

Full Length Research Paper

Molecular detection of canine parvovirus in Jos, Nigeria

**S. C. Chollom^{1,2}, E. J. Fyaktu², A. E. J. Okwori³, G. O. A. Agada⁴, G. Hashimu⁵, R. Y. Akele³,
E. I. Voumangai⁶, T. Dashe⁷ and D. Z. Egah³**

¹Viral Research Department, National Veterinary Research Institute, Vom, Nigeria.

²Department of Medical Microbiology, University of Jos, Nigeria.

³Federal College of Veterinary and Medical Laboratory Technology, National Veterinary Research Institute, Vom, Nigeria.

⁴Central Diagnostics Laboratory, National Veterinary Research Institute, Vom, Nigeria.

⁵Bacterial Vaccine Production Department, National Veterinary Research Institute, Vom Nigeria.

⁶Laboratory Department, Plateau State Specialist Hospital, Jos, Nigeria.

⁷Institute of Human Virology Nigeria, Plateau State Virology Research Centre, Plateau State Specialist Hospital Jos, Nigeria.

Accepted 28 November, 2012

Canine parvovirus (CPV) remains the most significant viral cause of enteritis in puppies over the age of two months. This study was meant to detect the virus by targeting the VP2 gene, a 583 bp gene (nucleotide 4003 to 4585) of the capsid protein. The detection of the virus was carried out by conventional polymerase chain reaction (PCR) on one hundred and nine samples. Seventy five of these were rectal swabs while thirty four were necropsy tissues, all from dogs presenting with symptoms suggestive of parvovirus enteritis from veterinary clinics in Jos metropolis. Results revealed that 47.70% of the samples were found to be positive for the virus. The rate of detection was more in necropsy tissues (64.71%) relative to rectal swabs (40.00%). A breakdown of the incidence of the infection across breeds of dogs showed that the Rottweillers had 60.00%, Doberman pinchers had 54.54%, Pitbulls had 53.33% while Tan coloured and local breeds of dogs had 50.00 and 21.42%, respectively. With a prevalence rate of 47.70%, the findings have confirmed that the virus is circulating in Jos, Nigeria. As such, stake holders must quickly intervene to arrest the situation given the high economic losses associated with the disease.

Key words: Canine parvovirus, polymerase chain reaction (PCR), Jos.

INTRODUCTION

Canine parvovirus (CPV) is a highly contagious virus mainly affecting dogs. The disease, commonly called parvovirus enteritis is highly infectious and is transmitted from dog to dog by direct or indirect contact with contaminated feces (Appel et al., 1979). Parvovirus enteritis is a relatively new disease that appeared in the late 1970s. It is highly contagious and often fatal in both domestic and wild dog populations with high morbidity (100%) and frequent mortality of up to 10% (Appel, 1978). It is charac-

terized by vomiting, bloody diarrhea, myocarditis and leucopenia (Streck et al., 2009). CPV was first recognized in 1978 and spread worldwide in one to two years (Carmichael, 2005). The virus is a small, non enveloped single stranded DNA virus belonging to the Parvoviridae family and parvovirus genus under the Parvovirinae sub-family (Hong et al., 2007). The virus, which first appeared between 1977 to 1978, probably arose from a very closely related virus in cats, feline panleukopaenia virus (FPLV) through a small number of mutations in the single capsid protein; a species jump which may have involved intermediate passage in other carnivores such as mink or raccoons (Truyen et al., 1996). As early as 1979 the first

*Corresponding author. E-mail: cholloms_12@yahoo.com. Tel: 2348036574286.

Table 1. Percentage prevalence according to sample type.

Sample type	PCR positive (%)
Swabs (n = 75)	30 (40.00)
Tissues (n = 34)	22 (64.71)
Total (T = 109)	52 (47.70)

Table 2. Percentage positivity of samples based on breed of dog.

