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■ RESEARCH ARTICLE

Battling Canine Parvovirus with Antiviral Peptide Therapy

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ABSTRACT: Canine Parvovirus (CPV) is a gastrointestinal virus transmitted through feces that causes severe illness in infected individuals and remains incurable. Unlike its original counterpart, CPV-1, which only affected puppies, CPV-2 has crossed the species barrier numerous times and affects wild animals of all ages. CPV-2 has developed significantly, transforming from CPV-2A to CPV-2B and CPV-2C. Finding an effective treatment method is critical to limit the evolution of the virus and conserve endangered species. While there are preventative vaccines for domestic animals, efforts to address outbreaks in wild animals remain inadequate. This includes current therapies being tested, like monoclonal antibodies, which cannot serve as a long-term solution to combat the adaptive nature of the pathogen. Antiviral Peptide Therapy (APT) uses peptides, or short amino acid chains, designed to target specific viruses and prevent their replication. Here, we propose the novel application of APT for the global treatment of CPV-2 in endangered species and compare it with other therapies developed today. While APT has never been used on animals or to address CPV-2, this method may be more effective than current therapies being explored, and its potential to revive endangered species is very promising.

KEYWORDS: Translational Medical Sciences; Antiviral Peptide Therapy; Canine Parvovirus; Disease Treatment and Therapies; Endangered Species.

■ Introduction

The first CPV strain, CPV-1, only affected young, domesticated dogs around three weeks old. In 1978, the first case of the second, more prominent strain (CPV-2) was discovered in Germany, causing a widespread epidemic among numerous species. It is still a worldwide concern and is considered endemic, especially among susceptible populations like immuno-compromised species or areas with low wildlife vaccination rates.[1] CPV-2 outbreaks among such populations can impact reproductive success, population growth, and genetic variability.[1] Efforts have been made to protect these endangered animals, including disease surveillance, reduction in contact with other species, vaccinations, and development of antibody therapies. However, a more effective measure is necessary to fully combat the destructive effects of CPV-2 fully. In younger animals, CPV-2 has exhibited a mortality rate of up to 80%, resulting in substantial declines in endangered species populations.[2] The highly contagious nature of CPV-2 and its ability to spread rapidly among vulnerable animal populations makes it a pressing concern for conservation efforts.

This is why antiviral peptide therapy (APT) is a novel yet promising proposal for the pressing concern of CPV-2. In recent years, APT has emerged as an effective approach in the battle against other viral infections such as influenza, herpes simplex virus, SARS-CoV-2, and human immunodeficiency virus. APT involves short chains of amino acids, known as peptides, specifically designed to target and disrupt viral structures or processes. These peptides can interfere with viral entry into host cells, inhibit viral replication, or modulate the host's immune response to combat the infection.[8] Their ability to target specific viral components makes them particularly effective against a wide range of viruses, opening up the possibility that they can be effective against CPV. This modern therapy offers a promising avenue for developing antiviral treatments with potentially fewer side effects and lower resistance risk than traditional drugs.

Here, the focal point of our research centers on the development and application of APT as a potential treatment for CPV-2 in endangered species. With a specific emphasis on its comparative effectiveness against existing treatments, our research seeks to unravel the intricacies of APT application in conservation. We discuss how the unique challenges of treating endangered species can be addressed through the innovative application of APT and how its efficacy measures up against current conventional treatments. This investigation aims to shed light on the benefits of APT for endangered species and contribute insights into the broader landscape of CPV-2.

