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Research Article

Comparison of Immunochromatographic Test and Conventional Polymerase Chain Reaction as Diagnostic Methods for Canine Parvovirus Infection

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ABSTRACT

Canine parvovirus type 2 infection is one of the aetiological agents of contagious gastroenteritis in dogs associated with high morbidity and mortality. Early diagnosis and treatment is very crucial for the survival of infected dog. This study was conducted to compare two diagnostic methods of canine parvovirus. Namely; Immunochromatographic (IC) test and Conventional Polymerase Chain Reaction (PCR). Eighty-two (82) faecal specimens were collected *per rectum* from diarrhoeic dogs presented to various Veterinary Clinics and Hospitals in South Eastern Nigeria between the months of June 2017 – March 2018. Three states, namely Abia, Anambra and Enugu were randomly selected and purposive/convenience sampling method was used to select two clinics/hospitals in each state. The faecal samples were both subjected to IC test and PCR simultaneously. IC test revealed that 83% positivity (68/82), while PCR showed 96% positivity (79/82). The sensitivity of IC test over PCR was 86%, with specificity of 100%, while the positive predictive value 100%, negative predictive value 21%, positive likely hood ratio 0.86 and negative likely hood ratio 0.14. McNemar test's showed a significant difference between the two diagnostic methods. PCR was found to be more sensitive than IC test, though IC test is comparable with PCR and can be used in routine daily clinical practice.

Introduction

Canine parvovirus (CPV-2) infection occurs worldwide in domestic and wild canids (Nandi and Kumar, 2010). CPV-2 infection is a life-threatening contagious disease that mainly infects younger dogs from six weeks to six months (Kalli et al., 2010; Mylonakis et al., 2016). The virus has ubiquitous nature and can survive in the environment for more than a year, enabling the exposure of susceptible dogs to infected faeces, vomitus or formites (Sykes, 2014). However, it's unclear the exact climatic conditions that favour the survival of the organism in the environment (Jinag, 2018). The virus is extremely resistant to temperature changes and commonly used disinfectants (Decaro and Buonavoglia, 2012).

The incubation period of the disease is 4-5 days and clinical signs usually surface within 3-7 days of initial infection, depending on the infectious dose of the virus (Aiello et al., 2012). The disease manifests in two major forms namely the cardiac or myocarditis and the gastroenteric form or gastroenteritis. The cardiac form, affect mainly puppies under 3 months of age (Appel et al., 1979), and is associated with heart failure and sudden death in young puppies of about 4 -8 weeks of age (Mochizuki et al., 1996). The gastroenteritis form affects older puppies and often develops into vomiting and haemorrhagic diarrhoea with a foul smelling or distinct odour (Aiello et al., 2012; Johnson, 2014), which results in severe dehydration, electrolyte imbalance, villus atrophy, bacteria translocation and even death (Macintire, 2000; Goddard and Leisewitz, 2010).

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Survival rate depends on how early diagnosis is made and treatment is instituted (Prittie, 2004), it may be as low as 9% if no treatment is instituted, but may exceed 80% with treatment and good nursing care (Otto et al., 1997; Mylonakis et al., 2016). Diagnosis of CPV-2 infection based on clinical signs may be misleading, as most pathogens have similar signs of vomiting and diarrhoea (Sundaran *et al.,* 2015). Various techniques have been described for the diagnosis of CPV-2, which includes highly sensitive electron microscopy (EM) and molecular methods like conventional Polymerase Chain Reaction (PCR). These techniques are cumbersome and expensive and are mainly used for research purposes rather than in routine diagnosis of canine parvovirus infection. Viral isolation is very effective, but is quite laborious and time consuming and not employed in routine clinical diagnosis (Desario et al., 2005). Hemagglutination test (HI) and Haemagglutination (HA) test though seems to be acceptable in routine diagnosis, they are relatively simple, rapid, inexpensive, and less sensitive and specific (Muzyczka and Berns, 2001; Silva et al., 2013). Immunochromatogtaphic (IC) test also known as lateral flow is a point of care test and user friendly and inexpensive compared to other methods (Jamshidi et al., 2013; Al-Tayib, 2014). The technique is very easy to perform, with minimal cost on dog owners. Outbreaks of CPV-2 infections are devastating and huge economic losses due to cost of treatment and emotional stress on dog owners. Finding alternative safe, easy and reliable diagnostic method that requires no expertise, will be of immense help in keeping the outbreaks CPV-2 infections on check. This work was therefore, designed to compare IC test and PCR as diagnostic methods of canine parvovirus infection in dogs.

