CANINE PARVOVIRUS: A REVIEW

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ABSTRACT

Canine parvovirus (CPV) is a single stranded non-enveloped DNA virus belonging to the family of parvoviridae that requires rapidly dividing cells for its replication. The virus is however, extremely tough, surviving exposure to many routine disinfectants and surviving from months to years in soil or on fomites. There are currently three widely recognised strains of canine parvovirus: CPV-2a, CPV-2b and CPV-2c. Canine parvovirus is highly infectious and is transmitted from dog to dog by direct or indirect contact through feco-oral route and has been reported in many countries. The predisposing factors associated with the development of clinical parvovirus disease include stressors (such as early weaning, overcrowding and parasite load), insufficient passive or active immunity, geographical region and the presence of other pathogens. The disease has been reported to be more severe in puppies than in adult dogs. There are two common clinical forms: gastro-enteritis form common in adults and myocarditis form common in puppies. The disease is characterized by lethargy, leucopenia, dehydration, anorexia, fever, vomiting and diarrhea, which may contain mucus or blood with a very strong foul smell. Control of the disease is mainly adoption of vaccination and by hygienic measures. Interference by maternally derived antibodies is regarded as a major cause of canine parvovirus vaccination failures in young dogs. Veterinarians and researchers have come to the conclusion that the surest way to know that a puppy has adequately responded to vaccination or to confirm the immune status in a mature dog is to check the antibody levels in the dog's serum.

KEY WORDS: Dogs, Canine parvovirus, Bloody diarrhea, Vomiting, Leucopenia

INTRODUCTION

Canine parvovirus (CPV) belongs to in the family *parvoviridae* and subfamily *parvovirinae* that affects vertebrates (Dogonyaro, 2010). The *parvovirinae* is further split into three genera namely: *Parvovirus, Erythrovirus* and *Dependovirus* (Berns, 1990; Tattersal and Cotmore, 1990). Canine parvovirus is within the feline parvovirus sub group of the genus *Parvovirus* (Siegl *et al.,* 1985). The virus is very similar to feline panleukopenia virus (FPLV) and is 98 % identical to it, differing only in 6-7 amino acids of the viral capsid protein VP_2 (Carter and Wise, 2006; Hoelzer and Parrish, 2010; Mittal *et al*., 2014; Chollom *et al.,* 2013). It is also closely related to mink enteritis virus (MEV) raccon parvovirus (RPV) and blue fox parvovirus (BFPV) (Jones *et al*., 1997). The minute virus of canine and bovine parvovirus also belongs to the family parvoviridae (Dogonyaro, 2010).

Canine parvovirus was designated type 2 to distinguish it from a previously recognized parvovirus of dogs known as minute virus of canines (Binn *et al.,* 1980; Carmichael *et al*., 1994). According to Tilley and Smith (2011), the original canine parvovirus underwent genetic

alterations developing into CPV-1 and CPV-2, but canine parvovirus type 2 is antigenetically unrelated to canine parvovirus type 1 (Carmicheal *et al*., 1994).

Many authors stated that canine parvovirus type 2 originated from feline parvovirus and continued to undergo mutation giving rise to other variants of canine parvovirus type 2 (Dogonyaro, 2010; Cholom *et al.,* 2013; Sherry *et al.,* 2012; Mittal *et al.,* 2014). Canine parvovirus type 2 developed further into canine parvovirus type 2a in 1978 which varied from canine parvovirus type 2 by seven amino acid substitutions and one epitope (Hoelzer and Parrish, 2010; Parrish *et al.,* 1991). Canine parvovirus type 2a essentially replaced canine parvovirus type 2 worldwide by 1982 (Parrish *et al.,* 1988; Chollom *et al.,* 2013). Another variant, canine parvovirus type 2b emerged in 1984 and became the predominant canine parvovirus variant worldwide by 1988 (Tilley and Smith, 2001; Parrish *et al.,* 1991).

Canine parvovirus type 2b differs from canine parvovirus type 2a only in the changed residue 426-ASN to ASP (Sherry *et al.,* 2012; Hong *et al.,* 2007). In 2000, the latest variant, canine parvovirus type 2c was first detected in Italy and subsequently in other countries (Nakamura *et al.,* 2004; Buonavoglia *et al.,* 2001; Decaro *et al.,* 2006a; Perez *et al.,* 2007; Kapil *et al.,* 2007; Hong *et al.,* 2007).

Canine parvovirus type 2c differs from both canine parvovirus type 2b and canine parvovirus type 2a in the same condon for residue 426 which changed to GLU (Hoelzer and Parrish, 2010). Thus there is not a great deal of antigenic difference among CPV-2a, CPV 2b and CPV-2c compared to the difference between CPV-2 and the three variants (Sherry *et al.,* 2012). The relative proportion of the variant varies from country to country (Truyen *et al.,* 1996; Chinchkar *et al.,* 2006; Pereira *et al.,* 2007; Martella *et al.,* 2004).

EPIDEMIOLOGY

Distribution

In the late 1970s, a new infectious disease of puppies was observed worldwide (Appel *et al.,* 1979). Within 12 months, CPV-2 was identified as the aetiological agent of severe haemorrhagic gastroenteritis in dogs (Kelly, 1978; Appel *et al.,* 1979) and spread rapidly all over the world (Dogonyaro, 2010). The disease was almost simultaneously also reported in Canada (Thompson and Gagnon 1978; Gagnon and Povey, 1979), Australia (Kelly, 1978), United Kingdom (Jefferies and Blackmore 1979; McCandlish *et al.,* 1979), New Zealand (Grumbrell, 1979) and Belgium (Burtonboy *et al.,* 1979). The out breaks were shown to be due to a novel pathogenic canine parvovirus of dogs which was first described in 1970 (Binn *et al.,* 1970) and designated canine parvovirus type 1 (CPV-1) (Dogonyaro, 2010). According to Tilley and Smith (2011), the original canine parvovirus underwent genetic alterations developing into canine parvovirus type 1 and canine parvovirus type 2. Canine parvovirus type 2 is antigenically unrelated to canine parvovirus type 1 (Carmichael *et al.,* 1994; Sherry *et al.,* 2012).

