The deduced amino acid from the nucleotides revealed residues that confirm antigenic types of CPV-2a (asparagine N426) and CPV-2c (glutamic acid E426) at position 426 of the capsid protein VP2 (Fig. 2). Other mutations were at position D305Y (Aspartic-Tyrosine) CPV-2a/2c, D305N (Aspartic-Asparagine) CPV-2a, Y324I (Tyrosine-Isoleucine) CPV-2a/2c, Q370R (Glutamine-Arginine) CPV-2c, N375D (Asparagine-Aspartic acid) CPV-2a/2c, and G440A (Glycine-Alanine) CPV-2c.

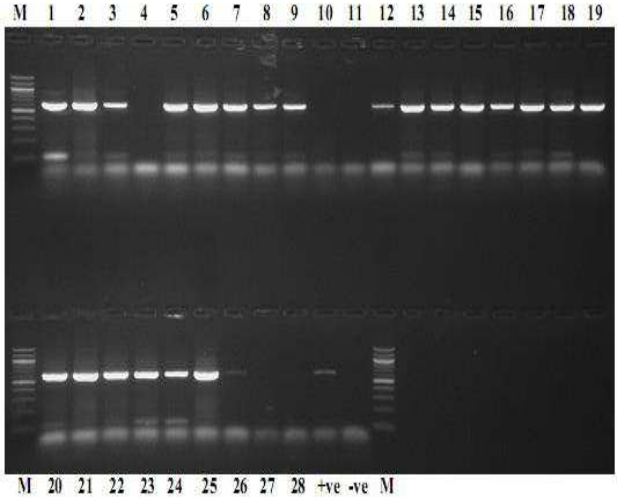

Fig. 1: Gel electrophoresis of CPV-2 amplicons at 629 bp of dogs in South Eastern Nigeria using Hfor/Hrev primer (Timurkan and Oguzoglu, 2015). Lines 1-3, 5-9 and 12-26 are Positive field samples, Lines 4, 10-11, 27-28 are Negative field samples, +ve and –ve: Positive and negative controls respectively, and M: 100 bp molecular maker

Table 1: Summary of canine parvovirus isolates obtained from the GenBank and from our study

Strain	Name	Origin	Accession No.
2a	331/05	Italy	FJ005254
2a	67/05	Italy	FJ005253
2a	96/02	Italy	FJ005252
2b	G82/97	Italy	FJ005260
2b	29/97	Italy	FJ222823
2b	SAH	Italy	FJ222822
2c	56/00	Italy	FJ222821
2c	GR09/09	Greece	GQ865519
2c	04S19	France	DQ025988
CPV-2	Isolate	USA	M74852
CPV-2	133	USA	EU659116.1
2c	Seq1	Nigeria	MH908630
2a	Seq2	Nigeria	MH908631
2c	Seq3	Nigeria	MH908632
2c	Seq4	Nigeria	MH908633
2c	Seq5	Nigeria	MH908634
2c	Seq6	Nigeria	MH908635
2c	Seq7	Nigeria	MH908636
2c	Seq8	Nigeria	MH908637
2c	Seq9	Nigeria	MH908638
2c	Seq10	Nigeria	MH908639
2c	Seq11	Nigeria	MH908640
2c	Seq12	Nigeria	MH908641
2a	Seq13	Nigeria	MH908642
2c	Seq14	Nigeria	MH908643
2a	Seq15	Nigeria	MH908644
2c	Seq16	Nigeria	MH908645
2c	Seq17	Nigeria	MH908646
2a	Seq18	Nigeria	MH908647
2c	Seq19	Nigeria	MH908648
2c	Seq20	Nigeria	MH908649