Materials and Methods

Study area

This study was carried out in three states in South Eastern Nigeria namely; Abia, Anambra and Enugu States. South-East is one of the six geo-political zones in the country consisting of Abia State, Anambra State, Ebonyi State, Enugu State and Imo State (Figure 1). Dogs are kept as part of culture of the people for breeding, hunting and security. Dog meat is also a normal delicacy among some population in that region.

Figure 1. Map of Nigeria showing the three states (Abia, Anambra and Enugu) occupying South Eastern part of the country (Bomah, 2014), where sampling were conducted for the current study

Sample collection

Eighty-two (82) faecal specimens were collected per rectum from diarrhoeic dogs presented to various Veterinary Clinics and Hospitals in South Eastern Nigeria (Abia, Anambra and Enugu) between the months of June 2017-March 2018. The patient profiles and the history including the feeding, housing and other management practices were carefully evaluated before the commencement of the study (Table 1).

Immunochromatographic (IC) test

Immunochromatographic (IC) test (J & G Biotech Ltd. UK), was carried out as described by Esfandiari and Klingeborn, (2000). Briefly, faecal swabs were collected from the rectum or from freshly voided faeces and mixed with the assay diluents. The mixture was stirred evenly, and four (4) drops of supernatant were added into the sample hole of the IC test device. The test result was interpreted after 5-10 minutes. The presence of only one band within the result window indicates a negative result, while the presence of two colour bands (T and C) within the result window, no matter which band appears first, indicates a positive result. If the purple colour band is not visible within the result window after performing the test, the result is considered invalid. Positive samples were collected in 2 ml cryovial containing virus transport medium (VTM) and store at -20^{o}C until further analysis.

Conventional Polymerase Chain Reaction

DNA extraction from collected samples was carried out using the Quick-DNA^{TM} Miniprep Kit (Zymo Research USA) as described by the manufacturer's instructions with some minor modifications. Genomic lysis buffer was used to re-suspend the field samples and PCR graded water was used as negative control. The commercial CPV-2 based vaccine Biocan (Bioveta, Czech Republic), was used as positive control in this study. The PCR was assay was performed at the National Veterinary Research Institute, Vom, Nigeria, according to the methods described by Buonavoglia et al. (2001). The primer pair used were 555for 5'-CAGGAAGATATCCAGAAGGA-3' and 555rev 5'-GGTGCTAGTTGATATGTAATAAACA-3'.

The PCR amplicons were evaluated after electrophoresis on 1.5% agarose in Tris-borate EDTA (TBE) buffered gels stained with ethidium bromide. Amplified products were viewed under ultraviolent (UV) light (Bio-Rad Doc™ XR system). CPV-2 amplicons of band size 583 bp were considered positive.

Results

Table 1. Summary of patient profile, Immunochromatogtaphic test and Conventional Polymerase Chain Reaction. *Immunochromatographic IC test showed that 68/82 (83%) of the collected sample were positive for CPV-2, while conventional PCR revealed that 79/82 (96%) of the samples were positive for CPV-2*