Genetic Diversity and Evolution of CPV :

While CPV-2 is the most significant of the canine parvoviruses, a large variety of strains and modifications of this particular virus make its effects much more widespread. The virus is believed to have developed and evolved from the feline panleukopenia virus (FPV) in the late 1970s, with the first CPV-2 appearing in the early 1980s.[3] CPV-2 has also begun to affect wild animals and deteriorate their already critically endangered statuses due to contact between domestic dogs and wild animals, whether physically or by their feces. Detection of CPV-2 in wild animals has been reported in scientific literature since around the late 1900s. Alongside the well-known CPV-2 strain, researchers at Cornell University's Wildlife Health Lab reported related strains, including RPV (Raccoon Parvovirus)

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and BFPV (Blue Fox Parvovirus), originating from the same Feline Panleukopenia lineage. These strains have specific genetic distinctions enabling their infectivity in an even greater diversity of wildlife populations, including raccoons, foxes, big cats, and other felids such as lions, tigers, leopards, cougars, and civets.[3] CPV-2 has spread among various families and species, including several endangered species, as seen in Figure 1.

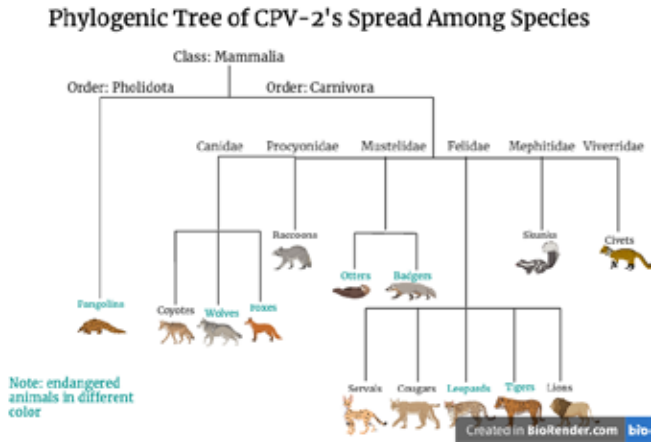

Figure 1: Phylogenetic Tree of CPV-2's Spread Among Species. This is a phylogenetic tree, not to scale, meant to showcase the spread of CPV-2 among species, with endangered species in blue. It shows that close to half of the species affected by CPV-2 are endangered.

Aside from having other branches from the common ancestor, CPV-2 has diversified into smaller variants like 2a, 2b, and 2c.[1] Each variant possesses distinct genetic traits influencing its impact and adaptation to different hosts. CPV-2a, the original variant, initiated the early outbreaks. Subsequently, CPV-2b emerged, exhibiting improved resilience against antibodies, eroding the effectiveness of existing vaccines. The most recent variant, CPV-2c, has unique genetic features and widespread prevalence in various regions and is still being studied today.[1] The development of different strains over time is described in Figure 2.

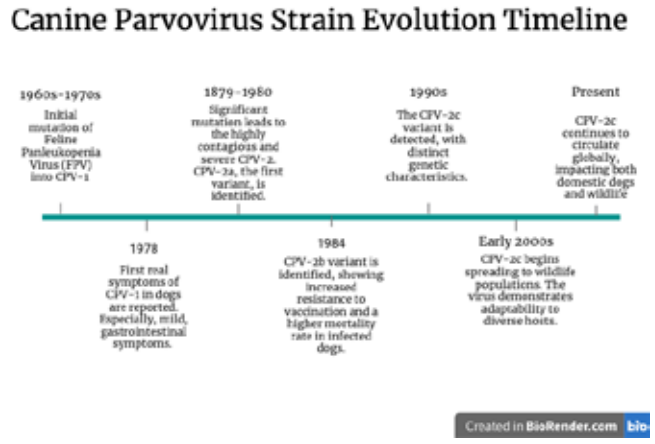

Figure 2: Canine Parvovirus Strain Evolution Timeline. This timeline from the 1960s to the present is a visual representation of the information presented in research done by Gael Darren Maganga *et al.*, 2023 regarding the different adaptations the virus underwent before becoming CPV-2.

Specifically, the recent variant displays changes in certain amino acids and proteins.[4] The diverse genetic makeup of these variants dictates many characteristics such as their host cell recognition, environmental stability, and evasion of the host's immune response. This genetic diversity emphasizes the need for a comprehensive, adaptive vaccine and treatment development strategy. A treatment method targeted at one variant can open the door to addressing the entire spectrum of CPV variants and related viruses, a vital step in safeguarding many species. Therefore, a closer look at viral evolution is an important aspect of finding and proposing a treatment for CPV.

