Impact of emerging strains of canine parvovirus

by

Jennafer Moore

B.S., Kansas State University, 2014

A REPORT

submitted in partial fulfillment of the requirements for the degree

MASTER OF SCIENCE

Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine

KANSAS STATE UNIVERSITY
Manhattan, Kansas

2021

Approved by:

Major Professor
Dr. Alison Paige Adams

Copyright

Abstract

Canine parvovirus 2 (CPV-2) is an extremely contagious virus that causes high morbidity and mortality in canines, especially pediatrics. The virus was first discovered in 1978, while it was in the midst of a global pandemic. Since this time, it has remained at the forefront of veterinary medicine. Canine parvovirus 2 causes acute hemorrhagic enteritis and myocarditis, both of which are very difficult for canines to recover from, making it one of the leading causes of death. Viral shedding occurs through the feces of infected animals that occurs during the incubation period, and it spreads through contact with individuals and fomites. Canine parvovirus 2 is a robust virus, having the ability to resist many disinfectants and survive in contaminated soil for up to 5 months or longer. Additionally, there are ongoing issues that include the failure of available vaccines in providing adequate protective immunity and the lack of some in-clinic diagnostic assays to positively identify the virus. New strains of the virus, such as CPV-2c, have evolved over the last 30 years, and they have also spread all over the world. All of these factors have led to the inability to contain the virus and its disease manifestation. The purpose of this report is to understand how strains of canine parvovirus have emerged over the years as well as to describe the impact of new strains on canine health.

Table of Contents

Chapter 1 - Introduction to Canine Parvovirus

Canine parvovirus (CPV-2), commonly known as "parvo", causes acute hemorrhagic enteritis in puppies and adult dogs. In Latin, the word "parvo" means small (Nandi et al., 2010). It is a significant veterinary disease because of the high morbidity, mortality, transmissibility, and resistance to sanitation. This report will focus on how new strains of canine parvovirus have emerged and review the resultant impacts on canine health. Before focusing on this, Chapter 1 will first review the most important features of canine parvovirus, including the molecular structure, epidemiology, clinical disease presentation, diagnostics, treatment, and prevention strategies. There are challenges in preventing this disease that will also be discussed.

Taxonomy

Viruses have their own system of classification, separate from that of cellular organisms (Kuhn, 2017). Canine parvovirus 2 is in the realm *Monodnaviria,* the kingdom *Shotokuvirae,* the phylum *Cossaviricota,* the class *Quintoviricetes,* and the order *Piccovirales* (National Center for Biotechnology Information, 2020). It has been classified in the family *Parvoviridae,* which is divided into two subfamilies: *Parvovirinae* and *Densovirinae.* *Parvovirinae* is further divided into five genera: *Amdovirus, Bocavirus, Dependovirus, Erythrovirus,* and *Protoparvovirus,* formerly known as *Parvovirus* (Halder et al., 2012; Yip et al., 2020). For CPV-2, it is considered part of the genus *Protoparvovirus* and the species *Carnivore protoparvovirus* 1 (Battilani et al., 2019).

Characterization of canine parvovirus

Canine parvovirus 2 is a non-enveloped, single-stranded DNA virus, and is suspected to have evolved from feline panleukemia virus (FPV) (Halder et al., 2012). The evolution of CPV-2 will be further discussed in Chapter 2. The virus is small in size (approximately 25 nanometers

in diameter) and icosahedral in shape (Halder et al., 2012; Decaro et al., 2012). An icosahedron is described in geometry as a shape that has 20 sides. The virus has a linear, single-stranded, and negative-sense DNA genome with approximately 5,000 nucleotide bases (Halder et al., 2012).

Canine parvovirus genomic organization

The genomic structure of parvovirus includes two open reading frames (ORFs) with palindromic sequences that are 120-550 nucleotides in length, which fold into hairpin structures (Halder et al., 2012). These are critical for replication. The sequence of the 5' end encodes for proteins that are not structural (NS), which are important for packaging and genome replication. There are two nonstructural proteins (NS1 and NS2). The 3' end encodes for the two structural viral proteins (VP1 and VP2) that form the capsid (Halder et al., 2012). Viral protein 1 is the primary cause of viral infectivity. Viral protein 2 is the main component in the capsid of CPV-2, and the slight changes in the amino acid sequence are what define the three variant strains: CPV-2a, CPV-2b, and CPV-2c. Canine parvovirus 2b and Canine parvovirus 2c are defined by amino acid changes in VP2. Residue 426 of the VP2 sequence is the location that with a single amino acid change distinguishes it as either CPV-2b or CPV-2c. For strains CPV-2 and CPV-2a, asparagine is present at residue 426, but for CPV-2b, it is aspartic acid and for CPV-2c, it is glutamic acid (Markovich et al., 2012). These single amino acid changes greatly affect monoclonal antibody binding to the viral capsid as well as polyclonal antibody binding (Markovich et al., 2012).

The capsid structure that contains these nonstructural and viral proteins is important to the virus's pathogenicity as well. The surface of the capsid has a long, raised region, referred to as a "spike" on the threefold axes of the capsid (Halder et al., 2012). This threefold axis of the capsid is the most antigenic region on the capsid structure and is home to epitope A, which is

home to many of the residues that change between parvovirus species and CPV-2 variant strains (Halder et al., 2012). These will be discussed in more detail throughout Chapters 1 and 2. The threefold axes is also the target binding location for monoclonal and polyclonal antibodies (Halder et al., 2012). There is also a deep canyon that surrounds some cylindrical structures at the fivefold axes, and lastly, there is a deep dimple at the twofold axes of the capsid (Halder et al., 2012).

History and epidemiology

In the mid to late 1970's, a novel virus emerged that caused acute hemorrhagic enteritis and myocarditis in canines known today as canine parvovirus type 2 (CPV-2). The virus was officially isolated and identified in 1978 in the United States (Parrish et al., 1988). The virus swept through the entire canine population in the Americas, Asia, Australia, Europe, and New Zealand so quickly and devastatingly that a pandemic was declared from 1979-1980 (Parrish et al., 1988). Dog sera from Greece and Belgium were later tested for antibodies, and the results showed that CPV-2 had emerged in these countries between 1974 and 1976, but in Australia, Japan, and the United States only sera from 1978 and later tested positive for CPV-2 (Parrish et al., 1999). Ever since 1978, CPV-2 has been omnipresent globally. In 1979, wild coyotes became widely infected throughout the United States as well (Parrish et al., 1999). The first variant strain of CPV-2, CPV-2a, emerged in 1979 in the United States. A few years later, the next variant strain, CPV-2b, emerged in the United States in 1984. Several years later, the last prominent variant strain, CPV-2c, emerged in Italy in 2000.

It has been nearly 45 years since the CPV-2 virus first emerged. In that time, three new variant strains have emerged, and the four strains have disseminated throughout 42 countries, on six continents worldwide (Decaro et al., 2012; Miranda et al., 2016). The original CPV-2 strain

is now much less common than the three antigenic variant strains but does still cause cases of disease to this day. The variant strains are dominant in fluctuating proportions in countries worldwide. Awareness about the dominant variant strain depends on how actively CPV-2 is being monitored or how many CPV-2 research studies are actively taking place in a given place at a given time.

Canine parvovirus 2a has been shown to be present in 37 countries and to be the main variant in Asia, Australia, and Europe, as well as the only variant present in New Zealand, Nigeria, Hungary, Czech Republic, Slovenia, and Romania (Battilani et al., 2019: Decaro et al., 2007; Decaro et al., 2011; Decaro et al., 2012; Miranda et al., 2016). Canine parvovirus 2b has been found across five continents, 31 countries, and it is the dominant variant strain in Africa, many Asian countries, many African countries, Ireland, the U.K., and the U.S. (Battilani et al., 2019). Canine parvovirus 2a and canine parvovirus 2b were equally present in Belgium, Switzerland, and Austria (Truyen et al., 2000). Canine parvovirus 2c was found in 21 countries across the world and determined to be the most prominent variant strain in many European countries and South America (Battilani et al., 2019).