Age	Sex	Breed	Location	Vacination status	IC test	PCR
6weeks	M	Boerboel	Abia	Unvaccinated	Postive	Postive
6weeks	M	Rottweiler	Abia	Vaccinated	Postive	Postive
4months	F	German shepherd	Abia	Vaccinated	Postive	Postive
2months	F	Boerboel	Abia	Unvaccinated	Postive	Postive
6momths	M	Boerboel	Abia	Vaccinated	Negative *	Postive
7weeks	F	German shepherd	Abia	Vaccinated	Postive	Postive
3months	F	German shepherd	Abia	Unvaccinated	Postive	Postive
4months	M	German shepherd	Abia	Vaccinated	Negative*	Negative*
3months	F	German shepherd	Abia	Unvaccinated	Postive	Postive
2months	F	German shepherd	Abia	Vaccinated	Postive	Postive
7weeks	M	Boerboel	Abia	Vaccinated	Postive	Postive
7months	F	German shepherd	Abia	Unvaccinated	Postive	Postive
5months	F	Rottweiler	Abia	Vaccinated	Postive	Postive
3months	M	Caucasian	Abia	Unvaccinated	Postive	Postive
7months	M	German shepherd	Abia	Unvaccinated	Postive	Postive
11months	F	Lhasa	Abia	Unvaccinated	Negative*	Postive
3months	F	German shepherd	Abia	Unvaccinated	Postive	Postive
5months	F	Pitbull	Abia	Vaccinated	Postive	Postive
1year	M	German shepherd	Abia	Unvaccinated	Postive	Postive
5months	M	Cacuasian	Abia	Vaccinated	Negative*	Postive
6months	M	Local	Abia	Unvaccinated	Postive	Postive
7weeks	F	German shepherd	Abia	Unvaccinated	Postive	Postive
5months	M	Boerboel	Abia	Unvaccinated	Negative*	Postive

Age	Sex	Breed	Location	Vacination status	IC test	PCR
7weeks	F	Rottweiler	Abia	Vaccinated	Postive	Postive
2months	M	Cucassian	Abia	Vaccinated	Postive	Postive
3months	M	German shepherd	Abia	Vaccinated	Postive	Postive
2months	F	German shepherd	Abia	Vaccinated	Postive	Postive
6weeks	M	German shepherd	Anambra	Unvaccinated	Postive	Postive
3months	M	German shepherd	Anambra	Unvaccinated	Postive	Postive
3months	M	Cuccasian	Anambra	Vaccinated	Negative*	Postive
7weeks	F	Caucasian	Anambra	Unvaccinated	Postive	Postive
2months	M	Rottweiler	Anambra	Vaccinated	Postive	Postive
2months	M	Rottweiler	Anambra	Vaccinated	Negative*	Negative*
2years	F	Boerboel	Anambra	Unvaccinated	Negative*	Postive
3years	F	Rottweiler	Anambra	Vaccinated	Postive	Postive
5months	M	German shepherd	Anambra	vaccinated	Postive	Postive
3months	M	Cuacasian	Anambra	Unvaccinated	Postive	Postive
6months	M	Boerboel	Anambra	Vaccinated	Negative*	Postive
5months	M	Caucasian	Anambra	Unvaccinated	Postive	Postive
