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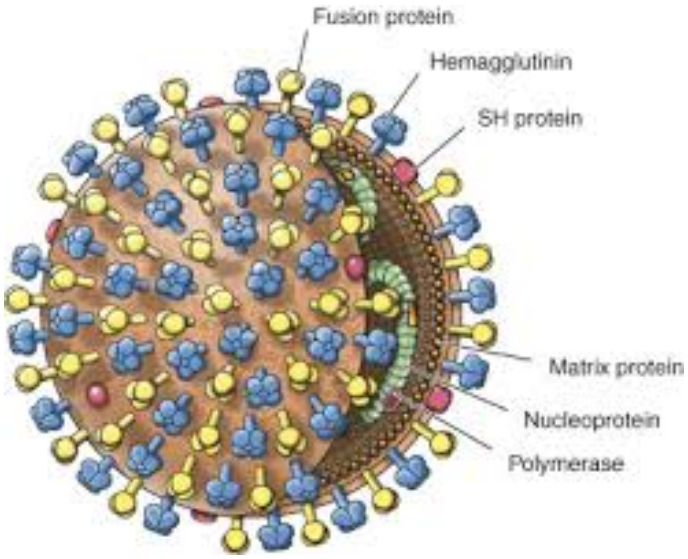

PERPUSTAKAAN BALAI BESAR PERAKITAN DAN MODERNISASI VETERINER

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Kata Pengantar

Bibliografi Canine Distemper Virus: Dinamika, Evolusi, dan Tantangan Pengendalian

Puji syukur kita panjatkan ke hadirat Tuhan Yang Maha Esa, karena atas rahmat dan karunia-Nya bibliografi bertema Canine Distemper Virus (CDV) ini dapat tersusun. Bibliografi ini disusun sebagai upaya menyediakan sumber informasi ilmiah yang komprehensif mengenai penyakit *Canine Distemper*—salah satu penyakit menular penting pada anjing dan berbagai spesies satwa liar yang disebabkan oleh virus dari genus *Morbillivirus*.

Kumpulan referensi dalam bibliografi ini mencakup hasil-hasil penelitian terkini yang membahas berbagai aspek CDV, meliputi:

- Deteksi dan identifikasi molekuler virus dengan teknik PCR, RT-PCR, hingga *digital chip PCR*.
- Studi epidemiologi pada populasi domestik dan satwa liar di berbagai negara.
- Analisis genetik, evolusi, serta hubungan antara strain vaksin dan strain lapangan.
- Peranan reseptor seluler seperti *nectin-4* dan *PVRL4* dalam proses infeksi dan transmisi virus.
- Pengembangan vaksin baru dan evaluasi efektivitasnya terhadap berbagai varian virus.
- Infeksi lintas spesies, patogenesis neurologis, dan mekanisme kekebalan inang terhadap CDV.

Bibliografi ini diharapkan dapat menjadi sumber rujukan yang bermanfaat bagi peneliti, akademisi, mahasiswa, serta praktisi kesehatan hewan dalam memahami perkembangan riset dan upaya pengendalian *Canine Distemper Virus*. Melalui pemanfaatan literatur ini, diharapkan dapat muncul inovasi dan strategi baru untuk memperkuat program vaksinasi, meningkatkan kewaspadaan epidemiologis, dan melindungi populasi hewan domestik maupun satwa liar dari ancaman penyakit ini.

Bibliografi ini dapat diakses secara gratis di Perpustakaan Balai Besar Perakitan dan Modernisasi Veteriner (BRMP Veteriner).
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Akhir kata, kami menyampaikan terima kasih kepada semua pihak yang telah mendukung penyusunan bibliografi ini. Semoga karya ini dapat memberikan kontribusi nyata bagi pengembangan ilmu pengetahuan dan peningkatan kesehatan hewan di Indonesia.

Perpustakaan BRMP Veteriner

Daftar Isi

1. **Establishment of a chip digital PCR detection method for canine circovirus**,Xiaoxiao Lei, Qiao Lv, Yan Qin, Wei Chen, Yanqing Hu, Chenchen Zhao, Xinyu Zhang, Haixin Huang, Yuying Li, Jingyi Lu, Tian Lan, Wenchao Sun, Min Zheng,Heliyon,Volume 10, Issue 9,2024,e30859,https://doi.org/10.1016/j.heliyon.2024.e30859.

Abstract:

Canine circovirus (CanineCV), which is a new mammalian circovirus first reported in the United States in 2012, mainly causes diarrhea and vomiting in dogs. As CanineCV evolves and new subtypes emerge, there is an urgent need for new detection technologies to improve the sensitivity and detection rates of viruses in complex scenarios. A chip digital PCR(cdPCR) assay was established for the detection of CanineCV in this study. The results showed good reproducibility, specificity and a linear relationship; the minimum detection limit of CanineCV by cdPCR was 6.62 copies/μL, which is 10 times more sensitive than quantitative real-time PCR (qPCR). The qPCR-positive detection rate was 1 %, while CanineCV cdPCR (2.1 %) exhibited a greater positive detection rate. Fifteen complete genomes were sequenced and subdivided into CanineCV-1 and CanineCV-3. In conclusion, we developed a rapid, reliable, and specific cdPCR method for screening and monitoring canine CV.

Keywords: Canine circovirus (CanineCV); Chip digital PCR (cdPCR); Quantitative real-time PCR (qPCR)

2. **Interaction of a synthetic peptide corresponding to the N terminus of canine distemper virus fusion protein with phospholipid vesicles: a biophysical study**,Francisco J Aranda, José A Teruel, Antonio Ortiz,Biochimica et Biophysica Acta (BBA) - Biomembranes,Volume 1618, Issue 1,2003,Pages 51-58,https://doi.org/10.1016/j.bbamem.2003.10.005.

Abstract:

The F protein of canine distemper virus (CDV) is a classic type I glycoprotein formed by two polypeptides, F1 and F2. The N-terminal regions of the F1 polypeptides of CDV, measles virus and other paramyxoviruses present moderate to high homology, supporting the existence of a high conservation within these structures, which emphasises its role in viral-host cell membrane fusion. This N-terminal polypeptide is usually termed the fusion peptide. The fusion peptides of most viral fusion-mediating glycoproteins contain a high proportion of hydrophobic amino acids, which facilitates its insertion into target membranes during fusion. In this work we report on the interaction of a 31-residue synthetic peptide (FP31) corresponding to the N terminus of CDV F1 protein with phospholipid membranes composed of various phospholipids, as studied by means of various biophysical techniques. FTIR investigation of FP31 secondary structure in aqueous medium and in membranes resulted in a major proportion of α-helical structure which increased upon membrane insertion. Differential scanning calorimetry (DSC) showed that the presence of concentrations of FP31 as low as 0.1 mol%, in mixtures with l-α-dimyristoylphosphatidylcholine (DMPC), l-α-dipalmitoylphosphatidylcholine (DPPC) and l-α-distearoylphosphatidylcholine (DSPC), already affected the thermotropic properties of the gel to liquid-crystalline phase transition. In mixtures with the three lipids, increasing the concentration of peptide made the pretransition to disappear, and lowered and broadened the main transition. This effect was slightly stronger as the acyl chain length of the phospholipid grew larger. In the corresponding partial phase diagrams, no immiscibilities or critical points were observed. FTIR showed that incorporation of 1 mol% of peptide in DPPC shifted the antisymmetric and symmetric CH2 stretching bands to higher values, indicating the existence of an additional disordering of the acyl chain region of the fluid bilayer. FTIR studies of the C□O stretching band indicated that incorporation of FP31 into phosphatidylcholine membranes produced

a strong dehydration of the polar part of the bilayer. In mixtures with l-α-dielaidoylphosphatidylethanolamine (DEPE), increasing FP31 concentrations broadened and shifted to lower temperatures the lamellar to hexagonal-HII phase transition, indicating that this peptide destabilized the bilayer and promoted formation of the hexagonal-HII phase. The results are discussed in terms of lipid–peptide hydrophobic mismatch and its influence on the role of the N-terminal polypeptide of CDV F1 protein in viral membrane fusion.

Keywords: Canine distemper virus; Viral peptide; Phospholipid membrane; DSC; FTIR

3. **LRP6 Is a Functional Receptor for Attenuated Canine Distemper Virus,**Vaiva Gradauskaite, Marine Inglebert, John Doench, Melanie Scherer, Martina Dettwiler, Marianne Wyss, Neeta Shrestha, Sven Rottenberg, Philippe Plattet,mBio,Volume 14, Issue 1,2023,https://doi.org/10.1128/mbio.03114-22.

Abstract:

Wild-type canine distemper virus (CDV) is an important pathogen of dogs as well as wildlife that can infect immune and epithelial cells through two known receptors: the signaling lymphocytic activation molecule (SLAM) and nectin-4, respectively. Conversely, the ferret and egg-adapted CDV-Onderstepoort strain (CDV-OP) is employed as an effective vaccine for dogs. CDV-OP also exhibits promising oncolytic properties, such as its abilities to infect and kill multiple cancer cells in vitro. Interestingly, several cancer cells do not express SLAM or nectin-4, suggesting the presence of a yet unknown entry factor for CDV-OP. By conducting a genome-wide CRISPR/Cas9 knockout (KO) screen in CDV-OP-susceptible canine mammary carcinoma P114 cells, which neither express SLAM nor nectin-4, we identified low-density lipoprotein receptor-related protein 6 (LRP6) as a host factor that promotes CDV-OP infectivity. Whereas the genetic ablation of LRP6 rendered cells resistant to infection, ectopic expression in resistant LRP6KO cells restored susceptibility. Furthermore, multiple functional studies revealed that (i) the overexpression of LRP6 leads to increased cell-cell fusion, (ii) a soluble construct of the viral receptor-binding protein (solHOP) interacts with a soluble form of LRP6 (solLRP6), (iii) an H-OP point mutant that prevents interaction with solLRP6 abrogates cell entry in multiple cell lines once transferred into recombinant viral particles, and (iv) vesicular stomatitis virus (VSV) pseudotyped with CDV-OP envelope glycoproteins loses its infectivity in LRP6KO cells. Collectively, our study identified LRP6 as the long sought-after cell entry receptor of CDV-OP in multiple cell lines, which set the molecular bases to refine our understanding of viral-cell adaptation and to further investigate its oncolytic properties.