Incidence

Canine parvovirus 2 infects canines of all ages, but particularly pediatrics (younger than 12 weeks) and juveniles (12 weeks to 6 months) that are between 6 weeks old and 4 months old (Serpell et al., 2016). This is due to insufficiencies in immunological protection as well as being an ideal host because of their rapid growth and development. Canine parvovirus 2 can cause 100% morbidity in canine patients of all ages, with mortality rates at about 90% in pediatrics and juveniles and 10% in adults (Tosaris, 2018). Crossbred canines tend to be less susceptible to the virus than purebreds (Houston et al., 1996). The virus causes acute hemorrhagic enteritis, and in

many cases, causes subacute myocarditis (Decaro et al., 2012). Many patients succumb to a secondary infection due to the damage to the immune system, primarily the bone marrow and white blood cells that act as a primary line of defense against infection. White blood cell (WBC) counts can typically drop as low as 2,000-3,000 cells/μL of blood. Often, patients may succumb to malabsorption due to the significant damage to the small intestines and the loss of their nutrient absorption capabilities. The small intestines are the primary site of all nutrient absorption from food, so when they are severely damaged, malabsorption occur.

In some cases of CPV-2, myocarditis can occur in patients, usually pediatrics 3 months of age or younger, and they can succumb to subsequent heart failure (Hayes et al., 1979). Often when puppies survive the first 3-4 days of the viral infection, they will make a full recovery in about a week. Around 70-90% of patients with enteritis from CPV-2 can survive with the correct supportive care and derive long term, if not lifelong, immunity following recovery (Gallagher, 2020).

Pathogenesis

Parvovirus is typically transmitted through the fecal-oral route from contact with feces of an infected animal or through the oronasal route through contact with a fomite (Goddard et al., 2010). Infected canines shed the virus in their feces starting at around 4-5 days post exposure, and they can continue to shed the virus for up to 3-4 weeks following subclinical or clinical infection (Goddard et al., 2010). Once the virus has been ingested, it travels to the lymph nodes of the gastrointestinal tract, the lymph nodes of the throat, or the tonsils where it incubates and multiplies for about two days. Once it has replicated, the virus spreads to the bloodstream. From there it travels to the bone marrow, targeting the WBCs as well as to both the large and small intestines, where it replicates in the intestinal villi and later causes atrophy of the villi. In the

small intestines, the virus eventually critically damages the enterocytes of the crypts of the intestine, otherwise known as the crypts of Lieberkühn (Tosaris, 2018). This damage and necrosis destroy the capability of the intestines to absorb nutrients. Due to this damage in the intestinal lining, blood and protein are leaked into the intestinal lumen, causing anemia and protein loss, respectively (Goddard et al., 2010). In pediatrics, the virus may also travel from the lymph nodes to the heart to target the rapidly regenerating cells that are present there. This is what can lead to myocarditis and heart complications like inflammation of heart muscle, poor function, and arrhythmias in pediatrics younger than three months of age (Hayes et al., 1979). Endotoxins can also be absorbed into the bloodstream from intestinal bacteria, thereby causing endotoxemia (Isogai, et al., 1989). The absorbance of these bacteria can also lead to septic shock.

Parvovirus replicates in cells that rapidly divide, which is why pediatric and juvenile canines are more of an ideal host than adults since their cellular regeneration is much higher due to growth and development. Mitotic cells, like those that are rapidly dividing in growing canines, are necessary for successful viral replication due to the fact that CPV-2 needs to utilize a polymerase to effectively proliferate (Tu et al., 2015). Canine parvovirus 2 can replicate independently, without coinfection, which is also known as an autonomous parvovirus.

Canine parvovirus 2 replicates inside of the nucleus of the invaded host cell. This means that the virus must navigate through three barriers within the cell in order to reach the nucleus and DNA replication machinery: the cellular membrane, cellular plasma, and nuclear membrane. The virus attaches to the host's cell surface receptors in order to invade a cell. Canine parvovirus 2 is able to invade the cell via clathrin-dependent endocytic pathway (Tu et al., 2015). The CPV-2 VP2 is an "anti-receptor" that binds to the transferrin receptors (TfR) on the cell's surface and

is internalized through the cell membrane via clathrin-coated pits (Tu et al., 2015; Cureton et al., 2012). Canine parvovirus 2 is then moved through the endocytic plasma via endosomes at a lower pH. As it gets closer to the nucleus, it is internalized by lysosomes, that continue to transport it until it activates phospholipase A_2, which releases the virus from the lysosome directly into the cytoplasm near the nucleus (Tu et al., 2015). The VP1 of the virus has a nuclear localization signal (NLS) that helps to guide the virus to the nucleus, where it is moved through the nuclear pore complex (NPC) and accesses the DNA replication machinery (Tu et al., 2015). Once the virus has replicated, the mature virions are moved out of the NPC to continue the life cycle, replicating in cells throughout the animal's body (Tu et al., 2015).

Gross pathology and histopathology

For gross pathology in fatal cases, the walls of the intestines are typically thickened and discolored (Gallagher, 2020). Lesions can be observed with multifocal necrosis of the crypt epithelium, loss of crypt architecture, and villous blunting and sloughing (Gallagher, 2020). Watery, mucoid, or hemorrhagic intestinal contents may also be observed (Gallagher, 2020). The Peyer's Patches and mesenteric lymph nodes of the small intestines of affected animals are typically enlarged (Decaro et al., 2012). Abdominal and thoracic lymph nodes often display edema and congestion, and the thymus may also be atrophied (Decaro et al., 2012). For infected canines with myocarditis, pale streaks indicating fibrosis can usually be observed in the myocardium (Gallagher, 2020).

For histopathologic lesions of the gastrointestinal tract, hemorrhagic enteritis may be observed as well as intranuclear inclusion bodies (Decaro et al., 2012). The Peyer's Patches, mesenteric lymph nodes, abdominal and thoracic lymph nodes of affected animals may be completely depleted of cortical lymphocytes and lymphoid tissue (Gallagher, 2020). The spleen

is also usually completely depleted of cortical lymphocytes and lymphoid tissue (Gallagher, 2020). Bone marrow hypoplasia may also be observed (Gallagher, 2020).

For canines that die of complicating acute respiratory distress syndrome, systemic inflammatory response syndrome, endotoxemia, or septicemia, histopathological examination may reveal pulmonary edema, alveolitis, and bacterial colonization of the lungs and liver (Gallagher, 2020).

Symptoms and clinical signs

Canine parvovirus (CPV-2) incubates within the body for 3-7 days after transmission. Then, the onset of symptoms begins with lethargy, pain in the abdomen, abdominal distension, fever, and loss of appetite. This quickly leads to weight loss, dehydration, vomiting, and mucoid or hemorrhagic (bloody) diarrhea with a distinctive and indecent odor. Patients sometime present with hypothermia (American Veterinary Medical Association, 2021). Patients may also experience septic shock or endotoxemia as a result of bacteria entering the bloodstream or endotoxins from disintegrated bacteria entering the bloodstream respectively (American Veterinary Medical Association, 2021). Death usually occurs within the first 2-3 days of clinical signs (American Veterinary Medical Association, 2021).

Pediatric dogs can experience infection in the heart that leads to many complications, such as inflammation of the heart muscle (myocarditis), poor cardiac function, and arrhythmias (Hayes et al., 1979). In these rare cases, if an unvaccinated pregnant bitch is infected with the virus or if puppies younger than 8 weeks of age from an unvaccinated bitch are infected with the virus, then these offspring may develop myocardial infection (Gallagher, 2020). This leads to myocarditis, ultimately resulting in cardiopulmonary failure, usually within 24 hours of clinical symptoms (Goddard et al., 2010; Gallagher, 2020).

Clinical pathology

There is diagnostic value in analyzing the blood of canine patients with symptoms of CPV-2. Not only can a WBC count help to support a CPV-2 diagnosis, but leukopenia, specifically acute lymphopenia, is a common finding with WBC counts as low as 2,000-3,000 cells/mL of blood, where the normal reference range is about 6,000-14,000 cells/mL (Markovich et al., 2012). This is an important finding indicating the need to treat as soon as possible; however, sometimes the WBC count may appear to be within normal range if an opportunistic bacterial coinfection is also present. Even though the lymphocyte count may be low due to the viral infection, neutrophils may be increased in the blood (i.e., neutrophilia) due to the bacterial infection. This would give the false interpretation of a normal WBC count (Markovich et al., 2012). This is also an important distinction to be aware of regarding treatment since antimicrobials may also be necessary in addition to the supportive care for the viral infection.