In 1979 and 1980, an antigenic variant of canine parvovirus type 2 was identified in several different countries using monoclonal antibodies and the variant was termed canine parvovirus type 2a (CPV-2a). In the 1980s the virus underwent a further antigenic change and the new variant that emerged was referred to as canine parvovirus type 2b (CPV-2b) (De Ybanez *et al.,* 1995). These new variants replaced canine parvovirus type 2 and have continued to circulate in dogs today (Hong *et al.,* 2007).

Canine parvovirus type 2 enteritis was reported in Nigeria in 1985 (Kamalu, 1985). The occurrence of canine parvovirus type 2 in Nigerian mongrel dogs raised questions as to the source of the infection (Kamalu, 1985). Canine parvovirus type 2 has been described genetically and serologically in South Africa (Steinel *et al.,* 1998). Currently, the prevalence of canine parvovirus type 2a and type 2b are at varying levels in different countries worldwide (Truyen *et al.,* 2000). Canine parvovirus type 2b is the

predominant antigenic type in USA, South Africa (Parrish *et al.,* 1991, Steinel *et al.,* 1998, Doyongarro 2010) and Turkey (Yilmaz *et al.,* 2005). Canine parvovirus type 2a is more common in Italy (Sagazio *et al.,* 1998) and other European countries (Buonavoglia *et al.,* 2000, 2001; Martin *et al.,* 2002, Mochizuki *et al.,* 1993a).

A new antigenic variant has been reported in dogs in Europe and Southern Asia (Buonavoglia *et al.,* 2001; Nakamura *et al.,* 2004; Decaro *et al.,* 2006a). This new antigen variant is currently circulating together with canine parvovirus types 2a and 2b in Europe and South America (Martella *et al.,* 2004, Perez, 2007). These canine parvovirus type 2 mutants previously designated as Glu-426 mutant and now named cannel parvovirus type 2c (CPV-2c) has also been detected in Vietnam (Nakamura *et al.,*2004) and its pathogenicity has been investigated (Decaro *et al.,* 2005b). There are presently no documented cases of canine parvovirus type 2c infection from Africa (Dogonyaro, 2010). In Nigeria, only canine parvovirus type 2a has been reported (Dogonyaro, 2010).

Susceptible Host

All sexes, ages and breeds of dogs have been found to be susceptible to CPV-2 infection (Castro *et al.,* 2007 and Gombac *et al.,* 2008). In Slovenia, Gombac *et al.* (2008) stated that 83.3% of dogs that died in a study were males while 16.7% were females. The observed difference in the susceptibility was statistically significant. Castro *et al.* (2007) reported that there was no significant difference among the sexes of dogs with CPV infection in a study done in Rio de Janeiro.

An investigation into ages of dogs prone to canine parvovirus infection in Brazil showed that infection occurred mostly in 2-4 months old puppies (Cubel Garcia *et al.,* 2000, Castro *et al.,* 2007). In Slovenia, the highest percentage (67.6%) of death due to CPV infection was noticed in dogs below six months of age, followed by dogs aged between six months to one year, (25.7%) and was lowest, (6.8%) in dogs one year old and above (Gombac *et al.,* 2008). Tilley and Smith (2011) stated that many cases of CPV infection in dogs are seen between six weeks and six months of age with the disease being more severe in younger puppies.

It has also been reported that Doberman pinscher, Rottweiler and German shepherd dogs appear to be under greater risk of developing parvoviral enteritis (Glickman *et al.,* 1985; Houston *et al.,* 1996) than Pit bulls, Labrador retrievers, English springers, Spaniels and Alaskan sled dogs (Tilley and Smith, 2011). Local breeds of dogs were the least susceptible to the infection when compared with the foreign breeds (Chollom, *et al.,* 2013). Local breeds have a greater degree of resistance against the virus and have been regarded as healthy carriers of CPV (Nelson and Couto, 1998). This is of great epidemiological relevance as they play important roles in distribution of the virus indiscriminately to other breeds due to their free ranging habits (Chollom *et al.,* 2013). Local breeds pose a great danger to the foreign breeds of dogs which are more susceptible to the agent (Nelson and Couto, 1998).Canine parvovirus mostly affects dogs (Tilley and Smith, 2011). Several other species of wild carnivores, such as coyotes, raccoons, red foxes and wolves are also susceptible to canine parvovirus infection (Barker *et al.,* 1993; Truyen *et al.,* 1998). Canine parvovirus infection has also been reported in the bat eared fox, honey badger, cheetah, African wild cat and Siberian tiger (Steinel *et al.,* 2000).

Transmission

Canine parvovirus is highly contagious (Dogonyaro, 2010). Its transmission from infected to susceptible dogs takes place indirectly by the feaco-oral route but dogs can also become infected from viruses present on formites such as shoes, clothing, human hands, food bowls and other utensils (Pollock and Carmichael, 1982; Carmicheal, 1994; Decaro *et al.,* 2005b). This is the major means of transmission. The virus is also transmitted by direct contact with the infected dogs (Kahn and Line, 2005). The

incubation period of canine parvovirus in the field is 4-5 days but with experimental infection; it is three days (Dogonyaro, 2010). Unlike most other viruses, canine parvovirus is stable in the environment and resistant to the effects of heat, detergent, alcohol and many disinfectants (Ernest, 2009).