IMPORTANCE

Oncolytic viruses (OV) have gathered increasing interest in recent years as an alternative option to treat cancers. The Onderstepoort strain of canine distemper virus (CDV-OP), an enveloped RNA virus belonging to the genus Morbillivirus, is employed as a safe and efficient vaccine for dogs against distemper disease. Importantly, although CDV-OP can infect and kill multiple cancer cell lines, the basic mechanisms of entry remain to be elucidated, as most of those transformed cells do not express natural receptors (i.e., SLAM and nectin-4). In this study, using a genome-wide CRISPR/Cas9 knockout screen, we describe the discovery of LRP6 as a novel functional entry receptor for CDV-OP in various cancer cell lines and thereby uncover a basic mechanism of cell culture adaptation. Since LRP6 is upregulated in various cancer types, our data provide important insights in order to further investigate the oncolytic properties of CDV-OP.

Keywords: attenuated CDV; CRISPR/Cas9 KO screen; LRP6; cell entry; receptor

4. **Epidemiological investigation of canine coronavirus infection in Chinese domestic dogs: A systematic review and data synthesis,**Bo Dong, Xiaodong Zhang, Junyu Bai, Gaoqiang Zhang, Chengyu Li, Weiming Lin,Preventive Veterinary Medicine,Volume 209,2022,105792,https://doi.org/10.1016/j.prevetmed.2022.105792.

Abstract:

Canine enteric coronavirus (CCoV) is a pathogenic virus that infects dogs worldwide, causing enteric issues and causing harm to the dog industry and dogs. Although CCoV is not recognized as a highly lethal canine intestinal pathogen, it has been reported that CCoV is significantly associated with canine diarrhea in dogs. CCoV is a common health problem in dogs, attracting major concern from veterinarians and dog owners across China. In this study, we summarized the prevalence and epidemiological characteristics of CCoV in dogs in mainland China. The study revealed that the pooled prevalence of CCoV infection was 33%, and which associated with age, but not with sex, season and immunization status. In addition, the study also further suggested that CCoV-II was the predominant CCoV subtype in Chinese dogs. This study will provide valuable information for CCoV infections across China and other countries. Furthermore, this study also suggested that continuous surveillance and epidemiological studies of CCoV are necessary.

Keywords: Canine coronavirus; China; Epidemiology; Prevalence; Meta analysis; Sequence

5. **Influence of vaccine strains on the evolution of canine distemper virus,**Renata da Fontoura Budaszewski, André Felipe Streck, Matheus Nunes Weber, Franciele Maboni Siqueira, Rafael Lucas Muniz Guedes, Cláudio Wageck Canal,Infection, Genetics and Evolution,Volume 41,2016,Pages 262-269,https://doi.org/10.1016/j.meegid.2016.04.014.

Abstract:

Canine distemper virus (CDV) is a major dog pathogen belonging to the genus Morbillivirus of the family Paramyxoviridae. CDV causes disease and high mortality in dogs and wild carnivores. Although homologous recombination has been demonstrated in many members of Paramyxoviridae, these events have rarely been reported for CDV. To detect potential recombination events, the complete CDV genomes available in GenBank up to June 2015 were screened using distinct algorithms to detect genetic conversions and incongruent phylogenies. Eight putative recombinant viruses derived from different CDV genotypes and different hosts were detected. The breakpoints of the recombinant strains were primarily located on fusion and hemagglutinin glycoproteins. These results suggest that homologous recombination is a frequent phenomenon in morbillivirus populations under natural replication, and CDV vaccine strains might play an important role in shaping the evolution of this virus.

Keywords: Canine distemper virus; Dog; Recombination; Phylogeny; Evolution

6. **Infection of ferrets with wild type-based recombinant canine distemper virus overwhelms the immune system and causes fatal systemic disease,**Brigitta M. Laksono, Dagmar Roelofs, Anouskha D. Comvalius, Katharina S. Schmitz, Laurine C. Rijsbergen, Daryl Geers, Sham Nambulli, Peter van Run, W. Paul Duprex, Judith M. A. van den Brand, Rory D. de Vries, Rik L. de Swart, Rebecca Ellis Dutch,mSphere,Volume 8, Issue 4,2023,https://doi.org/10.1128/msphere.00082-23.

Abstract:

Canine distemper virus (CDV) causes systemic infection resulting in severe and often fatal disease in a large spectrum of animal host species. The virus is closely related to measles virus and targets myeloid, lymphoid, and epithelial cells, but CDV is more virulent and the infection spreads more rapidly within the infected host. Here, we aimed to study the pathogenesis of wild-type CDV infection by experimentally inoculating ferrets with recombinant CDV (rCDV) based on an isolate directly obtained from a naturally infected raccoon. The recombinant virus was engineered to express a fluorescent reporter protein, facilitating assessment of viral tropism and virulence. In ferrets, this wild type-based rCDV infected myeloid, lymphoid, and epithelial cells, and the infection resulted in systemic dissemination to multiple tissues and organs, especially those of the lymphatic system. High infection percentages in immune cells resulted in depletion of these cells both from circulation and from lymphoid tissues. The majority of CDV-infected ferrets reached their humane endpoints within 20 d and had to be euthanized. In that period, the virus also reached the central nervous system in several ferrets, but we did not observe the development of neurological complications during the study period of 23 d. Two out of 14 ferrets survived CDV infection and developed neutralizing antibodies. We show for the first time the pathogenesis of a non-adapted wild type-based rCDV in ferrets.

IMPORTANCE

Infection of ferrets with recombinant canine distemper virus (rCDV) expressing a fluorescent reporter protein has been used as proxy to understand measles pathogenesis and immune suppression in humans. CDV and measles virus use the same cellular receptors, but CDV is more virulent, and infection is often associated with neurological complications. rCDV strains in current use have complicated passage histories, which may have affected their pathogenesis. Here, we studied the pathogenesis of the first wild type-based rCDV in ferrets. We used macroscopic fluorescence to identify infected cells and tissues; multicolor flow cytometry to determine viral tropism in immune cells; and histopathology and immunohistochemistry to characterize infected cells and lesions in tissues. We conclude that CDV often overwhelmed the immune system, resulting in viral dissemination to multiple tissues in the absence of a detectable neutralizing antibody response. This virus is a promising tool to study the pathogenesis of morbillivirus infections.

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dissemination to multiple tissues in the absence of a detectable neutralizing antibody response. This virus is a promising tool to study the pathogenesis of morbillivirus infections.

Keywords: morbillivirus; canine distemper virus; immunopathogenesis; ferrets

7. **The administration of a single dose of a multivalent (DHPPiL4R) vaccine prevents clinical signs and mortality following virulent challenge with canine distemper virus, canine adenovirus or canine parvovirus**,Stephen Wilson, Joanna Illambas, Elisabeth Siedek, Anne Thomas, Vickie King, Catrina Stirling, Edita Plevová, Jeremy Salt, Gordon Sture,Trials in Vaccinology,Volume 32014,Pages 102-106,https://doi.org/10.1016/j.trivac.2014.05.002.

Abstract:

Four challenge studies following vaccination of dogs with a multivalent vaccine containing canine parvovirus (CPV-2b), adenovirus (CAV-1/-2) and distemper (CDV) are described. Six week old puppies received a single vaccination while non-vaccinated control dogs received water. In each respective trial, groups of dogs were challenged 21days after vaccination with heterologous viral isolates. Clinical observations, rectal temperature measurements, and blood and swab samples for analysis were collected throughout the study. Dogs in all studies had normal temperatures and general health up to challenge. Clinical signs of infection and temperatures outside the normal range were observed in non-vaccinated dogs challenged with CDV, CPV, CAV-1 and CAV-2; vaccinated dogs remained clinically normal after challenge. All dogs were sero-negative prior to vaccination, non-vaccinated dogs remaining negative until challenge. Vaccinated dogs all sero-converted by 21days after vaccination, with further increases seen after challenge. Non-vaccinated dogs sero-converted following challenge with CPV or CAV-2; no final blood samples were taken in the CDV and CAV-1 studies. Rectal swab analysis showed prevention of CPV shedding in vaccinated compared to non-vaccinated dogs, and nasal swab analysis following CAV-2 challenge showed longer duration and higher amount of viral shedding for non-vaccinated dogs. In conclusion, we demonstrated that a single administration of a minimum titre, multivalent vaccine to dogs of six weeks of age is efficacious and prevents clinical signs and mortality caused by CAV-1 and CDV; prevents clinical signs and significantly reduces virus shedding caused by CAV-2; and prevents clinical signs, leucopoenia and viral excretion caused by CPV.

Keywords: Canine; Vaccine; Distemper; Parvovirus; Adenovirus

8. **A study on canine distemper virus (CDV) and rabies epidemics in the red fox population via fractional derivatives**,Pushpendra Kumar, Vedat Suat Erturk, Abdullahi Yusuf, Kottakkaran Sooppy Nisar, Sayed F. Abdelwahab,Results in Physics,Volume 25,2021,104281,https://doi.org/10.1016/j.rinp.2021.104281.

Abstract:

Several deadly epidemics that have recognized as serious problems all over the world in the last few decades. Lassa hemorrhagic fever, coronavirus, dengue fever, malaria, and HIV are well-known deadly diseases in humans. In this research, we analysed the dynamics of the canine distemper virus (CDV) and rabies epidemics in the red fox population of the northern region of Italy with the help of time-fractional models. We performed our analysis in the new generalized Caputo non-classical derivative sense with the application of the Predictor–Corrector algorithm. We used the data of northern Italy for simulations and estimated the endemic equilibrium points for both CDV and rabies models. Also,

we presented the local stability of disease-free equilibrium points. Some theorems are mentioned for the purpose of existence and uniqueness analysis. Our results are perfect for giving an idea of the dynamics of the CDV and rabies epidemic in northern Italy. The dynamics of the given solutions are specified with the help of necessary graphical simulations. The projected algorithm is so effective in finding the solutions of complex dynamical systems. By this study, we give an idea of how applied mathematics is directly connected to biological studies. The major scientific aim of this study is to understand the outbreaks of CDV and rabies on the population of the red foxes by using the texture of fractional mathematical models.