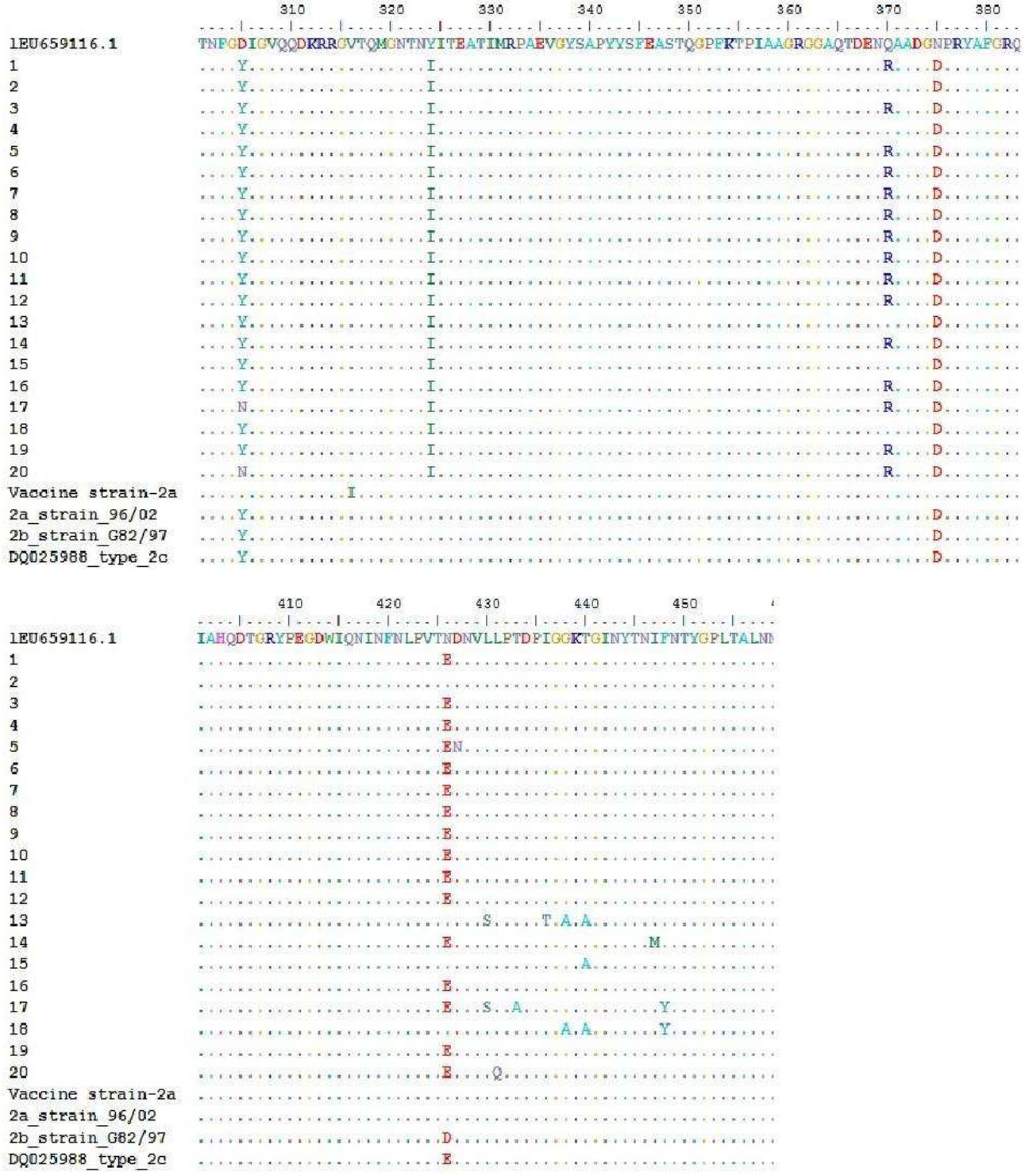

Fig. 2: Amino acid sequence alignment of partial *VP2* encoding gene of CPV-2 of South East Nigeria strains and a vaccine strain compared to the EU659116.1 strain. Samples of strain 2a, 2b, and 2c strains are also included from the GenBank. The field samples are numbered from 1-20