Breed	PCR positive (%)
Rottweillers (n = 25)	15 (60.00)
Doberman Pinchers (n = 33)	18 (54.54)
Pit Bulls (n = 15)	08 (53.33)
Tan Coloured (n = 04)	02 (50.00)
Local (n = 28)	06 (21.42)
Unidentified (n = 04)	03 (75.00)
Total (T = 109)	52 (47.70)

variants of CPV2 appeared, termed CPV2a, and they were quickly followed by the appearance of CPV2b in 1984 (Parrish et al., 1985, 1991). The original type 2 virus has now disappeared from the field having been replaced by the 2a and 2b variants; although the relative proportions of these two types varies from country to country (Truyen et al., 1996; Chinchkar et al., 2006; Pereira et al., 2007). However, in Africa, including Nigeria, no reports as regarding the circulation of the virus in the area is available (Kapil et al., 2007). This study is aimed at detecting the virus using polymerase chain reaction (PCR) in Jos, Nigeria.

MATERIALS AND METHODS

A total of one hundred and nine samples were collected. Seventy five of which were rectal swabs while thirty four were necropsy tissues. The rectal swabs were collected from dogs presenting with bloody diarrhea and vomiting signs while necropsy tissues of target organs (intestine, heart, lymph nodes, spleen and liver) were harvested from dogs that died from the disease.

Sample collection

Rectal swabs were collected by the insertion of the cotton bud of a swab stick (Steriline®) through the anal region of the animal into the rectum after the dog has been restrained. The swab stick was carefully turned clockwise and gently withdrawn. A scissors was used to cut off the swab bud into 2 ml cryovial containing virus transport medium (VTM) and transported to the laboratory in a cold box. Necropsy tissues of target organs were collected from dead dogs. They were aseptically delivered into a universal sample bottle (Steriline®) and transported to the laboratory in a cold box.

Polymerase chain reaction (PCR)

Total DNA was extracted using the QIAamp® DNA Mini Kit according to manufacturer's specifications. Different procedures were adopted for DNA extraction from swabs and DNA extraction from tissues.

CPV-2 specific master mix

The master mix contained 13.88 µl of nuclease free water (Promega®), 2.5 µl of 10x PCR reaction buffer, 0.62 µl of dNTP mixture (10 mM) (Fermentas®), 1.5 µl of magnesium chloride (25 nM), 0.5 µl of forward and 0.5 reverse primers (555 forward and 555 reverse, respectively) for CPV2 (Inqaba Biotech SA®) at 20 pmol/µl and 0.5 units of Taq DNA polymerase (Qiagen®). This gave a total volume of 20 µl master mix. 5 µl of the CPV-2 master mix (above) was added to 5 µl extracted DNA in a new tube for amplification.

Amplification of DNA extracts

The mixture was submitted to a thermal cycling profile of initial denaturation at 94°C for 5 min. This was followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 2 min, extension at 72°C for 2 min and 72°C for 10 min in an Applied Biosystem® 9700 PCR machine. The primer sequences used were as follows: 555 forward, 5'-CAGGAAGATATCCAGAAGGA-3' and 555 reverse, 5'-GGTGCTAGTTGATATGTAATAAACA-3'. The master mix composition and PCR program was as described by Streck et al. (2009) with minor modifications.

Electrophoresis and analysis

A 10 µl of PCR amplicons was electrophoresed in a 1.5% agarose gel stained with ethidium bromide in the presence of 2 µl of gel loading buffer (Fermentas®). Positive and negative controls were included. The electrophoresis was carried out at 80 volts for 50 min.

RESULTS

Amplicon band sizes of about 583 bp were considered positive for the virus. Results revealed that 52 (47.70%) of the one hundred and nine samples were positive for the virus.

From the seventy five rectal swabs examined, 30 (40.00%) of them were positive for CPV just as 22 (64.71%) out of the thirty four tissue samples examined and were positive for the virus by conventional PCR (Table 1). Statistically, there was no significant difference ($P > 0.05$) in the number of positive samples from the sample types surveyed (Table 1). Although the sample size was small for breed comparison, Rottweillers had 60.00% incidence, Doberman pinchers 54.54%, Pitbulls 53.33%, Tan coloured 50.00% while local breeds of dogs had only 21.40% (Table 2).