Keywords: Canine distemper virus; Rabies epidemic; New generalized Caputo-type fractional-order derivative; Mathematical model; Predictor–corrector method; Numerical simulations

9. **Serological and molecular survey of canine distemper virus in red foxes (Vulpes vulpes): Exploring cut-off values and the use of protein A in ELISA tests,** C. Muñoz-Hernández, A. Wipf, N. Ortega, G.G. Barberá, J. Salinas, M. González, C. Martínez-Carrasco, M.G. Candela,Preventive Veterinary Medicine,Volume 221,2023,106075,https://doi.org/10.1016/j.prevetmed.2023.106075.

Abstract:

The wide distribution and ecological plasticity of the red fox (Vulpes vulpes) make it a potential reservoir for many infectious diseases shared with domestic and wild carnivores. One of such diseases is canine distemper, which is caused by an RNA virus and its main domestic reservoir is the dog. However, other carnivores can also participate in its maintenance, as shown by the recent upsurge of reported cases in wildlife in many parts of the world, and by the fact that red foxes may act as true reservoirs for canine distemper virus (CDV). The lack of validated serological tests for wildlife or other non-target species may be a handicap for monitoring this virus. In this study, serological assays were compared in 147 red fox sera using a commercial ELISA validated for its use in dogs and a non-specific modified ELISA with Protein A peroxidase conjugate to detect bound antibodies. In addition, the presence of CDV RNA in brain, spleen, lung, and liver samples from 144 foxes was investigated by a RT-qPCR. Through the comparison of the results of both ELISAs and the use of a finite mixture model of the optical density values obtained by both techniques, we adjusted the cut-off point of the commercial ELISA to obtain the seroprevalence in foxes. The overall seroprevalence detected was 53.7% (79/147) and 57.1% (84/147) by the commercial and modified ELISA, respectively, with a moderate agreement according to Cohen's Kappa statistic ($\kappa = 0.491$, $z = 5.97$, $p < 0.0001$). CDV RNA was detected in 30 out of 144 foxes, which resulted in 20.8% of CDV-infected foxes. At individual level, the results obtained by relating the serological status and the presence/absence of RNA in different organs were explained in terms of the pathogenesis of the infection. Our results highlight the convenience of adjusting the cut-off point when using an ELISA assay developed in domestic dogs for its use in foxes. Moreover, Protein A is confirmed to be a good alternative to be used in red foxes, presenting a good reactivity towards its IgG.

Keywords: Protein A; ELISA; Vulpes vulpes; Morbillivirus; Wildlife

10. **The V domain of dog PVRL4 (nectin-4) mediates canine distemper virus entry and virus cell-to-cell spread** Sebastien Delpeut, Ryan S. Noyce, Christopher D. Richardson,Virology,Volumes 454–455,2014,Pages 109-117,https://doi.org/10.1016/j.virol.2014.02.014.

Abstract:

The entry of canine distemper virus (CDV) is a multistep process that involves the attachment of CDV hemagglutinin (H) to its cellular receptor, followed by fusion between virus and cell membranes. Our laboratory recently identified PVRL4 (nectin-4) to be the epithelial receptor for measles and canine distemper viruses. In this study, we demonstrate that the V domain of PVRL4 is critical for CDV entry and virus cell-to-cell spread. Furthermore, four key amino acid residues within the V domain of dog PVRL4 and two within the CDV hemagglutinin were shown to be essential for receptor-mediated virus entry.

Keywords: Nectin-4; PVRL4; Poliovirus receptor-like protein-4; Receptor; V domain; Epithelial cell; Canine distemper virus; CDV; Hemagglutinin; Virus entry; Syncytia formation

11. **Canine distemper virus with the intact C protein has the potential to replicate in human epithelial cells by using human nectin4 as a receptor**,Noriyuki Otsuki, Tsuyoshi Sekizuka, Fumio Seki, Kouji Sakai, Toru Kubota, Yuichiro Nakatsu, Surui Chen, Hideo Fukuhara, Katsumi Maenaka, Ryoji Yamaguchi, Makoto Kuroda, Makoto Takeda,Virology,Volume 435, Issue 2,2013,Pages 485-492,https://doi.org/10.1016/j.virol.2012.10.033.

Abstract:

Recent outbreaks in monkeys have proven that canine distemper virus (CDV) causes diseases in a wide range of mammals. CDV uses SLAM and nectin4 as receptors to replicate in susceptible animals. Here, we show that human nectin4, but not human SLAM, is fully functional as a CDV receptor. The CDV Ac96I strain hardly replicated in nectin4-expressing human epithelial NCI-H358 cells, but readily adapted to grow in them. Unsurprisingly, no amino acid change in the H protein was required for the adaptation. The original Ac96I strain possessed a truncated C protein, and a subpopulation possessing the intact C protein was selected after growth in NCI-H358 cells. Other CDV strains possessing the intact C protein showed significantly higher growth abilities in NCI-H358 cells than the Ac96I strain with the truncated C protein. These findings suggest that the C protein is functional in human epithelial cells and critical for CDV replication in them.

Keywords: Canine distemper virus; Nectin4; Receptor; Morbillivirus

12. **Post-vaccinal distemper-like disease in two dog litters with confirmed infection of vaccine virus strain**,Henna M. Pekkarinen, Veera K. Karkamo, Katri J. Vainio-Siukola, Maria K. Hautaniemi, Paula M. Kinnunen, Tuija K. Gadd, Riikka H. Holopainen,Comparative Immunology, Microbiology and Infectious Diseases,Volume 105,2024,102114,https://doi.org/10.1016/j.cimid.2023.102114.

Abstract:

Modified live canine distemper virus (CDV) vaccines are widely used and considered both safe and effective. Although there are occasional literature reports of suspected vaccine-induced disease, there

are none where the vaccine strain has been identified in affected tissues. Here we describe two such cases in different litters. In litter A, five of ten puppies presented with fever, anorexia, vomiting, and diarrhea a few days post-vaccination. Four puppies died or were euthanized, and autopsy revealed atypical necrosis of the lymphoid tissue. In litter B, two of five puppies developed typical neurological signs some months post-vaccination and autopsy revealed encephalitis. In all cases, affected organs tested positive for CDV on immunohistochemistry, and CDV RNA extracted from the lesions confirmed the presence of vaccine strain. Since multiple puppies from each litter were affected, it cannot be excluded without further studies that some undiagnosed inherited immunodeficiency disorder may have been involved.

Keywords: Canine distemper virus; Canine morbillivirus; Post-vaccinal infection; Fox terrier; German spaniel; Onderstepoort strain

13. **Adaptation of canine distemper virus to canine footpad keratinocytes modifies polymerase activity and fusogenicity through amino acid substitutions in the P/V/C and H proteins,**Jean-Paul Rivals, Philippe Plattet, Christine Currat-Zweifel, Andreas Zurbriggen, Riccardo Wittek,Virology,Volume 359, Issue 1,2007,Pages 6-18,https://doi.org/10.1016/j.virol.2006.07.054.

Abstract:

The wild-type canine distemper virus (CDV) strain A75/17 induces a non-cytocidal infection in cultures of canine footpad keratinocytes (CFKs) but produces very little progeny virus. After only three passages in CFKs, the virus produced 100-fold more progeny and induced a limited cytopathic effect. Sequence analysis of the CFK-adapted virus revealed only three amino acid differences, of which one was located in each the P/V/C, M and H proteins. In order to assess which amino acid changes were responsible for the increase of infectious virus production and altered phenotype of infection, we generated a series of recombinant viruses. Their analysis showed that the altered P/V/C proteins were responsible for the higher levels of virus progeny formation and that the amino acid change in the cytoplasmic tail of the H protein was the major determinant of cytopathogenicity.

Keywords: Canine distemper virus; Persistence; Cytopathic effect; Virus progeny

14. **Viral expression in experimental canine distemper demyelinating encephalitis,**W.J. Mitchell, B.A. Summers, M.J.G. Appel,Journal of Comparative Pathology,Volume 104, Issue 1,1991,Pages 77-87,https://doi.org/10.1016/S0021-9975(08)80090-4.

Abstract:

We have characterized the relationship between the expression of canine distemper virus (CDV) and demyelinating lesions in the white matter of the cerebellum of experimentally infected dogs. In animals which had demyelinating lesions, CDV proteins (N, P, F and H) were expressed and infectious virus could be recovered from brain tissue. Viral proteins (N, P, F and H) were detected by monoclonal antibodies and immunocytochemistry within demyelinating lesions as well as in scattered glial cells in areas of the white matter which lacked detectable lesions. Many cell types, including astrocytes, neurons, ependymal cells, choroid plexus cells, meningeal ceils and perivascular inflammatory cells were labelled for viral antigen. We conclude from our results that the mechanism of demyelination in

canine distemper virus-induced encephalitis involves expression of viral gene products at the lesion site.

15. **Phosphorylation of Canine Distemper Virus P Protein by Protein Kinase C-ζ and Casein Kinase II** Zheng Liu, Clayton C. Huntley, Bishnu P. De, Tapas Das, Amiya K. Banerjee, Michael J. Oglesbee,Virology,Volume 232, Issue 1,1997,Pages 198-206,https://doi.org/10.1006/viro.1997.8548.

Abstract:

Transcription by nonsegmented negative-strand RNA viruses is mediated by the viral RNA-dependent RNA polymerase and transcriptional cofactor P. The P protein is activated by phosphorylation, an event initiated by cellular kinases. The kinase used differs among this group of RNA viruses; vesicular stomatitis virus and respiratory syncytial virus utilize casein kinase II (CKII), whereas human parainfluenza virus type 3 utilizes PKC isoform zeta (PKC-ζ) for activation of its P protein. To identify the cellular kinase(s) involved in the phosphorylation of the canine distemper virus (CDV) P protein, we used recombinant CDV P in phosphorylation assays with native kinase activities present in CV1 cell extracts or purified CKII and PKC isoforms. Here, we demonstrate that the CDV P protein is phosphorylated by two cellular kinases, where PKC-ζ has the major and CKII the minor activities. In contrast, the P protein of another member of the morbillivirus genus, measles virus, is phosphorylated predominantly by CKII, whereas PKC-ζ has only minor activity. Selective inhibition of PKC-ζ activity within CV1 cells eliminated permissiveness to CDV replication, indicating anin vivorole for PKC-ζ in the virus replication cycle. The broad tissue expression of PKC-ζ parallels the pantropic nature of CDV infections, suggesting that PKC-ζ activity is a determinant of cellular permissiveness to CDV replication.