5months	F	German shepherd	Anambra	Vaccinated	Postive	Postive
7weeks	M	Rottweiler	Anambra	Unvaccinated	Postive	Postive
2years	F	Caucasian	Anambra	Vaccinated	Postive	Postive
2months	M	Rottweiler	Anambra	Vaccinated	Postive	Postive
2months	F	Caucasian	Anambra	vaccinated	Postive	Postive
6months	F	German shepherd	Anambra	Vaccinated	Negative*	Negative*
4months	F	Caucasian	Anambra	Vaccinated	Postive	Postive
7months	M	Rottweiler	Anambra	Unvaccinated	Postive	Postive
2months	M	Caucasian	Anambra	Vaccinated	Postive	Postive
2months	M	Caucasian	Anambra	Vaccinated	Postive	Postive
5months	F	German shepherd	Anambra	Vaccinated	Negative*	Postive
2months	M	Bull masstiff	Anambra	Vaccinated	Postive	Postive
2months	M	Boerboel	Anambra	Vaccinated	Postive	Postive
2months	F	German shepherd	Anambra	Vaccinated	Postive	Postive
2years	F	Boerboel	Anambra	Unvaccinated	Postive	Postive
6months	F	German shepherd	Anambra	Vaccinated	Postive	Postive
7weeks	F	Rottweiler	Enugu	Vaccinated	Postive	Postive
2months	F	Rottweiler	Enugu	Unvaccinated	Postive	Postive
3months	M	Bul mastiff	Enugu	Vaccinated	Postive	Postive
11months	F	German shepherd	Enugu	Unvaccinated	Postive	Postive
2months	M	Bull mastiff	Enugu	Unvaccinated	Postive	Postive
3years	F	German shepherd	Enugu	Unvaccinated	Postive	Postive
4months	M	German shepherd	Enugu	Vaccinated	Postive	Postive
4months	F	Local	Enugu	Unvaccinated	Postive	Postive
3months	F	Rottweiler	Enugu	Unvaccinated	Postive	Postive
4months	M	Rottweiler	Enugu	Unvaccinated	Negative*	Postive
3months	M	German shepherd	Enugu	Vaccinated	Postive	Postive
4months	M	German shepherd	Enugu	Unvaccinated	Postive	Postive
6months	M	Local	Enugu	Unvaccinated	Postive	Postive
3months	M	Pitbull	Enugu	Vaccinated	Postive	Postive
4months	F	Local	Enugu	Unvaccinated	Postive	Postive
1year	M	German shepherd	Enugu	Unvaccinated	Negative*	Postive
4months	F	Rottweiler	Enugu	Unvaccinated	Postive	Postive
3months	F	Rottweiler	Enugu	Unvaccinated	Postive	Postive
2months	M	Rottweiler	Enugu	Vaccinated	Postive	Postive
5months	F	German shepherd	Enugu	Vaccinated	Postive	Postive
5months	F	Rottweiler	Enugu	Vaccinated	Postive	Postive
3months	F	Caucasian	Enugu	Vaccinated	Postive	Postive
3months	F	Bull mastiff	Enugu	Unvaccinated	Negative*	Postive
3months	M	Rottweiler	Enugu	Vaccinated	Postive	Postive
2months	F	Caucasian	Enugu	Unvaccinated	Postive	Postive