Diagnostics

There are a handful of different diagnostic assays available for detecting CPV-2 from clinically obtained samples. Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) assay are the most common diagnostic tools that are utilized today for a rapid or absolute result, but there are a few other diagnostic assays that are available.

The ELISA test used for detection of CPV-2 is the most commonly used diagnostic assay for in-clinic detection of CPV-2. The SNAP Canine Parvovirus Antigen Test is an in-clinic rapid diagnostic ELISA test used for detection of CPV-2 and takes about 15 minutes to complete. It is a hemagglutination test based on ELISA technology (IDEXX Laboratories, Inc., n.d.). This rapid test is similar to many pregnancy tests. The design of the test increases the reliability of the result by helping to reduce user error in test preparation or interpretation by simplifying the

process and making the result easy to read in a window-like display. The manufacturer claims that no further testing should be needed to reach a definitive diagnosis if a positive result is displayed, but that a negative test result does not rule out a diagnosis of parvovirus disease (IDEXX Laboratories, Inc., n.d.).

The PCR assay is the most reliable diagnostic assay available, and it is used for confirming in-clinic positive results. Typically, fecal samples are collected in the clinic and sent to a diagnostic laboratory, where viral DNA is amplified for detection of CPV (Tosaris, 2018). The PCR assay is sensitive enough to identify and differentiate variant strains that are displayed on an agarose gel that utilizes specific primers for each variant strain of CPV (Tosaris, 2018).

Other diagnostic assays include the Canine Parvovirus-Coronavirus Antigen Test Kit (Anigen, Inc.), hemagglutination assays, virus isolation, immunohistochemistry, and electron microscopy. In addition, supportive diagnostic confirmation includes clinical pathological findings.

The Canine Parvovirus-Coronavirus Antigen Test Kit is an in-clinic diagnostic test used for the detection of CPV-2. It is a chromatographic immunoassay that gives a rapid result in the clinic, very similar to the SNAP test. The test results can be interpreted about 5-10 minutes (BioNote, n.d.). In-clinic rapid tests like this one can also be confirmed by collecting a sample for a PCR assay (Yip et al., 2020).

Hemagglutination assays (HA) are laboratory-based diagnostic tests that can, but not often are, utilized for CPV-2 diagnosis or diagnosis confirmation. Hemagglutination assays require fresh erythrocytes from donor pigs to execute the assay, which can be a roadblock to assay execution if there is an issue with the supply of these cells (Decaro et al., 2012).

Hemagglutination assays are more efficient than some of the other laboratory-based assays, as it can be executed in a 96-well plate and yields results in about four hours (Decaro et al., 2012).

Virus isolation is a laboratory-based diagnostic tests that can, but not often is, utilized for CPV-2 diagnosis or diagnosis confirmation. This is a cell culture-based assay that requires a laboratory with these capabilities, and it is also very time-consuming due to the incubation required (Decaro et al., 2012). Results can take 1-2 weeks to return which makes the use of this assay undesirable for emergent cases. This assay is also considered to have low sensitivity and can yield false negatives (Decaro et al., 2012).

Immunohistochemistry is a laboratory-based diagnostic tests that can, but not often is, utilized for CPV-2 diagnosis or diagnosis confirmation. This assay is also considered to have low sensitivity and can yield false negatives (Decaro et al., 2012).

Electron microscopy (EM) can also be used to examine fecal samples for CPV-2; however, this method is considered poorly sensitive and depends on the location of the virus within the sample (Decaro et al., 2010).

Lastly, clinical pathology can be used to support the findings of positive rapid tests. The veterinarian can take a blood sample to confirm lymphopenia that is typically associated with infection. Since CPV-2 targets the lymphocytes and bone marrow quickly after infection, a low WBC count is a clear co-indicator for the presence of this infection (Cornell University, n.d.). The combination of a positive rapid in-clinic test and a low WBC count provides a strong indication of CPV infection and the need for immediate medical treatment.

Treatment

Treatment of canine parvovirus patients primarily involves supportive care until the patient is in recovery. This care is expensive and not a guarantee that the patient will return to

perfect health if they survive. Infected animals are given intravenous electrolyte fluids to help replenish electrolytes, correct dehydration, and combat ongoing fluid loss. Colloid therapy is suggested for animals with severe gastrointestinal protein loss as a bolus and/or continuous intravenous administration (Goddard et al., 2010). Antibiotics are usually provided due to the high risk of bacteria invading the intestines and causing secondary infection. Beta-lactam antibiotics are usually prescribed to provide Gram positive and anaerobic bacterial coverage (Gallagher, 2020). If an infection is ongoing, the canine patients are administered an antibiotic targeting Gram-negative bacteria. Antiemetic medication can be recommended as appropriate to assist canine patients from continued vomiting, which helps to combat fluid and nutrient losses as well as weight loss. However, antidiarrheals are not recommended because the contaminated feces should not be retained within the body. Sometimes a feeding tube may be placed within 12 hours of admission to the hospital or clinic so that a continued flow of nutrients occurs with decreased disruption from vomiting. The use of a feeding tube can also assist in combatting weight loss. Gradual reintroduction to food and water once vomiting has ceased is important. Oral fecal microbiota transplants from a healthy adult has also been shown to be helpful to reduce the recovery time (Periera et al., 2018). Typically, a successful response to treatment takes about 6 days, but in ideal situations, it can be as few as 3 days after the onset of symptoms. With the correct combination of treatments, survival rates can reach up to 90% (American Veterinary Medical Association, 2021). The duration of immunity (DOI) following infection with CPV is usually life-long (Decaro et al., 2020).

Prevention

The main method of prevention of CPV-2 is vaccination. Vaccination protocols for this virus are very important in puppies, and there are core vaccines for CPV-2 available all over the

world (Decaro et al., 2020). Core vaccinations are vaccinations that should be administered to all dogs, no matter the circumstances, because the vaccines protect against diseases that have severe morbidity and mortality, are highly communicable, and are globally distributed (Decaro et al., 2020). The vaccine is often included as part of a multivalent vaccine that includes canine distemper virus, or it is available as a standalone CPV-2 vaccine. The vaccine is administered to canine patients either subcutaneously or intradermally.

In the U.S., vaccines that are currently licensed for protection against CPV-2 include modified live vaccines (MLVs) and inactivated (killed) vaccines. The inactivated vaccines are not usually recommended for canines, unless the animals are pregnant, because they require multiple booster injections to provide adequate immunological protection (Decaro et al., 2020).

The MLVs utilize the original CPV-2 strain of the virus as well as a CPV-2b strain. The MLVs are generally used because they "induce a strong, long-lasting (usually life-long) immunity by replicating within the host cells, without producing significant tissue damage or clinical signs" (Decaro et al., 2020). However, this type of vaccine has the potential revert to viremia and be shed in the feces. The ability to replicate in host cells is the main reason why the inactivated vaccine is administered to immunocompromised patients or pregnant females instead of the live-attenuated vaccine. Fetuses are very sensitive and susceptible to live modified viruses, and so, there is a significant risk that the fetus could be stillborn (Decaro et al., 2020). The MLV is given to dogs in a series of three vaccinations in the age ranges of 6-8 weeks, 10-12 weeks, and 14-16 weeks (Gallagher, 2020). Modified live vaccines for CPV are characterized by their early onset of immunity (OOI) with demonstrated protection as early as three days after receiving the vaccine (Decaro et al., 2020). They are also known for their lengthy duration of

immunity (DOI), resulting in protection for as long as 9 years after receiving the vaccine (Decaro et al., 2020).

Because CPV-2 is so hardy in the environment and so easily spread through contact, it is very important for owners or handlers to take preventive measures through contact very seriously as well. Owners of puppies must do their due diligence in keeping their animals away from other canines until they are vaccinated. Puppies should be reared and isolated in their own yards, and this environment should be clean and sanitized on a regular basis.