Phylogenetic analysis

All of the strains obtained from South Eastern region of Nigerian formed a separate cluster with a common ancestor. The group further divides into two different clusters comprising of 2a and 2c genotypes with a common ancestor, whereas the 2a strains from North-central Nigeria were grouped together with the 2a from Italy. Amongst all the GenBank sequences used for the analysis, only the 2c group cluster was close to the study sequence and shared a common root with the sequences of strains obtained from South Eastern Nigerian. The vaccine strain and CPV-2 reference strains from USA formed a monophyletic cluster. Likewise, the 2a strains from North-central Nigeria and the 2b strains from the GenBank form separate monophyletic clusters (Fig. 3).

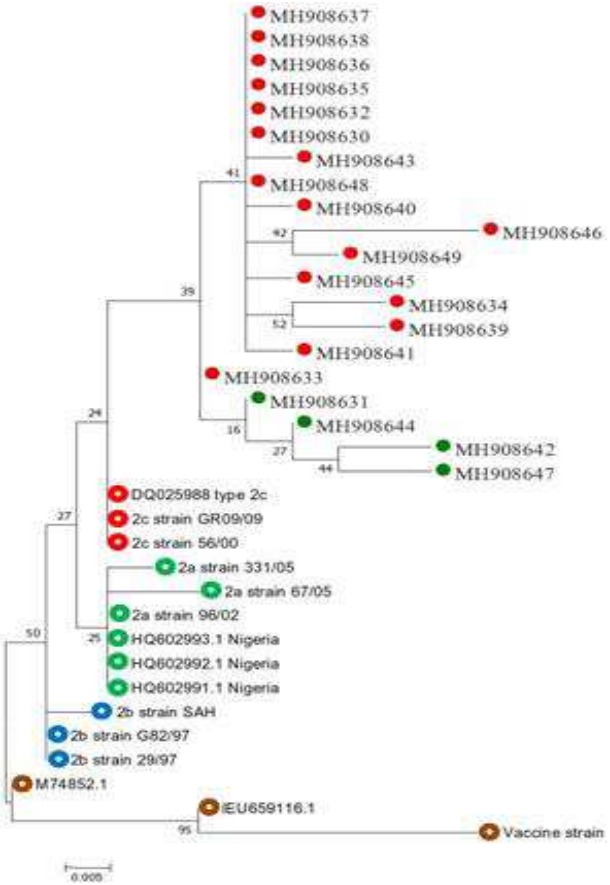

Fig. 3: Phylogenetic tree of the 35 CPV-2 strains in our study based on a 196 amino acid length of the partial *VP2* gene using MEGA 7. The tree was conducted using maximum likelihood based on the Poisson model. The reliability of the tree was assessed by 1000 bootstrap replicate. The genotypes CPV-2 and also the vaccine strain are indicated by brown colours (M74852.1, IEU659116.1, vaccine strain), 2a are shown in green colours (331/05, 67/05, 96/02, HQ602991.1, HQ602992.1, HQ602993.1), the 2b genotypes are shown in blue colours (29/97, G82/97, SAH), and the 2c genotypes are shown in red colours (56/00, GR09/09, DQ25988). The field isolates analyzed in this study are indicated by shaded red and green circle symbols while the strain sequences downloaded from the GenBank are indicated by non-shaded (empty) circle symbols

Discussion

Canine parvovirus is one of the major leading causes of morbidity and mortality in unvaccinated dogs and young dogs less the six months of age globally (Goddard and Leisewitz, 2010; Sykes, 2014). Molecular characterization and sequence analysis of *VP2* encoding genes of CPV-2, provides vital information on the circulating viruses in the study area and also shows the relationship of these strains with CPV-2 strains from other parts of the world. Canine parvovirus type 2 has been characterized and documented in several parts of the world including some African countries such as South Africa (Steinel *et al*., 2001; Dongonyaro *et al*., 2013), Tunisia (Touihri *et al*., 2009), and Nigeria (Dongonyaro *et al*., 2013; Apaa *et al*., 2016). In Nigeria CPV-2 characterization was only carried out in a few areas in North Central Nigeria (Jos metropolis) (Dongonyaro *et al*., 2013) and recently South Western region of Nigeria (Fagbohun and Omobowale, 2018).