Population at Risk

Puppies are the most susceptible to canine parvovirus infection due to lack of protective immunity from maternally derived antibodies or from ineffective responses to vaccinations (Patterson, 2007). The disease is usually prevalent in unvaccinated dogs due to ignorance of the owners, high costs of vaccines, poor husbandry and facilities for biosecurity practices (Muzaffar *et al.,* 2006). The continued presence of the pathogen therefore makes the disease endemic in particular areas (Dogonyaro, 2010).

PATHOPHYSIOLOGY OF CANINE PARVOVIRUS

After ingestion, the virus replicates in the lymphoid tissues of the oropharynx from where it spreads to the blood stream attacking rapidly dividing cells throughout the body especially those in the bone marrow, lymphopoietic tissues and crypt epithelia of the jejunum and ileum (Kahn and Line, 2005) and (in young dogs) myocardial cells (Ettinger and Feldman, 2005). Early lymphatic infection is accompanied by lymphopenia and precedes intestinal infection and gastrointestinal signs. Replication in the bone marrow and lymphopoetic tissue causes neutropenia and lymphopenia respectively and three days post infection, rapidly dividing intestinal crypt cells are infected leading to viral shedding in the faeces which peaks when clinical signs appear (Kahn and Line, 2005). Necrosis of the infected intestinal crypts leads to villi collapse and loss of intestinal epithelial integrity causing hemorrhagic diarrhea due mainly to increased intestinal permeability and mal-assimilation from abnormal mucosal function (Ettinger and Feldman, 2005). Normal enteric bacteria eg, *Clostridium perfringens* and *Escherichia coli* enter the denuded mucosa and may gain entry to the blood stream, resulting in bacteremia (Kahn and Line, 2005).

CLINICAL SIGNS OF CANINE PARVOVIRUS

Clinical features of infected dogs ranges from asymptomatic infection to fulminant disease and sudden death and the signs are seen in young and immuno-compromised dogs as well as predisposed breeds (Ettinger and Feldman, 2005) and may be exacerbated by concurrent infection (Kahn and Line, 2005). Canine parvovirus infection manifests in two clinical forms viz: myocarditis and gastroenteritis with the former seen in young puppies especially in the early neonatal period. Early infection in the life of puppies will lead to myocardial necrosis with either acute cardio-pulmonary failure (causing pulmonary edema, cyanosis and collapse) or scarring of the myocardium and progressive cardiac insufficiency. The canine parvovirus myocarditis is no longer seen because effective immunization of bitches protects pups during early period of life (Kahn and Line, 2005; Dogonyaro 2010).

The canine parvovirus gastroenteritis is most common in puppies 6-20 weeks of age when the maternal antibody protection wanes and vaccination has not yet adequately protected the puppies against infection (Hoskins, 1998 and Mosallanejad *et al*., 2008).

The most common clinical and haematological findings of canine parvovirus infection are vomiting, anorexia, depression, dehydration, foul smelling bloody diarrhea, hypothermia or fever marked thrombocytopenia and leucopenia (Yilmaz *et al.,* 2005). Large fluid and protein losses from vomiting and diarrhea can cause severe dehydration and hypovolemic shock. Prolonged capillary refill time, tachycardia, hypotension, cool extremities, and low rectal temperature are signs of shock and hypo-perfusion while abdominal pain secondary to acute gastroenteritis or intussusceptions may

be evident on palpation (Ettinger and Feldman, 2005).

MORBIDITY AND MORTALITY FOLLOWING CPV INFECTION

The morbidity and mortality rates reported for canine parvoviral enteritis in dogs have a wide range with the highest occurrences in young weaned pups (Eugster *et al.,* 1978; Nelson *et al.,* 1979). Death is usually due to the complications of the severe dehydration, circulatory shock, suppression of immune system and depression of bone marrow (Dogonyaro, 2010).

DIAGNOSIS

Diagnosis of canine parvoviral infection is very important, especially in kennels and shelters in order to isolate infected dogs and prevent transmission to susceptible contact animals. Diagnosis on the basis of clinical signs is not definitive, since several other pathogenic organisms can cause diarrhea in dogs. Therefore, a clinical diagnosis of canine parvovirus infections should always be confirmed with laboratory tests (Dogonyaro, 2010). Various laboratory methods have been developed to detect canine parvovirus in the faeces of infected dogs. They include electron microscopy (EM) (Alicia *et al.,* 1999), Enzyme-linked immunosorbent asssy (ELISA), immunochromatographic tests (IC), haemagglutination (HA) tests, viral isolation (VI), haemagglutination inhibition (HI) tests, conventional polymerase chain reaction (C-PCR) and real-time polymerase chain reaction (RT-PCR) (Desario *et al.,* 2005).

Electron Microscopy

Electron microscopy as a means of diagnosis, allows one to visualize minute objects as small as one nanometer. The specimens are not illuminated with light but bombarded by electrons as a source for image formation. Electron microscopy allows the identification and confirmation of CPV based on their size and morphology. Viruses are observed in groups or seen as single particles stained negatively with uranyl acetate, phosphotungstic acid or methylamine tungsten (Alicia *et al.,* 1999). The identification of the canine parvovirus type 2 viruses in faeces can be carried out only during the elimination period of the viruses, which occurs between the 3rd and the 9th day of infection. The sensitivity of electron microscopy is believed to be relatively low due to the large quantities of viruses required for a positive test result (Esfandiari and Klingeborn, 2000).