16. **Heritability and genome-wide association study of vaccine-induced immune response in Beagles: A pilot study**,Jeanna M. Blake, James Thompson, Harm HogenEsch, Kari J. Ekenstedt,Vaccine,Volume 42, Issue 12,2024,Pages 3099-3106,https://doi.org/10.1016/j.vaccine.2024.03.076.

Abstract:

Both genetic and non-genetic factors contribute to individual variation in the immune response to vaccination. Understanding how genetic background influences variation in both magnitude and persistence of vaccine-induced immunity is vital for improving vaccine development and identifying possible causes of vaccine failure. Dogs provide a relevant biomedical model for investigating mammalian vaccine genetics; canine breed structure and long linkage disequilibrium simplify genetic studies in this species compared to humans. The objective of this study was to estimate the heritability of the antibody response to vaccination against viral and bacterial pathogens, and to identify genes driving variation of the immune response to vaccination in Beagles. Sixty puppies were immunized following a standard vaccination schedule with an attenuated combination vaccine containing antigens for canine adenovirus type 2, canine distemper virus, canine parainfluenza virus, canine parvovirus, and four strains of Leptospira bacteria. Serum antibody measurements for each viral and bacterial component were measured at multiple time points. Heritability estimations and GWAS were conducted using SNP genotypes at 279,902 markers together with serum antibody titer phenotypes. The heritability estimates were: (1) to Leptospira antigens, ranging from 0.178 to 0.628; and (2) to viral antigens, ranging from 0.199 to 0.588. There was not a significant difference between overall

heritability of vaccine-induced immune response to Leptospira antigens compared to viral antigens. Genetic architecture indicates that SNPs of low to high effect contribute to immune response to vaccination. GWAS identified two genetic markers associated with vaccine-induced immune response phenotypes. Collectively, these findings indicate that genetic regulation of the immune response to vaccination is antigen-specific and influenced by multiple genes of small effect.

Keywords: Heritability; Vaccine Development; Immunity; Genome-Wide Association Study; Canine; Genetic Variation

17. **Phylogenetic analysis linked fatal neurologic disease in leopards (Panthera pardus) to Asia-5 lineage of canine distemper virus in Nepal** Amir Sadaula, Prajwol Manandhar, Bijaya Kumar Shrestha, Parbat Jung Thapa, Suresh Nepali, Janardan Dev Joshi, Babu Ram Lamichhane, Rachana Shah, Madhu Chetri, Kiran Raj Rijal, Kamal Prasad Gairhe, Naresh Subedi, Chiranjibi Prasad Pokheral, Roji Raut, Purushottam Pandey, Bikalpa Karki, Gita Pandey,Virus Research,Volume 350,2024,199463,https://doi.org/10.1016/j.virusres.2024.199463.

Abstract:

Canine distemper virus (CDV) is responsible for a highly contagious and often fatal neurological disease that affects various carnivores, including domestic dogs. In Nepal, recent reports of CDV exposure and illness in leopards (Panthera pardus) have raised concerns about the transmission of the virus among domestic dogs and wild carnivores. To investigate the genetic lineage and spread of CDV, our study utilized archived post-mortem samples from four leopards that exhibited clinical signs suggestive of canine distemper infection. These leopards were rescued in the Palpa, Dolakha, Kathmandu, and Parbat districts. Our phylogenetic analysis revealed that the CDV strains circulating among the leopards belong to the Asia-5 lineage, which is also prevalent among dogs and wild carnivores in Nepal and neighboring India. The genetic relatedness between the leopard CDV sequences and those from both dogs and other carnivores within the Asia-5 lineage suggests that leopards in Nepal may have acquired the virus from multiple sources, potentially facilitated by their generalist dietary habits preying on dogs and even mesocarnivores. Furthermore, we inspected specific amino acid substitution in the hemagglutinin gene of leopard CDV, which also suggests possible transmission from both domestic dogs and non-canid hosts, although further research is needed to draw definitive conclusions. Given the vulnerable state of the leopard population in Nepal, already threatened by poaching and retaliatory killing, the emergence of CDV as a potential novel threat is deeply concerning. Comprehensive surveillance studies are essential to understand the dynamics of CDV spillover and to develop informed interventions. Urgent measures, including vaccination programs and effective control of the dog population, are needed to mitigate the impact of this disease and safeguard the future of Nepal's leopards and other wild carnivores.

Keywords: Canine distemper virus; Leopards; Panthera pardus; Dogs; Spillover; Phylogenetic

18. **Cryo-EM structure of the prefusion state of canine distemper virus fusion protein ectodomain,**David Kalbermatter, Neeta Shrestha, Flavio M. Gall, Marianne Wyss, Rainer Riedl, Philippe Plattet, Dimitrios Fotiadis,Journal of Structural Biology: X,Volume 4,2020,100021,https://doi.org/10.1016/j.yjsbx.2020.100021.

Abstract:

Measles virus (MeV) and canine distemper virus (CDV), two members of the Morbillivirus genus, are still causing important global diseases of humans and animals, respectively. To enter target cells, morbilliviruses rely on an envelope-anchored machinery, which is composed of two interacting glycoproteins: a tetrameric receptor binding (H) protein and a trimeric fusion (F) protein. To execute membrane fusion, the F protein initially adopts a metastable, prefusion state that refolds into a highly stable postfusion conformation as the result of a finely coordinated activation process mediated by the H protein. Here, we employed cryo-electron microscopy (cryo-EM) and single particle reconstruction to elucidate the structure of the prefusion state of the CDV F protein ectodomain (solF) at 4.3 Å resolution. Stabilization of the prefusion solF trimer was achieved by fusing the GCNt trimerization sequence at the C-terminal protein region, and expressing and purifying the recombinant protein in the presence of a morbilliviral fusion inhibitor class compound. The three-dimensional cryo-EM map of prefusion CDV solF in complex with the inhibitor clearly shows density for the ligand at the protein binding site suggesting common mechanisms of membrane fusion activation and inhibition employed by different morbillivirus members.

Keywords: Canine distemper virus; Cryo-electron microscopy; Fusion protein; Morbillivirus cell entry; Single particle reconstruction

19. **Genotyping of canine distemper virus strains circulating in Brazil from 2008 to 2012,**Renata da Fontoura Budaszewski, Luciane Dubina Pinto, Matheus Nunes Weber, Eloiza Teles Caldart, Christian Diniz Beduschi Travassos Alves, Vito Martella, Nilo Ikuta, Vagner Ricardo Lunge, Cláudio Wageck Canal,Virus Research,Volume 180,2014,Pages 76-83,https://doi.org/10.1016/j.virusres.2013.12.024.

Abstract:

Canine distemper virus (CDV) is a major pathogen of dogs and represents a serious threat to both unvaccinated and vaccinated animals. This study surveyed dogs with or without clinical signs related to canine distemper from different regions of Brazil from 2008 to 2012. A total of 155 out of 386 animals were found to be CDV positive by RT-PCR; 37 (23.8%) dogs were asymptomatic at the time of sampling, and 90 (58%) displayed clinical signs suggestive of distemper. Nineteen (12.2%) dogs had a record of complete vaccination, 15 (9.6%) had an incomplete vaccination protocol, and 76 (49%) had no vaccination record. Based on the sequence analysis of the complete hemagglutinin gene of 13 samples, 12 of the strains were characterized as Genotype South America-I/Europe. Considering criteria of at least 95% nucleotide identity to define a genotype and 98% to define a subgenotype, South America-I/Europe sequences segregated into eight different phylogenetically well-defined clusters that circulated or co-circulated in distinct geographical areas. Together, these findings highlight the relevance of CDV infection in Brazilian dogs, demonstrate the predominance of one genotype in Brazil and support the need to intensify the current control measures.

Keywords: Canine distemper virus; Detection; Genotype; Phylogeny; South America

20. **Oligomerization and Cell Egress Controlled by Two Microdomains of Canine Distemper Virus Matrix Protein,**Matthieu Gast, Nicole P. Kadzioch, Doreen Milius, Francesco Origgi, Philippe Plattet, Michael J. Imperiale,mSphere,Volume 6, Issue 2,2021,https://doi.org/10.1128/msphere.01024-20.

Abstract:

The multimeric matrix (M) protein of clinically relevant paramyxoviruses orchestrates assembly and budding activity of viral particles at the plasma membrane (PM). We identified within the canine distemper virus (CDV) M protein two microdomains, potentially assuming α-helix structures, which are essential for membrane budding activity. Remarkably, while two rationally designed microdomain M mutants (E89R, microdomain 1 and L239D, microdomain 2) preserved proper folding, dimerization, interaction with the nucleocapsid protein, localization at and deformation of the PM, the virus-like particle formation, as well as production of infectious virions (as monitored using a membrane budding-complementation system), were, in sharp contrast, strongly impaired. Of major importance, raster image correlation spectroscopy (RICS) revealed that both microdomains contributed to finely tune M protein mobility specifically at the PM. Collectively, our data highlighted the cornerstone membrane budding-priming activity of two spatially discrete M microdomains, potentially by coordinating the assembly of productive higher oligomers at the PM. IMPORTANCE Despite the availability of efficient vaccines, morbilliviruses (e.g., canine distemper virus [CDV] and measles virus [MeV]) still cause major health impairments. Although antivirals may support vaccination campaigns, approved inhibitors are to date still lacking. Targeting late stages of the viral life cycle (i.e., the cell exit system) represents a viable option to potentially counteract morbilliviral infections. The matrix (M) protein of morbillivirus is a major contributor to membrane budding activity and is assumed to assemble into dimers that further associate to form higher oligomers. Here, we rationally engineered M protein variants with modifications in two microdomains that potentially locate at dimer-dimer interfaces. Our results spotlight the cornerstone impact of both microdomains in membrane budding activity and further suggest a role of finely tuned high-order oligomer formation in regulating late stages of cell exit. Collectively, our findings highlight two microdomains in the morbilliviral M protein as novel attractive targets for drug design.