CPV Pathology:

The most common symptoms of a wild animal infected by CPV-2 include lethargy, lack of appetite, and a fever, along with results of the virus's effect on the gastrointestinal system such as soft, foul smelling, mucous, or bloody feces, vomiting, and diarrhea. These symptoms and the context of the virus's path within the host are much better understood. Once inside, CPV-2 targets the body's fastest-multiplying cells and uses the cell's machinery to replicate its genetic material. Initially, the virus attacks the lymphoid tissues in the oropharynx, from where it spreads systemically via the bloodstream to several organs, including the bone marrow and other lymphoid organs. It begins by destroying the bone marrow, which is rich in crucial lymphocytes. By destroying these cells, CPV-2 suppresses lymphocyte production, resulting in lymphopenia, a dangerously low count of lymphocytes, which significantly weakens the immune response.

As the virus disseminates, it also reaches the gastrointestinal tract, where it preferentially infects the rapidly dividing cells in the small intestinal crypts which are crucial for replenishing the intestinal lining. The infection disrupts the epithelial lining of the intestine, which normally prevents fluid loss and bacterial invasion. This leads to severe symptoms like diarrhea and nausea. As the virus destroys the intestinal barrier, gut bacteria can breach this weakened defense, potentially entering the bloodstream and causing a life-threatening, systemic infection.

Throughout the infection, the CPV-2 virus uses the host cells' replication machinery to produce more viral particles, achieving viral entry at every stage—from the first infected cell to the last—ensuring its spread and persistence within the host.

■ Discussion

Global Impact of CPV-2 on Wildlife:

Global case studies conducted on a diverse range of animal species further demonstrate the profound impact of CPV-2. In Taiwan, from October 2017 to June 2019, researchers examined four Taiwanese pangolins that showed signs of gastrointestinal illness. They found that the sequences of nucleic acids from these four pangolins were identical to the CPV-2c strain found in China among domestic dogs. The team later used this information to identify the prevalence of the CPV-2 virus across all Taiwanese pangolins, a species already deeply affected by habitat destruction and animal trafficking.[5] The impact of CPV-2 on endangered species does not stop there;

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it was discovered to have affected other species, including the Asian small-clawed otter. In Japan, researchers Kenichi Temukai and Shohei Minami, among others, conducted a post-mortem examination on one of two otters that had been imported from Indonesia. The two displayed distinctive gastrointestinal issues, and the medics found a necrotic small intestine along with swollen lymph nodes. An oral swab of the animal confirmed it was the CPV-2a strain, the first to affect small-clawed otters.[6] In South Africa, effects of CPV-2 were observed during a case study on an unvaccinated serval from South Africa. The serval presented with vomiting, anorexia, and diarrhea before dying around two weeks later. While this was the first known case of CPV-2 among the serval species, extensive research has been done to explore further the virus's impact on non-domestic species on the African continent.[7] Fundamentally, studies on many species across different continents have furthered what is known about the effects of the virus, which is visually represented in Figure 3.

Figure 3: Global Map of Canine Parvovirus Cases. This map of the entire planet pinpoints different locations of species that have been presented with CPV-2 to show the global impact of the virus.

The presence of the virus across several species globally further enforces the increased urgency of the matter and the need for a solution.