Immunochromatography Test (ICT)

The SNAP Rapid Canine Parvovirus Antigen Kit (BioNote, Korea) for example is only one of the many commercialized IC tests, and is a rapid field diagnostic method used in clinical practice because the test procedure is simple. As a result, it can be performed by veterinarians as well as dog owners (Esfandiari and Klingeborn, 2000). However, a large amount of viral antigen is required to produce a clearly visible band and the interpretation of results may be affected by the subjectivity of the test operator. This is especially common when numbers of viruses are low (Mochizuki *et al.,* 1993b; Uwatoko *et al.,* 1995; Estandiari and Klingeborn, 2000; Desario *et al.,* 2005). This test detects all three viral variants (CPV-2a, -2b, and -2c). The test results are most accurate if the test is performed within 5 days of onset of clinical signs. Negative tests should be repeated daily on any dog suspected to have parvoviral infection based on signs (Ettinger and Feldman, 2005).

Enzyme-Linked Immunosorbent Assay (ELISA)

An easily accessible in-clinic procedure called immunoblot ELISA assay (rapid dot-ELISA assay or immunocomb ELISA test) has been developed for the semi-quantitative assay of CDV and CPV IgG antibody titres in the sera of vaccinated mature and young dogs using the enzyme-linked immunosorbent assay (ELISA) technology (Naveh *et al.,* 1995; Waner *et al.,* 1996; Waner *et al.,* 1998; Truyen, 2001; Eghafona

et al., 2007). The test is based on solid phase "dot"-ELISA technology, and antigens are applied to test spots on a comb-shaped plastic card (Biogal, 2014).

The blood samples to be tested are mixed with diluents in the first row of wells of a multi-chamber developing plate. The test spots on the comb are then incubated with the sample in the developing plate. Specific IgG antibodies from the samples, if present, bind to the antigens at the test spots.

After incubation unbound antibodies are washed from the antigen spots on the comb in the second well of the developing plate. In the third well the spots are allowed to react with an anti-dog IgG alkaline phosphate conjugate, which will bind to antigen-antibody complexes at the test spots. After two more washes in the fourth and the fifth well, the test spots are allowed to develop color by an enzymatic reaction in the last well. The intensity of the color directly corresponds to the level of antibodies in the test sample.

The immunity against CPV, CDV and ICH is scored individually on a scale from 0 to 6. The score of 0 means that the dog has no detectable antibodies against the disease, and scores of 1-2 mean a low level of antibodies not considered to be protective. Scores of 3-4, however, are consistent with a protective level of antibodies, and the score of 5-6 reflects a high level of humoral immunity. Thus, for dogs with scores of 3 or higher revaccination is not needed (Biogal, 2014).

According to the producer of the test (Biogal, 2014) the specificity and sensitivity for CPV are 100 % and 97 %, respectively.

Lateral Flow Immunoassay Test (LFAT)

The lateral flow assay (LFA) is a rapid and convenient diagnostic test which may be performed under most conditions and is especially useful for field application. It is easy, simple and rapid to use as a confirmatory test. The lateral flow assay is produced in a dipstick format. Lateral flow tests are a form of immunoassay in which the test sample flows along a solid substrate via capillary action. After the sample is applied to the test, it encounters a coloured reagent which mixes with the sample and transits the substrate encountering lines or zones which have been pretreated with an antibody or antigen. Depending upon the analytes present in the sample, the coloured reagent can become bound at the test line or zone (Dogonyaro, 2010).

Haemagglutination test (HA)

The haemagglutination assay (HA) is a method of quantification of viruses by means of haemagglutination. It is an easy, simple and rapid method which can be applied to large numbers of samples. Several viruses from different viral families, including the *Parvoviridae*, possess haemagglutinins on their surfaces that have the ability to agglutinate the red blood cells of several different animal species by binding to receptors on the surface of the red cells. Canine parvovirus is able to agglutinate porcine red cells. Red cells washed in phosphate buffered saline are added to a suspension of parvovirus with a pre-determined titre in a microtitre plate and observed for haemagglutination. The test is regarded as positive when the HA can be blocked by virus-specific antisera (Dogonyaro, 2010).

The advantages of HA are its speed and ease of performance, and the fact that living host systems are not required. Specific haemagglutination activity is detected in the faeces up to nine days post infection. Canine parvovirus strains lacking HA activity have been reported (Parrish *et al.,* 1988; Cavalli *et al.,* 2001). However, the HA test carried out in a 96-well plate format allows rapid processing of many samples in which results can be read after only four hours (Desaria *et al.,*2005).

Haemogglutination Inhibition (HI) Test

The haemagglutination inhibition (HI) test is mostly used to evaluate maternally derived antibodies and sero-conversion after CPV vaccination. Moreover, the haemagglutination

inhibition (HI) test is also carried out to determine the amount of antibody specific to antigenic characterization in a haemagglutination inhibition assay with a panel of MAbs (Buonavoglia *et al.,* 2001; Nakamura *et al.,* 2004; Desario *et al*., 2005; Martella *et al.,* 2006). The CPV strains can be typed as CPV-2 (original type), CPV-2a, CPV-2b or CPV 2c on the basis of MAb reactivity (Decaro *et al.,* 2006a).
The value of the HA and HI procedures lie in their applicability to two types of problems: the rapid identification of new virus isolates and the determination of the presence or absence of antibodies in sera obtained during the course of a disease. If a new isolate can be shown to agglutinate red cells, it can readily be determined whether any known antisera are capable of inhibiting agglutination. The real value of HI for CPV is to confirm the identity of CPV viruses as indicated under the HA section (Dogonyaro, 2010).