Age	Sex	Breed	Location	Vacination status	IC test	PCR
2months	F	Bull mastiff	Enugu	Vaccinated	Postive	Postive
3months	M	Bull astiff	Enugu	Vaccinated	Postive	Postive

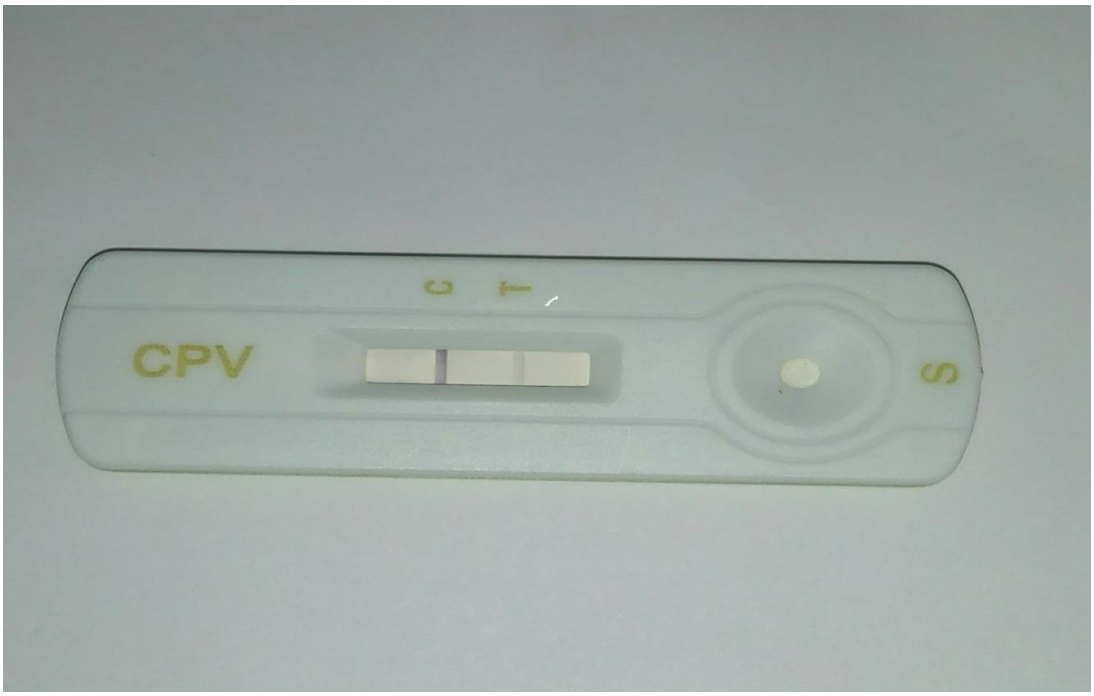

Figure 2. Immunochromatographic test (IC) kit/slide showing two lines on the Control band (C) and Test band (T), respectively, wheras S indicates the sample area

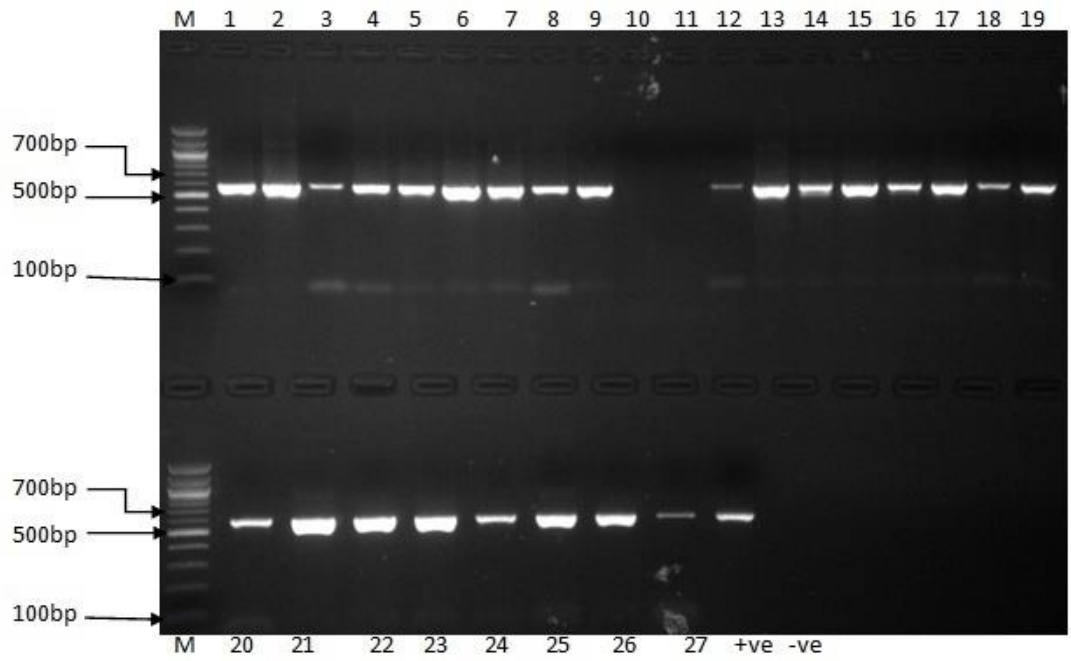

Figure 3. Gel electrophoresis of CPV-2 amplicons showing positive band of 583 bp of the target gene. The region of amplification was VP2 (Viral protein) gene. Lines 1-9 and 12-27 (both in upper and lower line) are positive field samples, whereas, lines 10 and 11 are negative field samples. Here +ve and –ve indicates positive and negative control, respectively and M is 100 bp molecular marker.