Control

The control measures of CPV-2 are very important, and good hygiene is the key. Veterinary clinics that receive canine patients as well as other communal canine areas, such as dog parks or kennels, are critical locations, because they are at the most risk of harboring the virus. Canine parvovirus 2 is highly robust on surfaces and objects, which makes thorough sanitization and sterilization very important. The virus is resistant to many disinfectants, lipid solvents, and trypsin, and it can survive on surfaces or contaminated soil for up to 5 months (Jacobs et al., 1980; Decaro et al., 2012). This is one of the properties of CPV-2 that allows it to be highly communicable.

Significant challenges in disease prevention

Maternal antibody interference is one of the main reasons why CPV-2 vaccine failures occur. Maternally derived antibodies (MDA) are part of passive immunity where pregnant or nursing mothers pass their own antibodies to their young via the placenta, colostrum, or milk. In canines, immunoglobulins do not pass through the placenta very well with only about 5-10% being transferred to the circulation of the fetus during pregnancy (Decaro et al., 2020). The majority of MDA are transferred to the young through the colostrum, where they are absorbed in

the small intestine, through the intestinal epithelium, to the blood. There is some continued transfer of MDA to the young through milk for at least 38 days after parturition, and this lactogenic immunity continues to protect the young from infection for limited amount of time (Decaro et al., 2020).

For CPV-2, MDA titers in the blood decline rapidly with the half-life ranging from 8.3-13.5 days, but they can persist for 13-15 weeks (Parrish et al., 1982). These MDA can block active immunization (vaccination) by neutralizing the viral antigens present in the vaccine, thereby preventing seroconversion. There is a period of time known as the "window of susceptibility" or "immunity gap" when the MDA can no longer protect the puppies from infection, but they can still interfere with immunization. This period of time usually lasts 2-3 weeks and is the reason why the vaccination and booster schedule for CPV-2 requires a series of three vaccinations during and after this period of time.

There have been many strategies suggested to help overcome MDA interference with vaccination. One way to do this is to perform a MDA titration at about 4-6 weeks of age to identify the ideal vaccination window of the puppy (Decaro et al., 2020). This can be performed using the hemagglutination inhibition (HI) assay for existing antibody levels, and then, using the titer results to extrapolate when the titers may fall low enough for the need of vaccination. This type of testing involves serum collection and transfer to a laboratory with the required specialized equipment. This makes this option fairly impractical until better technology exists.

Exploring alternate routes of vaccine administration is another strategy that has been suggested to potentially overcome MDA interference. Some experimental vaccines have shown some success in overcoming the MDA interference when administered intranasally (Buonavoglia et al., 1995). While the commercially available vaccines are only approved to be administered

subcutaneously and intramuscularly, some research has shown that these vaccines are effective at circumventing MDA interference when administered orally; however, it is less effective than the other routes of administration (Buonavoglia et al., 1995).

Another challenge in disease prevention is the lack of accuracy of existing diagnostic assays and vaccine failures due to the emergence of variant strains of CPV-2. In Chapter 2, the emergence of new strains of CPV-2 will be discussed in detail, including an in-depth discussion on the use of available diagnostic assays and the effectiveness of currently licensed vaccines in light of the circulation of new strains of CPV-2 in the U.S. and the world.

Chapter 2 – Emerging Variant Strains of Canine Parvovirus

As discussed in Chapter 1, canine parvovirus (CPV-2) is a significant disease in veterinary medicine estimated to cause over 250,000 cases of illness in the United States per year (Kindred Biosciences, Inc., 2021). Chapter 2 will review the historical aspects of CPV-2 with regard to the emergence of the virus as well as the recent variant strains of CPV-2. This chapter will also discuss important features and impacts of the different strains on canine health.

Evolution of canine parvovirus 2 and its variant strains

Canine parvovirus 2 surfaced in the mid to late 1970's, and while there is some debate about exactly when and where it first emerged, the virus sparked a pandemic in canines starting in 1978, when it was first officially detected in the United States, with the pandemic ending in 1979. It is widely regarded by researchers that CPV-2 evolved as a host range variant from the feline panleukopenia virus (FPV) (Yip et al., 2020; Truyen et al., 1995). Both CPV-2 and FPV belong to the genus *Protoparvovirus* (Yip et al., 2020). Some researchers suggest, based on phylogenetic data, it seems more likely that CPV-2 mutated from a different carnivorous parvovirus (Goddard et al., 2010) and that all parvoviruses were derived from a single common ancestor (Allison et al., 2012). Canine parvovirus 2 is 98% genetically similar to FPV (Truyen et al., 1995), and Yip et al. (2020) suggested that it may have "jumped the species barrier" in order to better replicate in canines. The virus has become a prime example of how viruses can successfully cross species barriers to infect new hosts (Miranda et al., 2014). This capability has led to the significant biodiversity present when evaluating the phylogenetic relationships among all parvoviruses that would not have existed if not for multiple cross-species transmission events (Miranda et al., 2014). Canine parvovirus 2 was distinguished as such to differentiate from canine parvovirus type 1 (CPV-1), which is distantly related to minute virus of canines, formerly

known as MVC (Carmichael et al., 1994). Interestingly, although CPV-2 is a DNA virus, it shows genomic substitution rates that are more similar to RNA viruses, with values of approximately 10^{-4} nucleotide substitutions per site per year when compared against FPV (Decaro et al., 2009). Canine parvovirus 2 became established by gaining the ability to bind to the canine transferrin receptor (TfR) (Truyen et al., 1996), which has been shown to be crucial for the predisposition of host cells to become infected by the virus (Decaro et al., 2012). Chang et al. (1992) determined that there are two important amino acid differences between FPV and CPV-2 that determine binding to the TfR. At the viral protein 2 (VP2) residue 93, lysine changed to asparagine in CPV-2, and at the VP2 residue 323, aspartic acid changed to asparagine in CPV-2 (Chang et al., 1992). These changes explain the expansion of the host range and are also CPV-2-specific antigenic epitopes (Chang et al., 1992). However, Miranda et al. (2016) points out eight amino acid differences between CPV-2 and FPV. According to these researchers, the VP2 changes at residues 93 and 323 are also joined by a change at residue 103 from valine in FPV to alanine in CPV-2, and that together, these three amino acid changes were necessary to gain the canine host range (Truyen et al., 1995). The changes at residues 323 and 375 are also thought to be responsible for determining the pH dependence of hemagglutination (HA) (Parrish et al., 1991). Three additional amino acid changes at VP2 have been proposed to cause the virus to lose the ability to replicate in felines: residue 80 changed from lysine in FPV to arginine in CPV-2, residue 564 changed from asparagine in FPV to serine in CPV-2, and residue 568 changed from alanine in FPV to glycine in CPV-2 (Truyen et al., 1994). Lastly, two more amino acid changes occurred at VP2: residue 232 changed from valine in FPV to isoleucine in CPV-2 and residue 375 changed from aspartic acid in FPV to asparagine in CPV-2 (Miranda et al., 2016). Due to the advancements in research and technology, the more recent analysis by

Miranda et al. (2016) may reflect improved accuracy when compared the analysis by Chang et al. (1992).

Canine parvovirus 2a, -2b, and -2c strains mainly differ by a single different amino acid at residue 426 at epitope A of the VP2 (Decaro et al., 2006). Both CPV-2 and CPV-2a express asparagine at residue 426 at epitope A of the VP2 gene, whereas CPV-2b expresses aspartic acid and CPV-2c expresses glutamic acid (Yip et al., 2020; Decaro et al., 2006). Canine parvovirus 2b and canine parvovirus 2c are thought to be antigenic variants of CPV-2a and are also commonly referred to as VP2 426Asp and VP2 426Glu, respectively (Miranda et al., 2016).

Canine parvovirus 2 can infect a variety of carnivorous species, depending on the strain. These species include dogs, coyotes, wolves, cats, bobcats, pumas, raccoons, and more (Miranda et al., 2014). There is concern that felines, in particular, may have an important epidemiological role in CPV-2 due to the fact that the species has historically been significant to the virus, and if infections continue to occur in this species, they could become a reservoir for new variants of CPV-2 (Miranda et al., 2014).