Viral isolation methods
Isolation of CPV requires cell culture facilities, capable and skilled personnel, and also the permissive cell lines to be used. Additionally, viral isolation is time-consuming. It requires a long incubation period (5-10 days) and additional testing by immunofluorescence (IF) assay using an anti-CUP conjugate (Decaro *et al.,* 2006a). Haemagglutination (HA) can also be used in order to detect viral antigens in the cell culture supernatant. The main disadvantage of viral isolation however, is low sensitivity. It has been demonstrated in natural and experimental infections that CPV-2 is detectable by viral isolation only for a few days post-infection (Desario *et al.,* 2005).

Molecular Detection and Identification of CPV
The diagnosis of CPV infection on the basis of clinical signs alone is inconclusive as mentioned earlier. Molecular methods are the methods of choice for CPV diagnosis because they are based on detection of DNA which has been shown to be highly sensitive (Buonavoglia *et al.,* 2001). The identification and characterization of CPV strains using the TaqMan assay and minor groove binder probe technology were described by Decaro *et al.,* (2005c; 2006b).

i) **Conventional polymerase chain reaction (PCR)**

In contrast to the various diagnostic methods discussed above, the conventional polymerase chain reaction (PCR) was demonstrated to be more sensitive for the detection of CPV (Buonavoglia *et al.,* 2001; Decaro, *et al.,* 2005a; Hong *et al.,* 2007). Sequence analysis provides ample information for CPV typing since the fragment amplified by PCR using primers 555 forward and 555 reverse, encodes at least two informative amino acids (residures 426 and 555 of the VP2 protein are encoded by nucleotides 4062-4064 and 4449-4451, respectively). These primers allow differentiation between CPV-2 (original type), CPV-2a, CPV-2b and the Glu-426 mutant (Desario *et al.,* 2005; Decaro *et al.,* 2005a; 2006b; Hong et al., 2007). By sequence analysis of the short fragment amplified with primers 555 forward and 555 reverse, discrimination between canine parvovirus types 2 and 2a is based only on a single nucleotide polymorphism (G-A) that determines the replacement of the amino acid Val (type 2) with IIe (type 2a) at residue 555 of the VP2 protein (Desario *et al.,* 2005).

ii) **Real-time polymerase chain reaction (RT-PCR) assay**

The RT-PCR assay, based on the TaqMan and Minor groove binders were demonstrated to be more sensitive than traditional techniques including the conventional PCR. The quantitative real-time PCR is sensitive, specific, and more reproducible and allows the detection and quantification of CPV-2 nucleic acid within a few hours, and it is less time consuming (Decaro *et al.,* 2005c; 2006b; Hong *et al.,* 2007). Also, there is less risk

of carry-over contamination than with the traditional and conventional PCR methods (Decaro *et al.,* 2005a; 2006b). The advantages of real-time PCR minor groove binder probes™ Technology (Kutyavin *et al.,* 2000) include:

a. The utilization of a minor groove binder (MGB) which attaches to single-stranded DNA probes that enhances the stability of the duplex formed between the probes and the target region of the CPV-2 genome.
b. Allowing an increase in the melting temperature (Tm) of the DNA duplex.
c. Enabling the use of smaller probes capable of detecting short conserved regions of the CPV-2 genome (Dogonyaro, 2010).

However, the molecular assays, especially the real-time PCR method, require expensive equipment, reagents and skilled personnel, thus, their routine use as diagnostic tests for the veterinary practice is limited (Desario *et al.,* 2005). Nevertheless, there are efforts by several companies to adapt molecular methods to clinical practice, taking advantage of microchip technology that would reduce the cost and size of the equipment necessary for testing on site (Desario *et al.,* 2005).

Postmortem Examination

The characteristic findings for CPV include segmental small intestinal enteritis with loss of villi, necrosis of intestinal crypt epithelial cells, intra-nuclear inclusion bodies in the tongue, small intestine, and Payer's patches, as well as severe lymphocyte depletion in lymphoid tissue (Ettinger *et al.,* 1995).

TREATMENT

Treatment of CPV mediated enteritis is often unsuccessful in spite of intense efforts by veterinarians. Survival rate depends on how quickly CPV is diagnosed, the age of the animal and how aggressively the treatment was administered. Treatment for severe cases that are not diagnosed early usually involves extensive hospitalization due to the severe dehydration and damage to the intestines and bone marrow (Dogonyaro, 2010).

Treatment ideally consists of intravenous fluids, suppression of vomiting and antimicrobial drugs (Macintire, 2004). Administration of crystalloid fluids such as Ringer's or 0.9 % saline at volumes sufficient to restore and maintain hydration despite ongoing fluid losses is a key element of therapy. Supplementation of fluids with potassium and dextrose may be necessary to maintain normal serum potassium and glucose concentration (Ettinger *et al.,* 1995). Once the dog can retain fluids, the IV fluids are gradually discontinued and very bland food slowly introduced. A puppy with mild clinical signs can recover in two or three days if the IV fluids are begun as soon as clinical signs are noticed. If more severe, depending on treatment, puppies can remain ill from five days up to two weeks. It is important to note that the last vaccine dose should be administered at 16 weeks of age. Untreated cases of parvovirosis have a mortality rate approaching 90 %, but with aggressive therapy survival rates may approach 80-95 % (Prittie, 2004). Other considerations in the supportive care of affected dogs include control of persistent vomition with antiemetic drugs such as metoclopramide, phenothiazine derivatives (chlorpromazine), serotonin antagonist (ondansetron), and NK-1 receptor antagonists (maropitant).