Comparative analysis

Data obtained were analysed using McNemar's statistical test and level of significance was accepted at $p < 0.05$. Immunochromatographic IC test showed that 68 (83%) of the collected sample were positive for CPV-2 (Figure 2), while conventional PCR revealed that 79 (96%) of the samples were positive for canine parvovirus (Figure 3). There was a significant difference ($p < 0.05$) between the two diagnostic methods. The sensitivity of IC test over PCR was 86%, specificity 100%, positive predictive value 100%, negative predictive value 21%, positive likelihood ratio 0.86, negative likelihood ratio 0.14 and disease prevalence of 96%.

Discussion

Early diagnosis and aggressive treatment is very essential for the survival from canine parvoviral enteritis. It gives the clinician and the client the prognosis of the disease, reduces cost and alleviates suffering for the patient (Seton, 2002). Among the diagnostic methods of CPV-2, IC test is more convenient and readily available for clinicians and dog owners to use even in their homes (Jamshidi et al., 2013). In this study sensitivity of IC test over PCR was 86%, which agrees with previous workers (Tinky et al., 2015) and (Jamshidi et al., 2013) who reported sensitivity of 72.22% and 84% respectively. The result is also in accordance with (Vakili et al., 2014), who stated that PCR is more sensitive than IC test. Specificity of 100% was also observed in this study, IC test has been previously described to be 100% specific unlike other non-molecular diagnostic methods like haemagglutination test (HA) and haemagglutination inhibition test (HI) (Schmitz et al., 2009; Vakili et al., 2014). Serologic tests are rarely used in diagnosis of CPV-2 infection because most dogs are vaccinated against CPV or have been exposed to the virus in some stage of their life. Moreover, haemagglutination inhibition (HI) is usually time consuming and not employed in acute condition, as the presence of high titre of haemagglutination inhibition (HI) in an unvaccinated dog may take up to three days post clinical illness in CPV-2 infection (Greene, 2012). In this study the positive predictive value of 100% and negative predictive value of 21% recorded is an indication that IC is a reliable diagnostic test for the detection CPV-2 antigen in faecal samples.

In this present study, McNemer's test showed significant difference between the two diagnostic methods IC test and PCR at p value < 0.05. This result disagrees with the previous works of Decaro *et al.* (2010) and Jamshidi et al. (2013) who did not find any significant different between Immunochromatographic test and molecular methods. The result is also attributable to the quantity of CPV-2 viral antigen in feaces at the time of testing, which is a major disadvantage of IC test, as it may likely affect the test result (Decaro et al., 2010; Jamshidi et al., 2013). It is proven that samples with viral load more than 109 DNA copies/mg faeces were generally detected by in-house assay (Decaro et al., 2010).

Therefore early testing is recommended whenever there is index suspicion of canine parvovirus infection. The high titre shedding of CPV in faeces often precedes the appearance of clinical signs which, has made the IC test a useful diagnostic technique. IC test is an antigen detection kit that captures the canine parvovirus in canine faeces. In monitoring CPV-2 infections with IC test, suspected cases with negative results need to be repeated to rule out false negative or a more sensitive diagnostic method employed. Potentially false negative results could be accounted in the late stages of the infection.

Conclusion

It was concluded from the study that PCR is more sensitive than IC test. However, they are comparable and IC test can be employed in routine clinical diagnosis and in screening for canine parvovirus in veterinary clinics and kennels on daily basis. Sensitivity of IC test depends on the quantity viral antigen in the faeces, early testing of suspected cases of CPV-2 infections is therefore recommended. To avoid any misinterpretation, the negative samples from the IC test may be sent for PCR testing.

Author Contribution

C. S. Ukwueze designed the work and collected samples, while B. M. Anene and R. C. Ezeokonwo evaluated the design and the results. C. I. Nwosuh was involved in the laboratory work and preparing the manuscript. All authors read the article and approved the final version to be published.

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Competing interests

The authors have declared that no competing interests exist.

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