Keywords: morbillivirus cell exit; matrix protein; M-M interaction; VLPs production; dynamics at the cell surface

21. **Persistent and Severe Viral Replication in PBMCs with Moderate Immunosuppression Served an Alternative Novel Pathogenic Mechanism for Canine Morbillivirus,**Chuchu Feng, Yan Bu, Jiaxi Cai, Guanyu Zhao, Zishu Li, Yuening Cheng, Xiaohao Zhang, Yijun Shi, Yang Gao, Xiangnan Li, Xuexing Zheng, Xianghong Xue,Microbiology Spectrum,Volume 11, Issue 1,2022,https://doi.org/10.1128/spectrum.04060-22.

Abstract:

Measles virus and canine distemper virus (CDV) cause lethal infections in their respective hosts characterized by severe immunosuppression. To furtherly acknowledge the attenuated mechanisms of the regionally ongoing epidemic CDV isolates and provide novel perspectives for designing new vaccines and therapeutic drugs, a recombinant CDV rHBF-vacH was employed with a vaccine hemagglutinin (H) gene replacement by reverse genetics based on an infectious cDNA clone for the CDV wild-type HBF-1 strain. Interestingly, unlike previously published reports that a vaccine H protein completely changed a pathogenic wild-type CDV variant to be avirulent, rHBF-vacH was only partially

attenuated by alleviating the degree of viral immunosuppression, and still caused 66.7% lethality in ferrets with a prolonged period of disease. Further comparisons of pathogenic mechanisms proved that the weaker but necessary invasions into peripheral blood mononuclear cells (PBMCs) of rHBF-vacH, and subsequently persistent viral replications in PBMCs and multiple organs, together contributed to its 66.7% mortality. In addition, despite significantly higher titers than the parent viruses, rHBF-vacH would not be a suitable candidate for a live vaccine, with great invasion and infection potentials of PBMCs from 16 tested kinds of host species. Altogether, sustained and severe viral replication in PBMCs with moderate immunosuppression was first proven to be an alternative novel pathogenic mechanism for CDV, which might help us to understand possible reasons for CDV fatal infections among domestic dogs and the highly susceptible wild species during natural transmission.

IMPORTANCE Despite widespread vaccine campaigns for domestic dogs, CDV remained an important infectious disease in vaccinated carnivores and wild species. In recent years, the regionally ongoing epidemic CDV isolates have emphasized conservation threats to, and potentially disastrous epidemics in, endangered species worldwide. However, little is known about how to deal with the CDV variants constantly regional epidemic. In this study, we employed a recombinant CDV rHBF-vacH with a vaccine H gene replacement in a CDV wild-type HBF-1 context to attenuate the epidemic CDV variant to design a new vaccine candidate. Interestingly, rHBF-vacH was only partially attenuated by alleviating the degree of viral immunosuppression, and still caused 66.7% lethality in ferrets by weaker but necessary invasions into PBMCs, and subsequently persistent and severe viral replications in PBMCs. Significantly higher virus titers of rHBF-vacH in vitro might indicate the rapid cell-to-cell spreads in vivo that indirectly contribute to fatal infections of rHBF-vacH in ferrets.

Keywords: canine distemper virus; immunosuppression; pathogenic mechanisms; viremia

22. **Disease Manifestations of Canine Distemper Virus Infection in Ferrets Are Modulated by Vitamin A Status1,2,**Carey Rodeheffer, Veronika von Messling, Sylvain Milot, François Lepine, Amee R. Manges, Brian J. Ward,The Journal of Nutrition,Volume 137, Issue 8,2007,Pages 1916-1922,https://doi.org/10.1093/jn/137.8.1916.

Abstract:

The measles virus (MV) causes half a million childhood deaths annually. Vitamin A supplements significantly reduce measles-associated mortality and morbidity. The mechanisms whereby vitamin A acts against MV are not understood and currently there is no satisfactory small animal model for MV infection. We report on the development of a ferret model to study antiviral activity of vitamin A against canine distemper virus (CDV). CDV is closely related to MV at the molecular level and distemper in ferrets mimics measles in humans. We infected vitamin A-replete (control) and vitamin A-depleted ferrets with CDV and assessed the ability of high-dose vitamin A supplements to influence CDV disease. In control ferrets, CDV infection caused fever, rash, conjunctivitis, cough, coryza, and diarrhea. In contrast, control ferrets that were given 30 mg of vitamin A did not develop typical distemper after infection and exhibited only a mild rash. The supplement did not negatively affect ferret health and resulted in a 100% increase in serum and liver vitamin A concentrations. We also found that profound vitamin A deficiency is inducible in ferrets and can be rapidly reversed upon high-dose vitamin A supplementation. Vitamin A deficiency caused anorexia, diarrhea, cataracts, behavioral abnormalities, and ultimately death, with or without CDV infection. All ferrets that received vitamin A supplements, however, recovered uneventfully from CDV infection. These results replicate many

aspects of the observations of vitamin A therapy in humans with measles and suggest that CDV infection in ferrets is an appropriate model for the study of the antiviral mechanism of vitamin A.

23. **A systematic review on global zoonotic virus-associated mortality events in marine mammals,**Katie Vigil, Huiyun Wu, Tiong Gim Aw,One Health,Volume 19,2024,100872,https://doi.org/10.1016/j.onehlt.2024.100872.

Abstract:

Marine mammals play a critical role as sentinels for tracking the spread of zoonotic diseases, with viruses being the primary causative factor behind infectious disease induced mortality events. A systematic review was conducted to document marine mammal mortality events attributed to zoonotic viral infections in published literature across the globe. This rigorous search strategy yielded 2883 studies with 88 meeting inclusion criteria. The studies spanned from 1989 to 2023, with a peak in publications observed in 2020. Most of the included studies were retrospective, providing valuable insights into historical trends. The United States (U.S.) reported the highest number of mortality events followed by Spain, Italy, Brazil and the United Kingdom. Harbor seals were the most impacted species, particularly in regions like Anholt, Denmark and the New England Coast, U.S. Analysis revealed six main viruses responsible for mortality events, with Morbillivirus causing the highest proportion of deaths. Notably, the occurrence of these viral events varied geographically, with distinct patterns observed in different regions. Immunohistochemistry emerged as the most employed detection method. This study underscores the importance of global surveillance efforts in understanding and mitigating the impact of viral infections on marine mammal populations, thereby emphasizing the necessity of collaborative One Health approaches to address emerging threats at the human-animal-environment interface. Additionally, the potential transfer of zoonotic viruses to aquatic organisms used in food production, such as fish and shellfish, highlights the broader implications for food safety, food security and public health.

Keywords: Marine mammal; Mortality; Virus; Zoonotic; Sentinel; Systematic review

24. **Morbillivirus: A highly adaptable viral genus,**Jane E. Libbey, Robert S. Fujinami,Heliyon,Volume 9, Issue 7,2023,e18095,https://doi.org/10.1016/j.heliyon.2023.e18095.

Abstract:

Over the course of human history, numerous diseases have been caused by the transmission of viruses from an animal reservoir into the human population. The viruses of the genus Morbillivirus are human and animal pathogens that emerged from a primordial ancestor a millennia ago and have been transmitting to new hosts, adapting, and evolving ever since. Through interaction with susceptible individuals, as yet undiscovered morbilliviruses or existing morbilliviruses in animal hosts could cause future zoonotic diseases in humans.

Keywords: Morbillivirus; Measles; Rinderpest; Canine distemper

25. **An appraisal of gene targets for phylogenetic classification of canine distemper virus: Is the hemagglutinin the best candidate?,** Alice Silveira Becker, José Valter Joaquim Silva Júnior, Rudi Weiblen, Eduardo Furtado Flores, Virus Research, Volume 325, 2023, 199043, https://doi.org/10.1016/j.virusres.2023.199043.

Abstract:

Sequence analysis of the canine distemper virus (CDV) hemagglutinin (H) gene may provide important insights on virus-host interactions and has also been frequently used for CDV phylogenetic classification. Herein, we performed an in silico analysis of CDV complete genomes (CGs) available in GenBank in order to investigate the suitability of H for CDV classification into lineages/genotypes. In addition, we analyzed the other viral genes for their potential use in CDV classification. Initially, we collected 116 CDV CGs from GenBank and compared their phylogenetic classification with that of their respective H nucleotide (nt) and amino acid (aa) sequences. Subsequently, we calculated the geodesic distance between the CG and H phylogenetic trees. These analyses were later performed with other CDV genes. All CDV CGs were also evaluated for possible recombination events. Nucleotide and aa analyses of H misclassified some Vaccine/America 1/Asia 3 lineage sequences compared to CG analysis, finding supported by both Maximum Likelihood (ML) and Bayesian Markov Chain Monte Carlo (B-MCMC) methods. Moreover, aa-based H analysis showed additional disagreements with the classification obtained by CG. The geodesic distance between the H and CG trees was 0.0680. Strong recombination signals were identified in the H gene, including Vaccine/America 1/Asia 3 lineage sequences. In contrast, C and P were the only genes that fully reproduced the CG classification (by ML and/or B-MCMC) and that did not show strong recombination signals. Furthermore, the P phylogenetic tree showed the lowest geodesic distance from the CG tree (0.0369). These findings suggest C and P as potential targets for CDV phylogenetic classification, especially when full genome sequencing is not possible. Finally, since our results were obtained considering the CDV CGs available to date, future analyses performed as more CDV sequences become available will be useful to assess probable issues of H-based phylogeny and to consolidate the suitability of the C and P genes for CDV classification.

Keywords: CDV; Lineages; Genotypes; C gene; P gene

26. **Host range and receptor utilization of canine distemper virus analyzed by recombinant viruses: Involvement of heparin-like molecule in CDV infection,** Kentaro Fujita, Ryuichi Miura, Misako Yoneda, Fusako Shimizu, Hiroki Sato, Yuri Muto, Yasuyuki Endo, Kyoko Tsukiyama-Kohara, Chieko Kai, Virology, Volume 359, Issue 2, 2007, Pages 324-335, https://doi.org/10.1016/j.virol.2006.09.018.