PREVENTION

Prevention is the only way to ensure that a puppy or dog remains healthy because the disease is extremely virulent and contagious. The virus is extremely hardy and has been found to survive in faeces and other organic materials such as soil for over a year (Dogonyaro, 2010). It survives extremely cold and hot temperatures. The only household disinfectants that kill the virus are

chlorine-based (Ettinger *et al.,* 1995). Disinfection of the area can only be accomplished by cleaning food bowls, water bowls, and other contaminated items with a solution of half cup of chlorine bleach in a gallon of water (133mls in 4 liters of water, Ernest, 2009). A dog that recovers successfully from CPV sheds the virus for a few days (Dogonyaro, 2010). Ongoing infection risk is primarily from faecal contamination of the environment. Puppies receive CPV vaccination as part of their multiple agent vaccine given at 8, 12 and 16 weeks of age after which annual booster vaccination should be given yearly (Ernest, 2009). The vaccine will take a few days to stimulate effective levels of immunity therefore the contagious individual should remain in quarantine until other animals are protected (Dogonyaro, 2010).

CONTROL

Control of CPV is a global challenge. The most effective method of control is vaccination (Ernest, 2009; Dogonyaro, 2010). Vaccines based on the original antigenic type CPV-2, have been shown to protect dogs against infection with the new (CPV-2a/2b) antigenic types (Yule *et al.,* 1997), and certain vaccines based on FPLV have been shown to protect cats from being infected with CPV-2b (Chalmers *et al.,* 1999). The ideal is for vaccines to contain the latest antigenic types of a given virus, as this confers the most complete protection, provided the new vaccines are as immunogenic as the old ones (Truyen, 2006). Puppies are generally vaccinated in a series of doses, extending from the earliest time that the immunity derived from the mother wears off until after that passive immunity is definitely gone (Nelson *et al.,* 1998). The duration of immunity produced by CPV vaccines has been tested for all major vaccine manufacturers in the United states and has been found to be at least three years after the initial puppy series and a booster one year later (Schultz, 2006).

VACCINATION AGAINST CANINE PARVOVIRUS

In the late 1970s and early 1980s, both live and inactivated FPLV vaccines were used to protect dogs against canine parvoviral disease due to the shared antigens which stimulated cross protection. However, the level of protection that they afforded was poor and the duration of the immunity was short (Dogonyaro, 2010). These vaccines were replaced by killed and attenuated canine parvoviral vaccines. The latter provided excellent protection and longer immunity (Spibey *et al.,* 2008). Currently the attenuated vaccines are derived from either canine parvovirus 2b isolates or the original type 2 viruses (Dogonyaro, 2010).

There have been reported cases of canine parvoviral infections (CPV type 2) after vaccinations. This poses a challenge to veterinarians and vaccine manufacturers. There are concerns that vaccines used currently to prevent canine parvoviral infections in dogs may fail to effectively protect pups against canine parvovirus type 2 antigenic variants (Truyen, 2006). In spite of the fact that the original canine parvovirus type 2 was completely replaced by CPV types 2a, 2b and 2c, it is still used in most commercial vaccines (Dogonyaro, 2010). Various studies have however, demonstrated that the Canine parvovirus V-2 vaccines are still effective in inducing protection against canine parvovirus 2 variants (Greenwood *et al.,* 1995; Carmicheal, 1994; Yule *et al.,* 1997; Spibey *et al.,* 2008; Larson and Schultz, 2008).

Canine parvovirus infection in 6-week-old pups born to vaccinated bitches is likely due to a failure of the maternally derived antibodies (MDA) to protect against canine parvovirus type 2. This could be due to sub-optimal transfer of MDA to the pups (Decaro *et al.,* 2006c). Morbidity and mortality in pups may also be because of to inadequate protection against the CPV-2 variants by MDA rather than to a failure in the transfer of MDA from the bitch to its offspring (Decaro *et al.,* 2004b). Due to the physicochemical properties of canine parvovirus 2 (high resistance in the environment with long

persistence in kennels and shelters), a good vaccine should prevent the disease as well as the viral shedding of the wild strains following infection in dogs (Dogonyaro, 2010). Dogs with HI MDA titres of < 1:80 are considered protected against disease and viral shedding after challenge with virulent canine parvovirus 2 (Pollock and Carmichael, 1982). However, it has been observed that pups with HI MDA titres of up to 1:160, originally considered protected against canine parvovirus 2 infection (Pollock and Carmichael, 1982), were infected by canine parvovirus 2b and shed virus in their faeces (Decaro *et al.,* 2005e). Consequently, the minimal MDA level required for protection from canine parvovirus 2 infection has to be reconsidered (Decaro *et al.,* 2005e). There have been a number of reports stressing the need to update the canine parvovirus 2 vaccines by replacing the original canine parvovirus types 2 (which are extinct) with the canine parvovirus 2 variants currently circulating in local canine populations (Dogonyaro, 2010). Polyvalent CPV vaccines could represent an alternative strategy to improve the effectiveness of prophylaxis against canine parvovirus (Martella *et al.,* 2005; Truyen, 2006; Cavali *et al.,* 2008).