Today, clinicians and researchers alike are struggling with the vaccine failure associated with currently available vaccines to protect canines as well as obtaining an accurate diagnosis when CPV-2 is present. In addition, traditional methods of diagnosing CPV-2 have been shown to be poorly sensitive, especially during the latter part of infection (Decaro et al., 2012).

Canine parvovirus 2 variant strains

Canine parvovirus 2

Canine parvovirus 2 was first identified in 1978 in the United States, where it caused severe, fatal cases of hemorrhagic gastroenteritis and subacute myocarditis of canines located in dog kennels and animal shelters (Decaro et al., 2012). As a result, thousands of canines died and

millions more were infected (Parrish et al., 2005). It was also discovered globally in Europe, Asia, and Australia within 6 months of its discovery in the U.S. (Carter et al., 1987). This rapid spread combined with the devastating loss of canines from CPV-2 resulted in a pandemic being declared until 1979 when the first canine parvovirus modified live vaccine was developed. Due to the fact that this virus was completely novel to the world, canines of all ages were highly susceptible to this disease, and therefore, this led to high morbidity and mortality. Canines were immunologically naïve, having neither protective antibodies nor immunity against the virus. While the virus was devastating for canines, the mutation of FPV that caused its emergence no longer allowed the virus to replicate in felines. The modified live vaccine that was developed for CPV-2 was able control further outbreaks; however, the virus was now spread worldwide by 1980 (Goddard et al., 2010).

Since the initial discovery of CPV-2, at least 75 variants of this virus have emerged (Decaro et al., 2009), but most notably are CPV-2a, CPV-2b, and CPV-2c that are much more virulent and pathogenic when compared to the other variant strains (Decaro et al., 2009). By the mid-1980's, CPV-2 was almost completely replaced in global distribution and disease by strains CPV-2a and CPV-2b (Hong et al., 2007). It has been posed by some researchers that the development of host immunity to CPV-2 may have been the catalyst for the gradual emergence of CPV-2 antigenic variants (Decaro et al., 2012).

Canine parvovirus 2a

Immediately following the CPV-2 pandemic, CPV-2a emerged in 1979 in the United States, and then in Japan, Belgium, and Australia (Parrish et al., 1999). Canine parvovirus 2a also spread to Denmark and France between 1979 and 1982 (Parrish et al., 1988). Infections with CPV-2a presented with the same symptoms of acute gastroenteritis and myocarditis as

CPV-2. It was prevalent through the mid 1980's worldwide, and it also became the most common virus in many other carnivores, including wolves, foxes, coyotes, many types of big cats, pandas, otters, etc. (Miranda et al., 2016).

The speed with which CPV-2a spread worldwide and replaced CPV-2 almost entirely indicates that this strain had major epidemiological advantages over CPV-2 (Parrish et al., 1988). When this strain of CPV-2 emerged, the virus had regained the ability to infect and replicate in felines (Truyen et al., 1996). Canine parvovirus 2a had six amino acid substitutions in the capsid protein (VP2) when compared to CPV-2. Some of these amino acid changes in VP2 were at residue 87, where methionine changed to leucine in CPV-2a, at residue 300 where alanine changed to glycine in CPV-2a, and at residue 305, where aspartic acid changed to tyrosine in CPV-2a (Parrish et al., 1991). These three amino acid changes gave the virus the ability to replicate in cats once again (Miranda et al., 2016). The other three amino acid changes that occurred were located at residue 101, where isoleucine changed to threonine in CPV-2a, at residue 297, where serine changed to alanine in CPV-2a, and at residue 555, where valine changed to isoleucine in CPV-2a (Truyen et al., 1996). With these antigenic mutations and epidemiological advantages of allowing CPV-2a to efficiently spread across geographical locations as well as between host species, this strain is suspected by many researchers to have given rise to variant strains CPV-2b and CPV-2c (Organtini et al., 2015). Recent research shows that CPV-2a has been reported in at least 37 countries, and it is co-circulating with CPV-2b and CPV-2c in 15 of those countries (Miranda et al., 2016).

Canine parvovirus 2b

Canine parvovirus 2b emerged in 1984 in the United States, and it is currently one of the more common variants observed in clinical disease today (Miranda et al., 2016). Recent

research shows that CPV-2b has been reported in at least 31 countries, cocirculating with CPV-2a and CPV-2c in 15 of those countries (Miranda et al., 2016). Canine parvovirus 2b is considered to be an antigenic variant of CPV-2a and differs from it with two amino acid substitutions in the major antigenic region (epitope A) of VP2 (Miranda et al., 2016). The first amino acid substitution is at residue 426 from asparagine to aspartic acid in CPV-2b and at residue 555 from isoleucine to valine in CPV-2b (Parrish et al., 1985). The latter substitution is a reversion to the original CPV-2 strain, but the substitution at residue 426 is the one that results in an altered antigenic epitope that is completely unique to CPV-2b (Parrish et al., 1991).

Along with the original CPV-2 strain, CPV-2b is the other main strain of the virus that is used in the vaccines that are available today (Yip et al., 2020). This variant of the virus is still fairly prevalent today and presents with the same clinical symptoms of acute gastroenteritis and myocarditis as CPV-2.

Canine parvovirus 2c

The last variant strain of CPV-2 that will be discussed is CPV-2c, which emerged in Italy in 2000 and is quickly becoming the most prominent variant strain seen in clinical disease today (Yip et al., 2020). After it was first discovered in Italy, CPV-2c spread to Vietnam, Spain, the United Kingdom, South America, North America, Portugal, and India (Decaro et al., 2006). However, research showed that CPV-2c was isolated in Germany four years prior to its official discovery in Italy, meaning that it had been circulating since at least 1996 (Decaro et al., 2007). Recent research shows that CPV-2c has been reported in 21 countries, cocirculating with CPV-2a and CPV-2b in 15 of those countries (Miranda et al., 2016).

Canine parvovirus 2c is considered by many to be an antigenic variant derived from CPV-2a. As discussed before, they differ by one amino acid change at residue 426, where CPV-

2a expresses an asparagine and CPV-2c expresses a glutamic acid (Markovich et al., 2012; Buonavoglia et al., 2001). As with the other variants of CPV-2, CPV-2c was able to spread across many countries and continents rapidly; however, it was able to achieve this more rapidly than the others. Due to this ability, researchers suggest that the VP2 mutation to glutamic acid at residue 426 may have provided this variant strain an epidemiological advantage (Markovich et al., 2012). It does not necessarily seem to correlate with this strain's ability to cause more severe disease in naïve or vaccinated dogs, but it does appear to indicate positive selection pressure for this particular strain of virus (Markovich et al., 2012). Researchers also suggest that CPV-2c may be capable of replicating more quickly and producing a greater volume of virus to shed and transmit to other canines than the other variant strains (Markovich et al., 2012). Canine parvovirus 2c is associated with more severe disease in adult dogs than its predecessors as well as more severe infection in fully vaccinated dogs (Decaro et al., 2009; Decaro et al., 2012). As a result, CPV-2c is associated with higher rates of mortality than the other variant strains (Decaro et al., 2009).

Current challenges of variant strains of canine parvovirus 2

Despite the fact that CPV-2 has been in circulation worldwide for nearly 45 years, there are many issues surrounding the diagnosis, control, and prevention of this disease that allow the virus to continue to wreak havoc on canine health. As discussed in Chapter 1, CPV-2 is resistant to disinfectants and hardy in surviving in a given environment. In addition to this, research and data have shown that there are many challenges associated with diagnostics and vaccine success. If positive cases fail to be detected by diagnostic assays or prevented by vaccination, then proper control, sanitization, and prevention measures alone will not effectively control the disease. This is of particular concern with CPV-2c, as dogs infected with this strain would shed the virus in

their feces and spread it to other dogs, but as discussed below, it may go undetected due to the lack of accuracy of diagnostic assays that are currently available.

Diagnostic accuracy

Access to effective and efficient diagnostic assays is crucial in the control and treatment of this disease. While there are very sensitive and accurate laboratory-based diagnostic assays available, they are inefficient in an emergency for immediate diagnosis because samples must be sent to an offsite laboratory. These laboratory-based assays are also valuable in confirming negative test results from in-clinic assays. Research has shown that there is a shortfall in the sensitivity of readily available, in-clinic diagnostics, which often leads to false negative test results (Decaro et al., 2012). This issue can result in either a delay in the necessary, life-saving treatments or a complete failure to administer treatment, both of which can result in fatality.