Applying Antiviral Peptide Therapy:

Using antiviral peptides to target viruses is an important research development and opens the door to new possibilities. These peptide chains can be designed specific to a single virus and target the virus's specific proteins, a quality known as being virucidal. When developing antiviral peptides, scientists target specific stages in the viral process, such as viral entry, viral synthesis, or assembly. Scientists view viral entry as the most crucial step in the CPV-2 process, making it the most optimal stage to target with APT. Specifically, as CPV-2 enters, it uses a lock and key mechanism to bind to the membrane.[8] Within this lock and key mechanism, the virus's capsid protein binds specifically to sialic acid residues present on the host cell membrane.[1] This interaction triggers endocytosis, allowing the virus to enter the cell. With the current research and technology, the antiviral peptide can be essentially coded to interfere with this lock and key mechanism. Precisely, the antiviral can mimic the host cell's cellular receptor and compete with the binding site in a process of competitive inhibition. For CPV-2, the primary cellular receptor is the canine transferrin receptor type 1 (TfR1), also known as the transferrin receptor protein 1 (TfR1).[8] The antiviral peptide would mimic the TfR1 to successfully guide the virus away from the cell, preventing it from replication and infecting the body. Additionally, because CPV-2 directly attacks rapidly dividing areas such as the bone marrow and epithelial cells, cell-penetrating peptides (CPPs) can be added to the antiviral chain to facilitate the entry of the antiviral into these areas during administration.[8]

Advantages of APT Over Current Therapies:

APT presents a targeted and potentially effective means to combat CPV-2 infections in these vulnerable populations. Today, the only significant therapy for CPV-2 being tested is an antibody-based solution known as Monoclonal Antibody Therapy. While antibodies are more efficient in developing, antivirals are much more specific and accurate in the long run. Unlike antibodies, which depend very heavily on cellular geometry and no longer work if the virus undergoes even the slightest of changes, antivirals target viral replication and reproduction. The reliability of antivirals is especially important considering the evolvability (or adaptability) of CPV-2 and its several strains. However, antivirals are much more complex to develop, hence why they can target complicated, distinct viruses. Peptides are biologically active molecules composed of a chain of amino acids, and they are also easily broken down by the enzyme peptidase, making them less likely to accumulate in areas and cause toxic side effects like pituitary damage. These peptides are small, easy to synthesize, and can be made to be very specific, especially with the help of advanced research. The antiviral peptide can be designed to attack a specific stage, typically the most crucial in the viral replication process, making it impossible for the virus to harm the animal further.[8] Overall, in light of the profound threat CPV-2 poses to endangered species and the pressing need for innovative solutions, the exploration of an APT targeting the CPV-2 cell entry stands as an incredible solution, offering hope in improving conservation efforts and mitigating the devastating impact of this pathogen and on the most vulnerable populations, with minimal negative side effects.

Potential Side Effects:

Because peptides are biological molecules, they are known to work cohesively with other bodily functions, often resulting in fewer side effects than traditional antiviral medications. They tend to have minimal impact on healthy cells, which reduces overall toxicity. While specific data on the side effects of antiviral peptides (AVPs) for CPV is lacking, peptide-based therapeutics for other viruses, such as HIV, have been associated with mild symptoms like headaches, rashes, and mild nausea, as well as localized allergic reactions or irritation at the administration site.[9] In clinical trials, for instance, Enfuvirtide (Enf) used to treat HIV showed skin reactions at the injection site in over 98% of patients as seen by Tessier *et al.*, in 2011. Additionally, peptides such as Boceprevir and Telaprevir, approved for Hepatitis C, have demonstrated side effects including fatigue, anemia, and dysgeusia.[8] Researchers have attributed the variation in side effects to factors such as gender, genetics, and age. In cases of endangered species suffering from CPV, the type of species may also influence the reac

tion to peptide treatments. Ongoing research aims to optimize these therapies by balancing their efficacy with potential side effects, ensuring they can be safely used against a wide range of viral infections.

Methods

This paper follows a retrospective analysis, synthesizing information from a range of peer-reviewed sources and case studies on Canine Parvovirus (CPV). The research process began by establishing a foundational understanding of CPV's etiology, pathology, and symptoms through an extensive literature review. Veterinary manuals and research papers provided comprehensive insights into the virus's history, the mechanism of infection, and clinical manifestations in canine populations. These resources were cross-referenced with global case studies to examine the virus's symptoms across diverse geographical regions and identify any variations in its global epidemiology.