Abstract:

We constructed recombinant viruses expressing enhanced green fluorescent protein (EGFP) or firefly luciferase from cDNA clones of the canine distemper virus (CDV) (a Japanese field isolate, Yanaka strain). Using these viruses, we examined susceptibilities of different cell lines to CDV infection. The results revealed that the recombinant CDVs can infect a broad range of cell lines. Infectivity inhibition assay using a monoclonal antibody specific to the human SLAM molecule indicated that the infection of B95a cells with these recombinant CDVs is mainly mediated by SLAM but the infection of 293 cell lines with CDV is not, implying the presence of one or more alternative receptors for CDV in non-lymphoid tissue. Infection of 293 cells with the recombinant CDV was inhibited by soluble heparin,

and the recombinant virus bound to immobilized heparin. Both F and H proteins of CDV could bind to immobilized heparin. These results suggest that heparin-like molecules are involved in CDV infection.

Keywords: Canine distemper virus; Host range; Receptor; Heparin; Glycosaminoglycan

27. **Establishment and evaluation of a multiplex real-time RT-PCR for quantitative and differential detection of wild-type canine distemper virus from vaccine strains,** Ping Sui, Yiyang Sun, Yijun Shi, Wei Ran, Ning Shi, Dongbo Sun, Jiasan Zheng, Jianjun Zhao,Heliyon,Volume 9, Issue 9,2023,e19344,https://doi.org/10.1016/j.heliyon.2023.e19344.

Abstract:

This study sought to establish a real-time reverse transcription (RT)-PCR method to differentially detect canine distemper virus (CDV) wild-type and vaccine strains. To this end, a pair of CDV universal primers and two specific minor groove binder (MGB) probes, harboring a T/C substitution in the hemagglutinin (H) gene, were designed. Using a recombinant plasmid expressing the H gene of the CDV wild-type or vaccine strain as standards, a sensitive and specific multiplex real-time RT-PCR was established for quantitative and differential detection of CDV wild-type and vaccine strains. The limit of detection for this multiplex assay was 22.5 copies/μL and 2.98 copies/μL of viral RNA for wild-type and vaccine strains, respectively. Importantly, the wild-type and vaccine MGB probes specifically hybridized different genotypes of wild-type CDV circulating in China as well as globally administered vaccine viruses, respectively, with no cross-reactivity observed with non-CDV viruses. Moreover, this method was successfully applied for the quantitative detection of CDV RNA in tissue samples of experimentally infected breeding foxes, raccoon dogs, and minks. Additionally, the multiplex real-time RT-PCR was able to detect the viral RNA in the whole blood samples as early as 3 days post-infection, 3 to 4 days prior to the onset of clinical signs in these CDV infection animals. Hence, the established multiplex real-time RT-PCR method is useful for differentiating wild-type CDV and vaccine strains in China, and for conducting canine distemper early diagnosis as well as dynamic mechanism of CDV replication studies in vivo.

Keywords: Canine distemper virus; Wild-type strains; Vaccine strains; H gene; Real-time RT-PCR; Differential diagnosis

28. **Genetic characterization of canine distemper virus in Serengeti carnivores,** Margaret A Carpenter, Max J.G. Appel, Melody E Roelke-Parker, Linda Munson, Heribert Hofer, Marion East, Stephen J O'Brien,Veterinary Immunology and Immunopathology,Volume 65, Issues 2–4,1998,Pages 259-266,https://doi.org/10.1016/S0165-2427(98)00159-7.

Abstract:

The lion (Panthera leo) population in the Serengeti ecosystem was recently afflicted by a fatal epidemic involving neurological disease, encephalitis and pneumonia. The cause was identified as canine distemper virus (CDV). Several other species in the Serengeti were also affected. This report presents CDV H and P gene sequences isolated from Serengeti lions (Panthera leo), spotted hyenas (Crocuta crocuta), bat-eared fox (Otocyon megalotis) and domestic dog (Canis familiaris). Sequence analyses demonstrated that the four Serengeti species carry closely related CDV isolates which are genetically distinct from other CDV isolates from various species and locations. The results are

consistent with the conclusions that: (1) a particularly virulent strain of CDV emerged among Serengeti carnivores within the last few years; (2) that strain has recognizable shared-derived (synapomorphic) genetic differences in both H and P genes when compared to CDV from other parts of the world; and (3) that the CDV strain has frequently crossed host species among Serengeti carnivores.

29. **Intravenous injection of a foamy virus vector to correct canine SCID-X1,**Christopher R. Burtner, Brian C. Beard, Douglas R. Kennedy, Martin E. Wohlfahrt, Jennifer E. Adair, Grant D. Trobridge, Andrew M. Scharenberg, Troy R. Torgerson, David J. Rawlings, Peter J. Felsburg, Hans-Peter Kiem,Blood,Volume 123, Issue 23,2014,Pages 3578-3584,https://doi.org/10.1182/blood-2013-11-538926.

Abstract:

Current approaches to hematopoietic stem cell (HSC) gene therapy involve the collection and ex vivo manipulation of HSCs, a process associated with loss of stem cell multipotency and engraftment potential. An alternative approach for correcting blood-related diseases is the direct intravenous administration of viral vectors, so-called in vivo gene therapy. In this study, we evaluated the safety and efficacy of in vivo gene therapy using a foamy virus vector for the correction of canine X-linked severe combined immunodeficiency (SCID-X1). In newborn SCID-X1 dogs, injection of a foamy virus vector expressing the human IL2RG gene resulted in an expansion of lymphocytes expressing the common γ chain and the development of CD3+ T lymphocytes. CD3+ cells expressed CD4 and CD8 coreceptors, underwent antigen receptor gene rearrangement, and demonstrated functional maturity in response to T-cell mitogens. Retroviral integration site analysis in 4 animals revealed a polyclonal pattern of integration in all dogs with evidence for dominant clones. These results demonstrate that a foamy virus vector can be administered with therapeutic benefit in the SCID-X1 dog, a clinically relevant preclinical model for in vivo gene therapy.

30. **Overcoming the Barrier of the Respiratory Epithelium during Canine Distemper Virus Infection,**Dai-Lun Shin, Elisa Chludzinski, Nai-Huei Wu, Ju-Yi Peng, Malgorzata Ciurkiewicz, Bevan Sawatsky, Christian K. Pfaller, Christine Baechlein, Veronika von Messling, Ludwig Haas, Andreas Beineke, Georg Herrler,mBio,Volume 13, Issue 1,2022,https://doi.org/10.1128/mbio.03043-21.

Abstract:

Canine distemper virus (CDV) is a highly contagious pathogen and is known to enter the host via the respiratory tract and disseminate to various organs. Current hypotheses speculate that CDV uses the homologous cellular receptors of measles virus (MeV), SLAM and nectin-4, to initiate the infection process. For validation, here, we established the well-differentiated air-liquid interface (ALI) culture model from primary canine tracheal airway epithelial cells. By applying the green fluorescent protein (GFP)-expressing CDV vaccine strain and recombinant wild-type viruses, we show that cell-free virus infects the airway epithelium mainly via the paracellular route and only after prior disruption of tight junctions by pretreatment with EGTA; this infection was related to nectin-4 but not to SLAM. Remarkably, when CDV-preinfected DH82 cells were cocultured on the basolateral side of canine ALI cultures grown on filter supports with a 1.0-µm pore size, cell-associated CDV could be transmitted via cell-to-cell contact from immunocytes to airway epithelial cultures. Finally, we observed that canine ALI cultures formed syncytia and started to release cell-free infectious viral particles from the

apical surface following treatment with an inhibitor of the JAK/STAT signaling pathway (ruxolitinib). Our findings show that CDV can overcome the epithelial barrier through different strategies, including infection via immunocyte-mediated transmission and direct infection via the paracellular route when tight junctions are disrupted. Our established model can be adapted to other animals for studying the transmission routes and the pathogenicity of other morbilliviruses.

IMPORTANCE Canine distemper virus (CDV) is not only an important pathogen of carnivores, but it also serves as a model virus for analyzing measles virus pathogenesis. To get a better picture of the different stages of infection, we used air-liquid interface cultures to analyze the infection of well-differentiated airway epithelial cells by CDV. Applying a coculture approach with DH82 cells, we demonstrated that cell-mediated infection from the basolateral side of well-differentiated epithelial cells is more efficient than infection via cell-free virus. In fact, free virus was unable to infect intact polarized cells. When tight junctions were interrupted by treatment with EGTA, cells became susceptible to infection, with nectin-4 serving as a receptor. Another interesting feature of CDV infection is that infection of well-differentiated airway epithelial cells does not result in virus egress. Cell-free virions are released from the cells only in the presence of an inhibitor of the JAK/STAT signaling pathway. Our results provide new insights into how CDV can overcome the barrier of the airway epithelium and reveal similarities and some dissimilarities compared to measles virus.

Keywords: canine distemper virus; air-liquid interface; cell-to-cell transmission

31. **Cross-sectional investigation and risk factor analysis of community-acquired and hospital-associated canine viral infectious respiratory disease complex,**Chutchai Piewbang, Anudep Rungsipipat, Yong Poovorawan, Somporn Techangamsuwan,Heliyon,Volume 5, Issue 11,2019,e02726,https://doi.org/10.1016/j.heliyon.2019.e02726.

Abstract:

Canine infectious respiratory disease complex (CIRDC) is associated with multiple factors. The possible transmission source can be via community-acquired infection (CAI) or hospital-associated infection (HAI), but the variable factors within these two routes are not well described. This study aimed to (i) investigate a cross-sectional incidence of canine respiratory viruses, including influenza (CIV), parainfluenza, distemper (CDV), respiratory coronavirus (CRCoV), adenovirus-2, and herpesvirus, in respiratory-diseased dogs, and (ii) analyze the possibly related risk factors. In total 209 dogs with respiratory illness, consisting of 133 CAI and 76 HAI dogs, were studied. Both nasal and oropharyngeal swabs were sampled from each dog and subjected for CIRDC virus detection using multiplex PCRs. Common six viruses associated with CIRDC were detected in both groups with CIV and CRCoV being predominantly found. Only CDV was significantly more prevalent in CAI than HAI dogs. Multiple virus detections were found in 81.2% and 78.9% of CAI and HAI dogs, respectively. Co-detection of CIV and CRCoV was represented the highest proportion and most often found with other CIRD viruses. Moreover, the clinical severity level was notably related to the age of infected dogs, but not to the vaccination status, sex and transmission route. Since healthy or control dogs were not included in this study, the prevalence of the CIRD virus infections could not be assessed.