Diagnostic assays fall short in accurately detecting different strains of CPV-2. CPV-2c is particularly difficult to detect, and this may be due to its pathogenesis. Canine parvovirus 2c infection can cause high antibody titers within the gut lumen, and these antibodies have been shown to interfere with the quantity of viral particles that are shed in the feces (Yip et al., 2020). Due to the low sensitivity of the in-clinic assays (Decaro et al., 2012), a heavy viral load may be required in order to obtain an accurate diagnosis. Also, some researchers have shown that CPV-2c causes feces to become more mucoid in consistency, as opposed to diarrhetic, which has been further shown to sequester the viral load within fecal samples (Yip et al., 2020). Another issue specific to the diagnosis of CPV-2c is that the amino acid change at residue 426 to glutamic acid has been shown to result in the lack of monoclonal antibody recognition of the epitope associated with this amino acid (Hong et al., 2007). This is a developing problem for the diagnostic assays that rely on monoclonal antibodies for specific detection of this strain (Hong et al., 2007). As

new strains of CPV-2 continue to emerge, this will be an important consideration for current in-clinic assays that rely on monoclonal antibodies for detection.

Vaccine failure can cause diagnostic failures as well. While dogs may still become infected with CPV-2 after being vaccinated, the antibodies formed from the vaccine may cause the viral load during natural infection to be dampened, particularly in fecal samples (Yip et al., 2020). This leads to the same issue, where there are not enough viral particles present in the feces to accurately detect the virus, leading to a false negative result (Yip et al., 2020).

Vaccine failures

Vaccine success is important for the prevention and control of disease. Maternally derived antibody (MDA) interference is the major cause of vaccine failure, but administration or handling error can cause this as well, and there are also some co-factors that can cause failure as well. The CPV-2 vaccine is a core multivariant modified live vaccine composed of strains CPV-2 and CPV-2b, and it is recommended for pediatric and juvenile canines as early as possible, with two additional boosters. There are a few different options available for this live-attenuated vaccine. NEOTECH's NEOPAR® was the first vaccine available in 1979. Now, Zoetis and Merck Animal Health both offer a version of the vaccine as well. These can either be just parvovirus live-attenuated or a combination vaccine with distemper, adenovirus type 1 and 2, and canine parainfluenza virus (Gallagher, 2020). As discussed in Chapter 1, the main issue surrounding CPV-2 vaccine success is the interference of MDAs in seroconversion following vaccination, since it is administered during the pediatric and juvenile stages when these MDAs are still in circulation.

In order to try to overcome MDA interference with vaccination seroconversion, pet owners must, first and foremost, take care to prevent and control potential exposures during the late booster period because this is the time that they are most vulnerable to infection due to low MDA titers and potential initial booster failure (Yip et al., 2020). The next most important action pet owners must take is to closely adhere to the recommended booster schedule. Pet owners must continue to be cautious about prevention and control for another couple of weeks after the final booster vaccine is administered in order to give their pet's immune system time to acquire the immunity.

Other causes of vaccine failure may include viral antigenic diversity, high environmental viral load challenge, genetic non-responders, breed, other bacterial or parasitic enteric infections, and immune incompetence (Yip et al., 2020). For most vaccines, some other common hurdles to vaccine success are inadequate storage temperatures, administration errors, and the strain of virus used in the vaccine is mismatched to strain of virus encountered (Yip et al. 2020). The latter point is especially important in the case of CPV-2c because this variant strain is not currently present in the vaccines and research suggests that there is a higher rate of vaccine failure when this variant strain is encountered.

There is a long history of CPV-2 infection in canines globally ever since the virus emerged in 1978. Even though there has been a vaccine available since nearly the beginning, there are many hurdles in the control and prevention of this virus. There are new, emerging strains, difficulties with readily-available diagnostic assays, and an unacceptable amount of vaccine failures. There is a lot of room for research and improvement moving forward.

Chapter 3 – Future Directions and Discussion

For nearly 45 years, CPV-2 has had a major effect on the health of canines and other carnivores worldwide. Costs for treatment are substantial to pet owners, costing an average of $1,200 in supportive care (Kindred Biosciences, Inc., 2021). There is still a lot of research and development to be executed, but as technology continues to evolve, there is hope for developing more effective diagnostic and prevention strategies. This report has outlined the deficiencies that currently exist in the diagnosis, control, and prevention of CPV-2, and it has discussed how these three issues contribute to an ongoing vicious disease cycle that perpetuates the virus and subsequent disease. This report has also highlighted the absence of an effective treatment for CPV-2 infection.

As mentioned, prevention methods are currently lacking. The strains used in the currently available live attenuated vaccines are the CPV-2 and CPV-2b (Decaro et al., 2020). While CPV-2b still circulates worldwide, CPV-2 is not nearly as common anymore. Canine parvovirus 2c has been, and still is, on the rise, and it has shown itself to be more evasive from prevention through vaccination. The fact that it infects and causes disease in both vaccinated puppies and adult dogs is an obvious problem (Decaro et al., 2009). This suggests that there is a need for vaccines that include the CPV-2c strain. Recent technological advances in the development of vaccines have been achieved for other viruses, namely coronavirus disease 2019 (COVID-19) for humans. It may be advantageous to explore these newer technologies for the development of vaccines that include CPV-2c and other emerging variant strains. Coronavirus disease 2019 vaccines are mRNA-based, which is a new type of vaccine, and it is being explored for its use in other diseases caused by influenza virus, Zika virus, rabies virus, and

cytomegalovirus (Centers for Disease Control and Prevention, 2021). Therefore, this vaccine strategy could be used for canine parvovirus and other impactful veterinary diseases.

The canine parvovirus has structural properties that allow it to withstand sanitization efforts and environmental conditions for extended periods of time. This is one of the most difficult hurdles to overcome, as it is one of the key lines of defense in any disease control. It is pertinent that scientists develop an effective, and yet environmentally friendly, sanitization product that can be used in veterinary clinics, kennels, homes, yards, and dog parks. Future research should focus on developing a product that breaks down specific structures of the virus since they are what make CPV-2 so hardy.

Treatment is also an area that has been lacking because there is neither a cure nor a particularly effective treatment available. Supportive care is the only recommendation once a canine presents with clinical symptoms. Unfortunately, this care is both costly and does not guarantee survival. This is a research area that has been lacking for nearly 45 years since CPV-2 first appeared in canines. This is not surprising, since research for treatments of gastroenteritis that are caused by viruses in humans (other than supportive care) have also not been successful (Freedman et al., 2020).

Most recently, researchers at the animal health company, Kindred Biosciences, Inc., have had some critical breakthroughs that could drastically improve both the prevention and treatment of CPV-2. According to a press release that the company issued in September 2020, a pivotal efficacy study showed 100% success in preventing the disease in canines challenged with CPV-2 when administered prophylactically (Kindred Biosciences, Inc., 2020). In another press release issued by the company in June 2021, another pivotal efficacy study yielded 100% success in treating the disease when administered once a positive test result was obtained (Kindred

Biosciences, Inc., 2021). Unfortunately, there was very little detail provided regarding the design of each study, including the numbers of animals used in each study. The product, currently named “Kind-030”, appears to be based on the use of monoclonal antibodies (Kindred Biosciences, Inc., 2021). The company is pursuing two indications for this product, including “prophylactic therapy to prevent clinical signs of canine parvovirus infection" and "treatment of established parvovirus infection” (Kindred Biosciences, Inc., 2020; Kindred Biosciences, Inc., 2021). According to information released about Kind-030, the product works by binding to “critical portions of the virus, preventing the virus from entering into cells” (Kindred Biosciences, Inc., 2021). In April 2021, the USDA approved the efficacy indication for this monoclonal antibody (Kindred Biosciences, Inc., 2021). This product should prove to be revolutionary in prevention as it enters the market. If this product is granted approval for the treatment indication, it will be the first time that a targeted and reliable treatment has been available for CPV-2. At this time, there is no information available on whether or not this product works equally well for all strains of CPV-2. The manufacturer also did not disclose which strain or strains they utilized for challenge in either pivotal efficacy study. This information will be valuable when they are released to the market, since as discussed previously for diagnostic assays and vaccines, what works for one strain of virus may not be as effective for the other strains of virus.