To explore current and potential treatment strategies, existing literature on antiviral therapies was analyzed, focusing particularly on why some proposed treatments, despite their promising findings, have not yet entered the market. This included a review of studies on monoclonal antibodies, along with antiviral peptide therapy—a treatment used in comparable viral diseases, such as human COVID-19. Findings were applied to CPV by mapping the virus's progression through the host body and identifying potential points of intervention with antiviral peptides.

The final stage of this research involved a critical evaluation of the drawbacks associated with antiviral peptide therapy, drawing on existing antiviral tests to understand side effects and consulting veterinary manuals for appropriate administration techniques. These findings were incorporated into a broader discussion about the practical considerations and challenges in developing new CPV treatments, including regulatory, logistical, and financial hurdles.

Open Questions:

As APT progresses in human medicine, it is important to consider the open questions it may raise. Wildlife conservation faces unique difficulties, such as global biodiversity loss, the looming threat of a sixth mass extinction, and the accelerated spread of infectious diseases due to human encroachment into natural habitats. The relationship between the anthropogenic and wildlife world is ever-changing and requires advanced therapy to prevent the catastrophes that the pathogen promises. Unfortunately, the lack of resources, research, demand, and attention to the animal world's problems hinder treatments' crucial and necessary progression towards the deadly pathogen. This is why APT is a significant and groundbreaking proposal to a pressing, frequently overlooked issue.

Additionally, the translation of APT from lab to wild settings comes with potential challenges and limitations that must be addressed before it can be developed into viable therapies for CPV. These limitations are primarily due to the peptide-based nature of APT. The synthesis process is complex and expensive, which may limit accessibility and scalability. Additionally, APTs are inherently unstable and sensitive to degradation, making their use in field conditions particularly challenging. They often require parenteral administration, which presents practical difficulties due to the inaccessibility and predictability of wildlife treatment. Moreover, there is a risk of allergenic reactions in some cases, even regardless of prior data.

Acknowledging potential errors and limitations in translating APT from lab to wild settings is important. A complex, intricate therapy such as APT may take years to reach promising lab stages. However, the proposal may raise questions about dosage optimization, delivery methods, and broader ecological conflicts. Administering such a complex therapy to wild animals may be complicated and tedious. Though the likelihood of developing resistance to APT is very low, especially compared to other treatment options, some risks remain. Especially in such a fragile world, and as with all well-meaning interventions, the unknown outcomes of using APT in wildlife heed a cautious and calculated approach. Regardless of the possible complications, this novel proposal is just as promising as it is crucial to solving a pressing and urgent issue in the animal world. These open questions press for future exploration and may guide the promising potential of such a therapy in the right direction.

Conclusion

Overall, APT poses a promising avenue in combating the harmful pathogen CPV. Currently, the most prevalent strain of the virus is CPV-2c, which has become increasingly common among domesticated animals and endangered species such as wolves, foxes, and more. Looking into the future, various efforts are being directed at conserving the species affected by CPV-2, including developing drugs, isolating infected species, improving supportive care, and enhancing vaccination strategies. The drastic impacts of CPV-2 make finding a long-lasting solution and combating its effects on endangered species before they reach extinction increasingly important. This is why antiviral peptide therapeutics appear to be an ideal solution. APT is being developed to target other impactful viruses, such as SARS-CoV-2, herpes simplex virus, and influenza virus, and the results are quite optimistic. The ability to adapt peptides specifically for the complexity and ever-changing nature of CPV-2 further makes it a reliable and stable possibility. By mimicking the lock and key mechanism of CPV, antiviral peptides pose a dependable method of protecting species from this still-incurable pathogen's often fatal and long-lasting effects. As we navigate the potential difficulties of APT, the well-being of the planet's wildlife is illuminated by the quest for innovative treatments and preventative strategies, striving for a brighter and healthier future for struggling species.

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