DISCUSSION

Canine parvovirus continues to be an important pathogen

of dogs and it is responsible for serious occurrences of morbidity and mortality despite the availability of safe and effective vaccines (Decaro et al., 2006a,b). This study revealed that 52 out of the 109 samples examined had the virus. This study therefore revealed a prevalence rate of 47.70% of the disease from this part of the world. The figure is less compared with seven positive samples of CPV 2c strain obtained from nine samples (77.78%) assayed in Brazil (Streck et al., 2009). Also, it is lower than the findings in Uruguay where 24 out of 25 faecal samples (96.00%) were found to be positive for one strain of the virus (Perez et al., 2007). The high percentage of positive tissues against rectal swabs is not unconnected with the fact that the selected tissues had earlier been established as the predilection sites of the agent as such, it is only natural that the virus concentration in such sites be higher (Lobetti, 2003).

Local breeds of dogs were the least susceptible to the infection as against their foreign counterparts. Authors had previously suggested that the former have a greater degree of resistance against the virus than the foreign breeds. They have also been fingered as healthy carriers of the virus (Nelson and Couto, 1998). This is of great epidemiological relevance as their role in the distribution of the virus is once again re-echoed. Since most of the local breeds are free rangers, it makes it possible for them to distribute the virus indiscriminately. This factor poses a great danger to the foreign breed of dogs which are more susceptible to the agent as their local counterparts (Nelson and Couto, 1998).

Although canine parvovirus was implicated in 52 (47.70%) of the samples as causative agent responsible for enteritis in these dogs, the cause of enteritis in 57 (52.29%) PCR-negative samples may be due to other pathogens such as *Leptospira* or *Eimeria* species. In general, these findings will no doubt interest both the local and international communities as they have waited all this while for a report on the agent from this part of the world (Kapil et al., 2007).

REFERENCES

- Appel MJG, Cooper BJ, Greisen H, Carmichael LE (1978). Status report: Canine viral enteritis. *J. Am. Vet. Med. Assoc.* 173:1516-1518.
- Appel MJG, Scott WF, Carmichael LE (1979). Isolation and immunization studies of canine parvo-like virus from dogs with haemorrhagic enteritis. *Vet. Rec.* 105(8):156-159.
- Carmichael L (2005). An annotated historical account of canine parvovirus. *J. Vet. Med. Infect. Dis. Vet. Public Health* 52(7-8):303-311.
- Chinchkar SR, Mohana SB, Hanumantha RN, Rangarajan PN, Thiagarajan D, Srinivasan, VA (2006). Analysis of VP2 gene sequences of canine parvovirus isolates in India. *Arch. Virol.* 151(9): 1881-1887.
- Decaro N, Desario C, Elia G, Campolo M, Lorusso E, Mari V, Martella V, Buonavoglia C (2006a). Occurrence of severe gastroenteritis in pups after canine parvovirus vaccine administration: Clinical laboratory diagnostic dilemma. *Vaccine* 25(7):1161-1166.
- Decaro N, Martella V, Desario C, Bellacicco AL, Camero M, Manna L, d'Aloja D, Buonavoglia C (2006b). First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. *J. Vet. Med. Infect. Dis. Vet. Public Health* 53:468-472.
- 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. *J. Clin. Microbiol.* pp. 4044-4047.
- Lobetti R (2003). "Canine Parvovirus and Distemper". Proceedings of the 28th World Congress of the World Small Animal Veterinary Association. <http://www.vin.com/proceedings/Proceedings.plx>
- Nelson RW, Couto CG, (1998). *Small Animal Internal Medicine* (2nd ed.) Mosby Publisher.
- Pereira CAD, Leal ES, Durigon EL (2007). Selective regimen shift and demographic growth increase associated with the emergence of high fitness variants of canine parvovirus. *Infect. Genet. Evol.* 7 (3):399-409.
- Perez R, Francia L, Romeo V, Maya L, Lopez I, Hernandez M (2007). First detection of canine parvovirus type 2c in South America. *Vet. Microbiol.* 124:147-152.
- Streck AF, de Souza CK, Goncalves KR, Zang L, Pinto LD, Canal CW (2009). First detection of canine parvovirus type 2c in Brazil. *Braz. J. Microbiol.* 40(3).
- Truyen U, Evermann JF, Vieler E, Parrish CR, (1996). Evolution of canine parvovirus involved loss and gain of feline host range. *Virology* 215: 186-189.