The finding of this study may be a new development, which implies that CPV-2c variant is now present in Nigeria, unlike the previous findings of Dongonyaro *et al*. (2013) and Apaa *et al*. (2016) that discovered only CPV-2a in Nigeria. Our results however, agrees with other researchers that discovered CPV-2c long ago in various in various countries of the world including Vietnam (Nakamura *et al*., 2004), European countries (Decaro *et al*., 2007), Italy (Decaro *et al*., 2009b), and Africa; Tunisia (Touihri *et al*., 2009). Our study showed that the new variant CPV-2c is widespread in South Eastern Nigeria, if not the entire country. This is possiblydue to the free movement of puppies across states. Ever since the emergence of CPV-2c in mid 2000 (Buonavoglia *et al*., 2001), there has been rapid displacement of old types by new antigenic variants (Decaro *et al*., 2007). Mutation occurring at capsid protein position 426 is the basefor major antigenic variation and classification of CPV-2 variants (Decaro *et al*., 2009b; Li *et al*., 2017). The existence of CPV-2c variantcan be attributed to the continuous antigenic evolution of the CPV-2 virus (Turyen, 2006). Previous studies have reported mutations affecting important residues of the capsid proteins at positions 297, 300, and 426 suggesting that canine parvovirus is still evolving (Martella *et al*., 2006; Truyen, 2006). Similarly, it is important to know which strain(s) of virus arecirculating in a region at a particular point in time, so that vaccine producers can consider and review the antigens included in their products for the effective control of CPV-2-induced disease.

In this study, it is worthy of note that the commercial canine parvovirus vaccine (Biocan®) (Bioveta, Czech Republic) popularly used in the South Eastern Nigeria contains the CPV-2a which may be extinct at present. Other commercial vaccines include either type 2a or 2b or both, though sequence differences of the strains may occur. Canine parvovirus type 2c is yet to be included in the present generations of commercial vaccines. Although several studies have demonstrated the efficacy of the current CPV-2 vaccine against other CPV-2 strains (cross protection) (Larson and Schultz, 2008; Spibey *et al*., 2008), some evidences suggest that dogs with the complete vaccination programme still suffer from CPV-2c (Decaro *et al*., 2007). In this present study, out of the 16 CPV-2c cases encountered, 14 dogs were fully vaccinated, and 11 dogs died. This may likely be due to the non-protective effects of the vaccines used. This result corroborates with Chiang *et al*. (2016) who observed, CPV-2c variants in dogs younger than six months of age despite full vaccination history. Similarly, Decaro *et al*. (2008 and 2009a) reported an outbreak of CPV-2c in adult dogs that had undergone full vaccination schedules including booster doses. In view of the forgoing findings, a new canine parvovirus vaccine containing the CPV-2c variants is not only a

requirement, but also an urgent necessity.

The rooted amino acid phylogenetic tree showed that all the South Eastern Nigerian strains formed a separate group having a common ancestor. In general, the resulting phylogenetic trees were not supported by high bootstrap values among the local samples; this could be due to the very low variability among the samples and could be expected, due to the close proximities between the sampled locations. However, it was evident by the reasons of far distance locations that the vaccine strain and the CPV-2 reference strains from USA not only formed a monophyletic cluster but were also supported by very high bootstrap values.

Canine parvovirus type 2 has been molecularly detected and characterized in South Eastern Nigeria, comprising of mainly CPV-2c strain. The CPV-2 vaccine used in the region is non protective to the dogs, and hence the likely need for a new CPV-2 vaccine with the latest antigenic variants circulating within the region. The phylogenetic tree of the protein analysis showed that all the South Eastern strains clustered together, whereas the other Nigeria isolates and other downloads from the GenBank formed different clusters.

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