Diagnostic assays are another major area in need of drastic improvement based on the research discussed in this report. The in-clinic, rapid tests utilized in most veterinary clinics have been shown to have low sensitivity, and they have also been shown to yield a high proportion of false negative test results. Using technology that is currently available, a new and different type of in-clinic assay with better sensitivity could be developed. Additionally, while

PCR is exponentially more sensitive at detecting CPV-2 than nearly all other available diagnostic assays, the levels of detection for existing ELISA-based assays show that there could be room for improvement.

There is still more research needed regarding several aspects of the virus itself. Yip et al. (2020) suggested that controlled studies comparing the pathogenicity of all CPV-2 strains should be performed, as this information is currently lacking and would provide substantial insight for several different areas of research. There is also a need to perform detailed molecular surveillance on all of the emerging CPV-2 strains that are in circulation worldwide (Yip et al., 2020). This information would be very beneficial from epidemiological standpoint, as it would help guide the analysis of the virus's distribution pattern data, as well as to inform decisions for the strategies for prevention and control in public health. There is also very little information about what co-factors are associated with a higher risk of CPV-2 infection, which could be used to reduce infection rates and minimalize the effects of the disease (Yip et al., 2020).

At the population level, it is important to make advancements in diagnostic assays and in control and prevention strategies. As different strains of CPV-2 are able to efficiently spread, mutate, and multiply, it is more likely that the world will see the emergence of new and significantly novel strains of CPV-2 in a population that is immunologically naive to these strains.

During the COVID-19 pandemic, BluePearl™ Specialty and Emergency Pet Hospital, Inc. reported that from their 90 clinics, they saw a 70% increase in CPV-2 cases relative to emergency cases when compared to the prior five years (2015-2019) (BluePearl™, 2020). Possible explanations for the increased numbers of CPV-2 cases may be that: 1) there was a rise in the supply and/or demand of shelter and foster pets, 2) canines moved through shelter systems

so quickly that proper precautions and protocols were not always followed, 3) dog owners took their dogs to new outdoor parks or other locations where CPV-2 survived in the environment, and 4) the pandemic caused physical or financial barriers to completing vaccination protocols. BluePearl™ is in the early stages of analyzing this data, but it does highlight how a major world event, like the COVID-19 pandemic, may contribute to the ongoing spread of CPV-2 and the potential for the emergence of new strains. Regardless of the reasons listed above, the ongoing transmission and the ability of CPV-2 to readily mutate to variant strains that are undetected by current in-clinic diagnostic assays and that cannot be prevented by currently licensed vaccines should be areas of future research efforts. Specifically, diagnostic assays should be quickly developed to accurately detect any emergent strains of CPV-2, and new vaccine development strategies could be based on those used more recently for human diseases. A new monoclonal antibody treatment shows promise for the future; however, until more data is collected, it is unclear whether it will be an effective treatment for disease caused by variant strains of CPV-2.

Bibliography

Allison, A. B., Harbison, C. E., Parrish, C. R., Pagan, I., Stucker, K. M., Kaeiber, J. T., Brown, J. D., Ruder, M. G., Keel, K.M., Dubovi, E. J., & Holmes, E. C. (2012). Role of multiple hosts in the cross-species transmission and emergence of a pandemic parvovirus. Journal of Virology, 86(2), 865–872.

America Veterinary Medical Association. Canine parvovirus. (2021). Retrieved 2021, from https://www.avma.org/resources-tools/pet-owners/petcare/canine-parvovirus

Battilani, M., Modugno, F., Mira, F., Purpari, G., Di Bella, S., Guercio, A., & Balboni, A. (2019). Molecular epidemiology of canine parvovirus type 2 in Italy from 1994 to 2017: Recurrence of the CPV-2b variant. BMC Veterinary Research, 15(1), 393.

BioNote. (n.d.). Rapid CPV/CCV Ag. Retrieved 2021, from http://bionote.co.kr/eng/board/rapid/board_view.asp?search_category=1&num=254

BluePearl™. (2020, July 6). BluePearl Finds Alarming Increase in Parvovirus Cases Amid COVID-19. Retrieved 2021, from https://bluepearlvet.com/press-releases/bluepearl-pet-hospital-finds-alarming-increase-in-parvovirus-cases-amid-covid-19/

Buonavoglia, C., Cavalli, A., Tempesta, M., Voight, V., Buonavoglia, D., Corrente, M., & Sagazio, P. (1995). Intranasal vaccination of pups in the presence of maternally derived antibodies to canine parvovirus (CPV). Evaluation of minimal immunizing dose. The New Microbiologica, 18(4), 371–375.

Buonavoglia, C., Martella, V., Pratelli, A., Tempesta, M., Cavalli, A., Buonavoglia, D., Bozzo, G., Elia, G., Decaro, N., & Carmichael, L. (2001). Evidence for evolution of canine parvovirus type 2 in Italy. Journal of General Virology, 82(12), 3021–3025.

Carmichael, L. E., Schlafer, D. H., & Hashimoto, A. (1994). Minute virus of canines (MVC, canine parvovirus type-1): Pathogenicity for pups and seroprevalence estimate. Journal of Veterinary Diagnostic Investigation, 6(2), 165-174.

Carter, B. J., & Tattersall, P. J. Chapter 19 Parvoviridae. In Congenital and Other Related Infectious Diseases of the Newborn; Elsevier BV: Amsterdam, The Netherlands, 1987; pp. 325–334

Centers for Disease Control and Prevention. (2021, March 4). Understanding mRNA COVID-19 Vaccines. Retrieved 2021, from https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-

vaccines/mRNA.html?s_cid=11344:how%20does%20mrna%20vaccine%20work:sem.ga:p:RG:GM:gen:PTN:FY21

Chang, S. F., Sgro, J. Y., & Parrish, C. R. (1992). Multiple amino acids in the capsid structure of canine parvovirus coordinately determine the canine host range and specific antigenic and hemagglutination properties. Journal of Virology, 66(12), 6858-6867.

Cureton, D. K., Harbison, C. E., Cocucci, E., Parrish, C. R., & Kirchhausen, T. (2012). Limited transferrin receptor clustering allows rapid diffusion of canine parvovirus into clathrin endocytic structures. Journal of Virology, 86(9), 5330-5340.

Decaro, N., Martella, V., Desario, C., Bellacicco, A. L., Camero, M., Manna, L., D'Aloja, D., & Buonavoglia, C. (2006). First detection of canine parvovirus type 2c in pups with haemorrhagic enteritis in Spain. Journal of Veterinary Medicine. Series B, 53(10), 468–472.

Decaro, N., Desario, C., Addie, D. D., Martella, V., Vieira, M. J., Elia, G., Zicola, A., Davis, C., Thompson, G., Thiry, E., Truyen, U., & Buonavoglia, C. (2007). The study molecular epidemiology of canine parvovirus, Europe. Emerging Infectious Diseases, 13(8), 1222–1224.

Decaro, N., Desario, C., Parisi, A., Martella, V., Lorusso, A., Miccolupo, A., Mari, V., Colaianni, M. L., Cavalli, A., Di Trani, L., & Buonavoglia, C. (2009). Genetic analysis of canine parvovirus type 2c. Virology (New York, N.Y.), 385(1), 5–10.

Decaro, N., Desario, C., Beall, M. J., Cavalli, A., Campolo, M., DiMarco, A. A., Amorisco, F., Colaianni, M. L., & Buonavoglia, C. (2010). Detection of canine parvovirus type 2c by a commercially available in-house rapid test. The Veterinary Journal (1997), 184(3), 373–375.

Decaro, N., & Buonavoglia, C. (2012). Canine parvovirus—A review of epidemiological and diagnostic aspects, with emphasis on type 2c. Veterinary Microbiology, 155(1), 1-12.

Decaro, N., Buonavoglia, C., & Barrs, V. R. (2020). Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? Veterinary Microbiology, 247, 108760.