FACTORS INFLUENCING VACCINE EFFICACY

Vaccine failure is usually due to problems with either client education or compliance with good animal management practices (Rashid *et al.,* 2009). It is important for clients to understand the proper timing and method of vaccine administration, what to realistically expect from vaccine administration and the importance of minimizing immunosuppressive factors and exposure to high doses of infectious agents in vaccinated animals (James, 1999). A safe vaccine is not simply one that has been manufactured, tested and found to be safe in clinical trials. Important as those aspects are, there are other possibilities for making immunization safer. These include safe transport to the point of administration, safe administration, safe disposal of the vial and injection equipment and post-marketing surveillance to detect any unexpected reactions as soon as possible (Clements *et al.,* 2004). These factors are:

1. Vaccine Factors

Veterinary vaccines whether attenuated or non-infectious from different manufacturers can vary in their potency, efficacy and duration of immunity. Attenuated vaccines tend to induce stronger and long-lasting immunity than non-infectious vaccines (Rashid *et al.,* 2009). Non-infectious vaccines which include killed, toxoid, subunit and DNA vaccines are safer and more stable than attenuated vaccines. However, due to risk of using live vaccines in pregnant or immunosuppressed animals as well as the risks of shedding vaccine virus, non-infectious vaccines are preferred for some diseases (James, 2007).

Vaccine, if used properly, induces protection from challenge in a high percentage of vaccinated animals. This is achieved by presenting the correct antigen in a safe manner to the host's immune system. However, wild type organisms change with time and place. Vaccines that were effective may become ineffective due to antigenic drift. Individual veterinary vaccines often incorporate different strains of organisms (Rashid *et al.,* 2009). For example, several strains of canine distemper virus may be found as ineffective vaccines. Some vaccines with poor efficacy may not be recognized until after their use. During a distemper outbreak in Finland, a disproportionate number of vaccinated dogs had been vaccinated with one popular vaccine, which was withdrawn from the market by the manufacturer once vaccine failure was recognized (Ek-Kommonen *et al.,*1997). Significant differences in antibody titres were demonstrated between this vaccine and three other distemper vaccines used. Fifty four percent of the dogs vaccinated with the poorly efficacious vaccine had no detectable antibodies

to this pathogen.

Some vaccines only contain specific strains of the virus or bacteria that cause disease. This is true for feline panleukopenia and canine leptospirosis infections in dogs. Vaccines against canine leptospirosis only protect against two types of the bacteria and would not protect an animal against the other types (Rashid *et al.,* 2009).

Annual Revaccination

Vaccine manufacturers often recommend that booster doses of vaccines should be given following a primary course (Ettinger *et al.,* 1995). In most cases, this advice is based on studies of duration of immunity showing that animals given a primary course of vaccination are protected when challenged 12 or 24 months later. From observations, on the persistence of antibody levels following vaccination, particularly of the canine virus, it has been suggested that dog do not require to be vaccinated annually, rather a period of 3 years between vaccinations has been suggested (Ramsey and Bryn,2001).

Adjuvants

All non-living vaccines require an adjuvant to provide an adequate immune response. A wide range of adjuvants are used in animal vaccines including aluminum salts and derivatives of the glycoside sapnin (Rashid *et al.,* 2009). The major theoretical advantage of non-living vaccine over living is that they are safer because they are incapable of replication. Their main disadvantages are that higher doses of the organisms have to be given and they do not present such a wide range of potential immunogens to the immune system (Rashid *et al.,* 2009). It has also been reported that the adjuvant contained in non-living vaccines may cause adverse reactions in the host (Ramsey and Bryn, 2001).

Degree of attenuation

Virulent living organisms cannot normally be used as vaccines. However, their virulence can be reduced so that they can no longer cause disease (Rashid *et al.,* 2009). The most common methods of attenuation involve adapting organisms to growth in unusual conditions. Virulent canine distemper virus preferentially attack lymphoid cells. Therefore, to produce a vaccine, this virus is cultured repeatedly in canine kidney cells until its virulence is lost (Tizard, 2000).

The cause of vaccine failures does not necessarily reflect on the quality of the vaccine. If stringent quality control tests were carried out and proper methods of storage and handling under tropical conditions have been observed, the vaccine quality factor can be eliminated (De Alwis, 1999).

2. **Host Factors**

All animals do not respond equally well to vaccination and some may not mount an effective immune response to a vaccine. The host factors most affecting vaccine efficacy are as follows;

Maternal Antibody

New born animals acquire immunoglobulins from their mother in the immediate perinatal period. Neonatal antibody titers are lower in larger litters or if suckling is impaired. The antibodies from the mother generally circulate in the newborn's blood for a number of weeks. There is a period of time from several days to several weeks in which the maternal antibodies are too low to provide protection against disease, but too high to allow a vaccine to work. This period is called the window of susceptibility (Rashid *et al.,* 2009). The length and timing of the window of susceptibility is different in every litter and between animals in the same litter. Maternal antibodies can interfere with the ability of vaccines to induce immunity. This is particularly true for live virus vaccines that contain relatively small amounts of infectious viruses and may be readily neutralized by maternal antibodies. For example distemper vaccines when given at conventional times left many puppies

unprotected as their levels of maternal antibodies were sufficient to neutralize the virus used in the vaccine (Ward, 2006).

Concurrent Disease

Infectious organisms require an incubation period before clinical signs of disease become apparent. This incubation period may be as short as a few hours or as long as a few years but in general, are a few days. If an animal is incubating an infectious disease at the time of vaccination then it may well develop clinical signs. Similarly, young animals from large multi-animal environments will be particularly likely to be incubating disease at the time of vaccination. Mixed infections are common in such environments. However, little is known about how concurrent infections affect the immunity such animals to infection (Rashid *et al.,* 2009). A range of antagonistic and synergistic interactions have been shown in hosts co-infected with helminth and protozoa which might have implications for successful vaccination (Helmby *et al.,*1998 and Christensen *et al.,*1987). It is also suspected that trypanosomiasis and theillerosis diminish immune responses to vaccination (Phan *et al.,* 1996).