Keywords: Zoology; Veterinary medicine; Respiratory system; Infectious disease; Clinical research; CIRDC; Community-acquired; Dogs; Hospital association; Respiratory virus; Thailand

32. **Dog nectin-4 is an epithelial cell receptor for canine distemper virus that facilitates virus entry and syncytia formation,**Ryan S. Noyce, Sebastien Delpeut, Christopher D. Richardson,Virology,Volume 436, Issue 1,2013,Pages 210-220,https://doi.org/10.1016/j.virol.2012.11.011.

Abstract:

Canine distemper virus (CDV) was shown to use dog nectin-4 as a receptor to gain entry into epithelial cells. RNA from dog placenta or MDCK kidney cells was isolated and cDNAs were prepared. Two splice variants of dog nectin-4 were identified. A deletion of 25 amino acids was found in the cytoplasmic domain of dog nectin-4 from MDCK cells, corresponding to a splice variant that is also seen in murine nectin-4, and did not affect its role as a receptor. Both dog nectin-4 and human nectin-4 could function as an entry factor for CDV containing an EGFP reporter gene. Inhibition of dog nectin-4 expression by RNAi or nectin-4 antibodies decreased CDV titers and EGFP fluorescence. Finally, dog nectin-4 also promotes syncytia formation, which could be inhibited by siRNA treatment. These data confirm that dog nectin-4 can be used by CDV to gain entry into epithelial cells, and facilitate virus spread.

Keywords: Nectin-4; PVRL4; Receptor; Epithelial cell; Canine distemper virus; CDV; MDCK

33. **Inoculation of raccoons with a wild-type-based recombinant canine distemper virus results in viremia, lymphopenia, fever, and widespread histological lesions,**Dagmar Roelofs, Katharina S. Schmitz, Geert van Amerongen, Laurine C. Rijsbergen, Brigitta M. Laksono, Anouskha D. Comvalius, Sham Nambulli, Linda J. Rennick, Peter van Run, W. Paul Duprex, Judith M. A. van den Brand, Rik L. de Swart, Rory D. de Vries, Shirit Einav,mSphere,Volume 8, Issue 4,2023,https://doi.org/10.1128/msphere.00144-23.

Abstract:

Raccoons are naturally susceptible to canine distemper virus (CDV) infection and can be a potential source of spill-over events. CDV is a highly contagious morbillivirus that infects multiple species of carnivores and omnivores, resulting in severe and often fatal disease. Here, we used a recombinant CDV (rCDV) based on a full-genome sequence detected in a naturally infected raccoon to perform pathogenesis studies in raccoons. Five raccoons were inoculated intratracheally with a recombinant virus engineered to express a fluorescent reporter protein, and extensive virological, serological, histological, and immunohistochemical assessments were performed at different time points post inoculation. rCDV-infected white blood cells were detected as early as 4 days post inoculation (dpi). Raccoon necropsies at 6 and 8 dpi revealed replication in the lymphoid tissues, preceding spread into peripheral tissues observed during necropsies at 21 dpi. Whereas lymphocytes, and to a lesser extent myeloid cells, were the main target cells of CDV at early time points, CDV additionally targeted epithelia at 21 dpi. At this later time point, CDV-infected cells were observed throughout the host. We observed lymphopenia and lymphocyte depletion from lymphoid tissues after CDV infection, in the absence of detectable CDV neutralizing antibodies and an impaired ability to clear CDV, indicating that the animals were severely immunosuppressed. The use of a wild-type-based recombinant virus in a natural host species infection study allowed systematic and sensitive assessment of antigen detection by immunohistochemistry, enabling further comparative pathology studies of CDV infection in different species.

IMPORTANCE

Expansion of the human interface supports increased interactions between humans and peridomestic species like raccoons. Raccoons are highly susceptible to canine distemper virus (CDV) and are considered an important target species. Spill-over events are increasingly likely, potentially resulting in fatal CDV infections in domestic and free ranging carnivores. CDV also poses a threat for (non-human) primates, as massive outbreaks in macaque colonies were reported. CDV pathogenesis was studied by experimental inoculation of several species, but pathogenesis in raccoons was not properly studied. Recently, we generated a recombinant virus based on a full-genome sequence detected in a naturally infected raccoon. Here, we studied CDV pathogenesis in its natural host species and show that distemper completely overwhelms the immune system and spreads to virtually all tissues, including the central nervous system. Despite this, raccoons survived up to 21 d post inoculation with long-term shedding, supporting an important role of raccoons as host species for CDV.

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Keywords: distemper; morbillivirus; pathogenesis; raccoon; canine distemper virus

34. **Establishment of a persistent mutant of canine distemper virus,**Kiyoko Iwatsuki, Yasuhiro Ikeda, Kenjiro Ohashi, Kazuya Nakamura, Chieko Kai,Microbes and Infection,Volume 1, Issue 12,1999,Pages 987-991,https://doi.org/10.1016/S1286-4579(99)80516-4.

Abstract:

We produced a B95a lymphoid cell line persistently infected with canine distemper virus (CDV), in which virus-specific antigens were present in nearly 100% of cells without causing cytopathic effect. The virus recovered from this cell line was able to infect fresh B95a cells persistently, indicating that a persistent CDV was established.

Keywords: CDV; persistent infection; B95a cells

35. **Cross-species transmission of canine distemper virus—an update,**Andreas Beineke, Wolfgang Baumgärtner, Peter Wohlsein,One Health,Volume 1,2015,Pages 49-59,https://doi.org/10.1016/j.onehlt.2015.09.002.

Abstract:

Canine distemper virus (CDV) is a pantropic morbillivirus with a worldwide distribution, which causes fatal disease in dogs. Affected animals develop dyspnea, diarrhea, neurological signs and profound

immunosuppression. Systemic CDV infection, resembling distemper in domestic dogs, can be found also in wild canids (e.g. wolves, foxes), procyonids (e.g. raccoons, kinkajous), ailurids (e.g. red pandas), ursids (e.g. black bears, giant pandas), mustelids (e.g. ferrets, minks), viverrids (e.g. civets, genets), hyaenids (e.g. spotted hyenas), and large felids (e.g. lions, tigers). Furthermore, besides infection with the closely related phocine distemper virus, seals can become infected by CDV. In some CDV outbreaks including the mass mortalities among Baikal and Caspian seals and large felids in the Serengeti Park, terrestrial carnivores including dogs and wolves have been suspected as vectors for the infectious agent. In addition, lethal infections have been described in non-carnivore species such as peccaries and non-human primates demonstrating the remarkable ability of the pathogen to cross species barriers. Mutations affecting the CDV H protein required for virus attachment to host-cell receptors are associated with virulence and disease emergence in novel host species. The broad and expanding host range of CDV and its maintenance within wildlife reservoir hosts considerably hampers disease eradication.

Keywords: Canine distemper virus; Spillover; H protein; Carnivores; Human health risk

36. **Clinical assessment of a point-of-care assay to determine protective vaccinal antibody titers to canine viral diseases**,Y. Ad, I.M. Halperin, E.C. Olstad, L.J. Gershwin, L. Sullivan, K.L. Reagan,The Veterinary Journal,Volumes 298–299,2023,106017,https://doi.org/10.1016/j.tvjl.2023.106017.

Abstract:

Guidelines recommend that dogs are vaccinated for canine distemper virus (CDV), canine parvovirus (CPV), and canine adenovirus (CAV) every 3 years. Alternatively, their antibody titers are measured and vaccines given when titers fall below a protective threshold. In this study, a point-of-care (POC) assay was compared to hemagglutination inhibition (for CPV) and virus neutralization (for CAV and CDV) assays to predict the need for revaccination Ninety-two dogs presented for vaccination were enrolled. The POC assay indicated protective titers against CDV in 79/80, CPV in 89/90, and CAV in 91/91 dogs with reference standard antibody measurements that were over a protective threshold. The sensitivity of the POC assay for to detect protective concentrations of CDV antibodies was 99% (95% confidence interval [CI 95%], 93.3–99.9%). Ten dogs were falsely considered protected against CDV by the POC assay with a specificity of 17% (CI 95%, 3.0–44.8%). The sensitivity of the POC assay for protective concentrations of CPV titers was 99% (CI 95%, 93.9–99.9%). The sensitivity of the POC assay to detect protective concentrations of CAV antibodies was 100% (CI 95%, 95.9–100%). Only classifying high-positive CDV and CPV titers on the POC assay as protective improved assay specificity to 100%, but sensitivity decreased to 51% and 76% respectively. This POC assay had a high sensitivity for the detection of protective antibody titers; however, some dogs were falsely categorized as protected, especially for CDV.

Keywords: Antibody; Canine adenovirus; Canine distemper; Canine parvovirus; Revaccination

37. **The fusion protein of wild-type canine distemper virus is a major determinant of persistent infection,**Philippe Plattet, Jean-Paul Rivals, Benoît Zuber, Jean-Marc Brunner, Andreas Zurbriggen, Riccardo Wittek,Virology,Volume 337, Issue 2,2005,Pages 312-326,https://doi.org/10.1016/j.virol.2005.04.012.

Abstract:

The wild-type A75/17 canine distemper virus (CDV) strain induces a persistent infection in the central nervous system but infects cell lines very inefficiently. In contrast, the genetically more distant Onderstepoort CDV vaccine strain (OP-CDV) induces extensive syncytia formation. Here, we investigated the roles of wild-type fusion (FWT) and attachment (HWT) proteins in Vero cells expressing, or not, the canine SLAM receptor by transfection experiments and by studying recombinants viruses expressing different combinations of wild-type and OP-CDV glycoproteins. We show that low fusogenicity is not due to a defect of the envelope proteins to reach the cell surface and that HWT determines persistent infection in a receptor-dependent manner, emphasizing the role of SLAM as a potent enhancer of fusogenicity. However, importantly, FWT reduced cell-to-cell fusion independently of the cell surface receptor, thus demonstrating that the fusion protein of the neurovirulent A75/17-CDV strain plays a key role in determining persistent infection.