Freedman, S. B., Xie, J., Nettel-Aguirre, A., Pang, X. L., Chui, L., Williamson-Urquhart, S., Schnadower, D., Schuh, S., Sherman, P. M., Lee, B. E., Gouin, S., Farion, K. J., Poonai, N., Hurley, K. F., Qiu, Y., Ghandi, B., Lloyd, C., & Finkelstein, Y. (2020). A randomized trial evaluating virus-specific effects of a combination probiotic in children with acute gastroenteritis. Nature Communications, 11(1), 2533–2533.

Gallagher, A. (2020, June). Canine parvovirus. Retrieved 2021, from https://www.merckvetmanual.com/digestive-system/diseases-of-the-stomach-and-intestines-in-small-animals/canine-parvovirus#v3266294

Goddard, A., & Leisewitz, A. L. (2010). Canine parvovirus. The Veterinary Clinics of North America. Small Animal Practice, 40(6), 1041-1053.

Halder, S., Ng, R., & Agbandje-McKenna, M. (2012). Parvoviruses: Structure and infection. Future Virology, 7(3), 253-278.

Hayes, M. A., Russel, R. G., Mueller, R. W., & Lewis, R. J. (1979). Myocarditis in young dogs associated with a parvovirus-like agent. Canadian Veterinary Journal, 20(5), 126.

Hong, C., Decaro, N., Desario, C., Tanner, P., Pardo, M. C., Sanchez, S., Buonavoglia, C., & Saliki, J. T. (2007). Occurrence of canine parvovirus type 2c in the United States. Journal of Veterinary Diagnostic Investigation, 19(5), 535–539.

Houston, D. M., Ribble, C. S., & Head, L. L. (1996). Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982-1991). Journal of the American Veterinary Medical Association, 208(4), 542-546.

IDEXX Laboratories, Inc. (n.d.). SNAP® Parvo. Retrieved 2021, from https://idexxcom-live-b02da1e51e754c9cb292133b-9c56c33.aldryn-media.com/filer_public/fa/e6/fae66802-7adc-476b-a319-fef522525d39/4414-00-parvo_uk.pdf

Isogai, E., Isogai, H., Onuma, M., Mizukoshi, N., Hayashi, M., & Namioka, S. (1989). Escherichia coli associated endotoxemia in dogs with parvovirus infection. Japanese Journal of Veterinary Science, 51(3), 597–606.

Jacobs, R. M., Weiser, M. G., Hall, R. L., Kowalski, J. J (1980). Clinic pathogenic features of canine parvoviral enteritis. Journal of the American Veterinary Medical Association, 16(4), 809–813.

Kindred Biosciences, Inc. (2020, September 16). Kindred Biosciences Announces Positive Results from Pivotal Efficacy Study of Parvovirus Monoclonal Antibody. Retrieved 2021, from https://ir.kindredbio.com/news-releases/news-release-details/kindred-biosciences-announces-positive-results-pivotal-efficacy

Kindred Biosciences, Inc. (2021, June 2). Kindred Biosciences Announces Positive Results from Pivotal Efficacy Study of Parvovirus Monoclonal Antibody for the Prevention of Deaths in Dogs Infected by Parvovirus. Retrieved 2021, from https://ir.kindredbio.com/news-releases/news-release-details/kindred-biosciences-announces-positive-results-pivotal-0

Kuhn, J. H. (2017). Virus taxonomy. Reference Module in Life Sciences, Reference Module in Life Sciences, 2017.

Markovich, J. E., Stucker, K. M., Carr, A. H., Harbison, C. E., Scarlett, J. M., & Parrish, C. R. (2012). Effects of canine parvovirus strain variations on diagnostic test results and clinical management of enteritis in dogs. Journal of the American Veterinary Medical Association, 241(1), 66-72.

Miranda, C., Parrish, C. R., & Thompson, G. (2014). Canine parvovirus 2c infection in a cat with severe clinical disease. Journal of Veterinary Diagnostic Investigation, 26(3), 462-464.

Miranda, C., & Thompson, G. (2016). Canine parvovirus: The worldwide occurrence of antigenic variants. Journal of General Virology, 97(9), 2043-2057.

Nandi, S., & Kumar, M. (2010). Canine parvovirus: Current perspective. Indian Journal of Virology, 21(1), 31-44.

National Center for Biotechnology Information. (2020). Parvoviridae. Retrieved 2021, from https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?id=10780

Organtini, L. J., Allison, A. B., Lukk, T., Parrish, C. R., & Hafenstein, S. (2015). Global displacement of canine parvovirus by a host-adapted variant: Structural comparison between pandemic viruses with distinct host ranges. Journal of Virology, 89(3), 1909-1912.

Parrish, C. R., Oliver, R. E., & McNiven, R. (n.d.). Canine parvovirus infections in a colony of dogs. Veterinary Microbiology, 7(4), 317–324.

Parrish, C. R., O'Connell, P. H., Evermann, J. F., & Carmichael, L. E. (1985). Natural variation of canine parvovirus. Science (American Association for the Advancement of Science), 230(4729), 1046-1048.

Parrish, C. R., Have, P., Foreyt, W. J., Evermann, J. F., Senda, M., & Carmichael, L. E. (1988). The global spread and replacement of canine parvovirus strains. Journal of General Virology, 69(5), 1111-1116.

Parrish, C. R., Aquadro, C. F., Strassheim, M. L., Evermann, J. F., Sgro, J. Y., & Mohammed, H. O. (1991). Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus. Journal of Virology, 65(12), 6544-6552.

Parrish, C. R. (1999). Host range relationships and the evolution of canine parvovirus. Veterinary Microbiology, 69(1), 29-40.

Parrish, C. R., & Kawaoka, Y. (2005). The origins of new pandemic viruses: The acquisition of new host ranges by canine parvovirus and influenza A viruses. Annual Review of Microbiology, 59(1), 553–586.

Pereira, G. Q., Gomes, L. A., Santos, I. S., Alfieri, A. F., Weese, J. S., & Costa, M. C. (2018). Fecal microbiota transplantation in puppies with canine parvovirus infection. Journal of Veterinary Internal Medicine, 32(2), 707–711.

Serpell, J. A., & Duffy, D. L. (2016). Aspects of juvenile and adolescent environment predict aggression and fear in 12-month-old guide dogs. Frontiers in Veterinary Science, 3, 49.

Tosaris, G. (2018). The outbreak of canine parvovirus in North America. Retrieved 2021, from https://microbewiki.kenyon.edu/index.php/The_Outbreak_of_Canine_Parvovirus_in_North_America

Truyen, U., Agbandje, M., & Parrish, C. R. (1994). Characterization of the feline host range and a specific epitope of feline panleukopenia virus. Virology (New York, N.Y.), 200(2), 494-503.

Truyen, U., Gruenberg, A., Chang, S-F., Obermaier, B., Veijalainen, P., & Parrish, C. R. (1995). Evolution of the feline-subgroup parvoviruses and the control of canine host range in vivo. Journal of Virology, 69(8), 4702-4710.

Truyen U., Evermann J. F., Vieler E., & Parrish C. R. (1996). Evolution of canine parvovirus involved loss and gain of feline host range. Virology (New York, N.Y.), 215, Virology (New York, N.Y.), Vol.215.

Truyen, U., Steinel, A., Bruckner, L., Lutz, H., & Möstl, K. (2000). Distribution of antigen types of canine parvovirus in Switzerland, Austria and Germany. Schweizer Archiv für Tierheilkunde, 142(3), 115–119.

Tu, M., Liu, F., Chen, S., Wang, M., & Cheng, A. (2015). Role of capsid proteins in parvoviruses infection. Virology Journal, 12(1), 114.

Yip, E., Hiu, Y., Peaston, A., Woolford, L., Khuu, S. J., Wallace, G., Kumar, R. S., Patel, K., Ahani Azari, A., Akbarzadeh, M., Sharifian, M., Amanollahi, R., Jafari Jozani, R., Khabiri, A., & Hemmatzadeh, F. (2020). Diagnostic challenges in canine parvovirus 2c in vaccine failure cases. Viruses, 12(9), 980.