Immune System Function

An animal must have an effective immune system if it is to respond appropriately to a vaccine. An animal's age may affect vaccine responses too. Old age has been suggested to suppress vaccine response, however, this is uncertain. In one study, older animals had lower titres after vaccination (Mansfield *et al.,* 2004). In another study, elderly pet dogs had higher pre-vaccination rabies titres than younger dogs (HogenEsch *et al.,* 2004). It was also reported that young and old dogs have similar post-vaccination rabies, distemper and CPV titers despite decreased proliferative responses of lymphocytes and other changes in immune parameters of older dogs.

Similarly, animals that are sick or receiving drugs (particularly glucocorticoids and cytotoxic agents) may have a reduced ability to respond appropriately to vaccination. Moreover, hyperthermic puppies (>39.8 ^{o}C or 103.6 ^{o}F) are unable to mount an effective immune response to canine distemper virus vaccination and will succumb to disease if subsequently challenged (Rashid *et al.,* 2009).

Anaesthetics have not been shown to influence vaccine efficacy on their own but the stress of surgical procedures may affect the ability of the immune system to respond effectively (Rashid *et al.,* 2009). Management practices that expose animals to severe stress following vaccination may result in an inadequate immune response, although Bock and De Vos (2001) could not find published evidence of this being significant under field conditions.

Poor nutrition can suppress immune responses by decreasing nutrient availability for cell division and protein (e.g. antibody and cytokine) synthesis (James, 2007).

Breed variation

Some breeds of cats and dogs are more susceptible to certain diseases. Studies in dogs have shown that Doberman and Rottweiler tend to be more susceptible to canine parvovirus and may need a different vaccination schedule than other dogs, if they are to be protected through vaccination (Rashid *et al.,* 2009).

3. Human Factors

There are several factors within the control of the vaccinator or farmer that may affect vaccine efficacy. First, vaccines should be stored at the appropriate temperature recommended by the manufacturer. This is especially true for live vaccines which might be inactivated at higher temperatures. Each vaccine has an expiry date printed on thevial which should be strictly adhered to. Vaccines should be reconstituted with the diluents with which they were supplied and once reconstituted, they should be used immediately (Rashid *et al.,* 2009). Similarly, vaccines are developed to be given by a certain route, either intranasally, subcutaneously or intramuscularly. If a vaccine is administrated by

a route different from the route for which it was developed, it may not be effective and could cause considerable harm. For example, studies in dogs suggest that antibody titers remain elevated longer after intramuscular than subcutaneous administration of attenuated rabies vaccines.

Sometimes, a needle inserted into the injectionsite to administer vaccines may pass close to the nerve. Irritant vaccines injected into or close to a nerve have been documented to be the cause of paralysis in some instances (Rashid *et al.,* 2009). For this reason, careful training is needed to ensure vaccines are injected at the appropriate depth and site. Moreover, syringes and needles are widely re-used in developing countries because of scarcity and re-sale value. More than 30 % of immunization injections may be unsafe, primarily due to re-use of needles (Farghaly and Barakat, 1993).

There are also several factors within the control of the owner that may affect vaccine efficacy. It is important that owners adhere to the vaccination schedules advised by their veterinarians as excessive or decreased delays between the first and second doses reduce these secondary antibody responses and therefore both the length and quality of the immunity produced (Rashid *et al.,* 2009). A particular owner may achieve a high coverage among his animals however, if these animals mingle with a large number of un-vaccinated animals in common pastures or household, they are at risk, particularly the few unvaccinated animals in his herd.

Incorrect handling or storage of the vaccine

Incorrect handling or storage of the vaccine, resulting in an ineffective vaccine being administered that will not provide protection e.g., the toxicity of dimethyl sulfoxide (DMSO) for Babesia parasites at temperatures above freezing is a serious constraint on the efficacy of the vaccine (Rashid *et al.,* 2009). After thawing the vaccine at between 37 and 40°C, it must be injected immediately (Rashid *et al.,* 2009). It has been shown that if the vaccine is thawed slowly in melting ice and kept in melting ice, it is still efficacy for upto 8 hours without showing significant changes in the prepatent period (De Waal, 1996). However, to maintain the margin of safety, it is recommended that the vaccine be used within 4 hours of thawing. Vaccines must be maintained at the correct cool or cold temperature during transport and storage as well as after reconstitution and during use. Their shelf life must not be exceeded.

Insufficient time between vaccination and exposure

A vaccine does not immediately provide protection. It takes from days to a week or more for an animal's body to respond to the vaccine. For some vaccines, an adequate level of immunity usually does not occur until 2-3 weeks after the second vaccination in the series. A young animal is susceptible to a disease if it is exposed to the disease before a vaccination has had time to stimulate the body's immunity (Rashid *et al.,* 2009).

4. Environmental Factors

Although vaccination programmes may be adequate to control infectious diseases under normal conditions of exposure, it should be remembered that they may not protect under severe conditions of challenge. This situation has been observed in kittens infected with feline parvovirus (FPV). In many cases, FPV was not suspected initially as a cause of death because vaccination was performed in the households in which diseases occurred. Disease was thought to develop as a result of accumulation of virus in an environment that either overcame vaccinal immunity in the affected kittens or infected the kittens in the period between the waning of maternal antibodies and the administration of the vaccination (Rashid *et al.,* 2009). Compliance with the manufacturers' instructions, safe transport and administration, screening the animals for concurrent infection as

well as reporting systems from farmer/breeder to veterinarian and from veterinarian to vaccine manufacturer can improve vaccine efficacy (Rashid *et al.,* 2009).

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