Keywords: Canine distemper virus; Persistence; Fusogenicity; Envelope glycoproteins; SLAM

38. **New world origin of canine distemper: Interdisciplinary insights,**Elizabeth W. Uhl, Charles Kelderhouse, Jane Buikstra, Jeffrey P. Blick, Brad Bolon, Robert J. Hogan,International Journal of Paleopathology,Volume 24,2019,Pages 266-278,https://doi.org/10.1016/j.ijpp.2018.12.007.

Abstract:

Objective

Canine distemper virus (CDV), human measles virus (HMV), and rinderpest virus (RPV) of cattle are morbilliviruses that have caused devastating outbreaks for centuries. This paper seeks to reconstruct the evolutionary history of CDV.

Materials and methods

An interdisciplinary approach is adopted, synthesizing paleopathological analysis of 96 Pre-Columbian dogs (750–1470 CE) from the Weyanoke Old Town, Virginia site, with historical reports, molecular analysis and morbilliviral epidemiology.

Results

Both measles (c.900CE) and rinderpest (c. 376 BCE) were first reported in Eurasia, while canine distemper was initially described in South America much later (1735 CE); there are no paleopathological indications of CDV in Weyanoke Old Town dogs. Molecularly, CDV is closely related to HMV, while viral codon usage indicates CDV may have previously infected humans; South American measles epidemics occurred prior to the emergence of canine distemper and would have facilitated HMV transmission and adaptation to dogs.

Conclusions

The measles epidemics that decimated indigenous South American populations in the 1500–1700 s likely facilitated the establishment of CDV as a canine pathogen, which eventually spread to Europe and beyond.

Significance

Understanding the historical and environmental conditions that have driven morbilliviral evolution provides important insights into potential future threats of animal/human cross-species infections.

Limitations

Interpreting historical disease descriptions is difficult and the archaeological specimens are limited. Molecular sequence data and codon usage analyses rely on modern viruses.

Suggestions for further research

Interdisciplinary approaches are increasingly needed to understand diseases of the past and present, as critical information and knowledge is scattered in different disciplines.

Keywords: Canine distemper; Human measles virus; Rinderpest virus; History of disease; Morbilliviral evolution; Codon usage bias

39. **Canine distemper virus neutralization activity is low in human serum and it is sensitive to an amino acid substitution in the hemagglutinin protein,** Xinsheng Zhang, Olivia L. Wallace, Arban Domi, Kevin J. Wright, Jonathan Driscoll, Omu Anzala, Eduard J. Sanders, Anatoli Kamali, Etienne Karita, Susan Allen, Pat Fast, Jill Gilmour, Matt A. Price, Christopher L. Parks,Virology,Volume 482,2015,Pages 218-224,https://doi.org/10.1016/j.virol.2015.03.035.

Abstract:

Serum was analyzed from 146 healthy adult volunteers in eastern Africa to evaluate measles virus (MV) and canine distemper virus (CDV) neutralizing antibody (nAb) prevalence and potency. MV plaque reduction neutralization test (PRNT) results indicated that all sera were positive for MV nAbs. Furthermore, the 50% neutralizing dose (ND50) for the majority of sera corresponded to antibody titers induced by MV vaccination. CDV nAbs titers were low and generally were detected in sera with high MV nAb titers. A mutant CDV was generated that was less sensitive to neutralization by human serum. The mutant virus genome had 10 nucleotide substitutions, which coded for single amino acid substitutions in the fusion (F) and hemagglutinin (H) glycoproteins and two substitutions in the large polymerase (L) protein. The H substitution occurred in a conserved region involved in receptor interactions among morbilliviruses, implying that this region is a target for cross-reactive neutralizing antibodies.

Keywords: Canine distemper virus; Measles virus; Plaque reduction neutralization test; Neutralizing antibody; Hemagglutinin; Pre-existing immunity; Cross-neutralization

40. **Unveiling the molecular epidemiology of canine distemper virus in Namibia: An expected pathogen showing an unexpected origin,**Giovanni Franzo, Lourens de Villiers, Lauren M. Coetzee, Mari de Villiers, Francis N. Nyathi, Maya Garbade, Chantal Hansen, Shadia Berjaoui, Paola Ripà, Alessio Lorusso, Umberto Molini,Heliyon,Volume 10, Issue 15,2024,e34805,https://doi.org/10.1016/j.heliyon.2024.e34805.

Abstract:

Objective

Canine distemper virus (CDV) is a highly infectious virus that represents a threat for domestic dogs and several wild species. Despite recognized in several African countries, current knowledge of its molecular epidemiology is scarce and poorly updated.

Design

Twenty-two hemagglutinin sequences, obtained from symptomatic Namibian dogs from 2020 to 2023, were analysed through phylogenetic and phylodynamic analysis to characterize the local CDV epidemiology and contextualize it in the international scenario.

Results

Two unrelated clades were identified, including strains sampled in different Namibian towns, in the absence of a strong geographical clustering. The ancestors of the two clades were estimated to have originated from South America, likely Brazil, and South Africa, approximately in 2000 and 2006, respectively. While the introduction from South Africa was predictable, the introduction from Brazil was unexpected. The mediation of other African countries, particularly Angola, appears to be the most likely importation pathway.

Conclusions

The occurrence of multiple introduction events, likely originating from cross-border illegal animal trade between African countries, and the absence of any geographical clustering within Namibian regions, suggest a need for further investigation into its spreading pattern, as well as improved biosecurity measures to limit foreign viral introduction into the country.

Keywords: Canine distemper virus; Namibia; Phylogenesis; Phylogeography; International

41. **Sustained Replication of Synthetic Canine Distemper Virus Defective Genomes In Vitro and In Vivo,**Natasha L. Tilston-Lunel, Stephen R. Welch, Sham Nambulli, Rory D. de Vries, Gregory W. Ho, David E. Wentworth, Reed Shabman, Stuart T. Nichol, Christina F. Spiropoulou, Rik L. de Swart, Linda J. Rennick, W. Paul Duprex, Benhur Lee,mSphere,Volume 6, Issue 5,2021,https://doi.org/10.1128/msphere.00537-21.

Abstract:

Defective interfering (DI) genomes restrict viral replication and induce type I interferon. Since DI genomes have been proposed as vaccine adjuvants or therapeutic antiviral agents, it is important to understand their generation, delineate their mechanism of action, develop robust production capacities, assess their safety and in vivo longevity, and determine their long-term effects. To address this, we generated a recombinant canine distemper virus (rCDV) from an entirely synthetic molecular clone designed using the genomic sequence from a clinical isolate obtained from a free-ranging

raccoon with distemper. rCDV was serially passaged in vitro to identify DI genomes that naturally arise during rCDV replication. Defective genomes were identified by Sanger and next-generation sequencing techniques, and predominant genomes were synthetically generated and cloned into T7-driven plasmids. Fully encapsidated DI particles (DIPs) were then generated using a rationally attenuated rCDV as a producer virus to drive DI genome replication. We demonstrate that these DIPs interfere with rCDV replication in a dose-dependent manner in vitro. Finally, we show sustained replication of a fluorescent DIP in experimentally infected ferrets over a period of 14 days. Most importantly, DIPs were isolated from the lymphoid tissues, which are a major site of CDV replication. Our established pipeline for detection, generation, and assaying DIPs is transferable to highly pathogenic paramyxoviruses and will allow qualitative and quantitative assessment of the therapeutic effects of DIP administration on disease outcome. IMPORTANCE Defective interfering (DI) genomes have long been considered inconvenient artifacts that suppressed viral replication in vitro. However, advances in sequencing technologies have led to DI genomes being identified in clinical samples, implicating them in disease progression and outcome. It has been suggested that DI genomes might be harnessed therapeutically. Negative-strand RNA virus research has provided a rich pool of natural DI genomes over many years, and they are probably the best understood in vitro. Here, we demonstrate the identification, synthesis, production, and experimental inoculation of novel CDV DI genomes in highly susceptible ferrets. These results provide important evidence that rationally designed and packaged DI genomes can survive the course of a wild-type virus infection.

Keywords: canine distemper virus; defective interfering viral particles; defective interfering genomes; defective genomes; next-generation sequencing; single-step attenuation; vaccines; ferret model

42. **Identification of enteric viruses circulating in a dog population with low vaccine coverage**,Christian D.B.T. Alves, Oscar F.O. Granados, Renata da F. Budaszewski, André F. Streck, Matheus N. Weber, Samuel P. Cibulski, Luciane D. Pinto, Nilo Ikuta, Cláudio W. Canal,Brazilian Journal of Microbiology,Volume 49, Issue 4,2018,Pages 790-794,https://doi.org/10.1016/j.bjm.2018.02.006.

Abstract:

Although the use of vaccines has controlled enteric diseases in dogs in many developed countries, vaccine coverage is still under optimal situation in Brazil. There is a large population of nonimmunized dogs and few studies about the identification of the viruses associated with diarrhea. To address this situation, stool samples from 325 dogs were analyzed by polymerase chain reaction for the detection of common enteric viruses such as Canine adenovirus (CAdV), Canine coronavirus (CCoV), Canine distemper virus (CDV), Canine rotavirus (CRV) and Carnivorous protoparvovirus 1 (canine parvovirus 2; CPV-2). At least one of these species was detected in 56.6% (184/325) of the samples. The viruses detected most frequently in either diarrheic or nondiarrheic dog feces were CPV-2 (54.3% of the positive samples), CDV (45.1%) and CCoV (30.4%), followed by CRV (8.2%) and CAdV (4.9%). Only one agent was detected in the majority of the positive samples (63%), but co-infections were present in 37% of the positive samples and mainly included CDV and CPV-2. The data presented herein can improve the clinical knowledge in regions with low vaccine coverage and highlight the need to improve the methods used to control these infectious diseases in domestic dogs.

Keywords: Distemper; Parvovirus; Diarrhea; Dog; Co-infection

43. **Influence of maternally-derived antibodies in 6-week old dogs for the efficacy of a new vaccine to protect dogs against virulent challenge with canine distemper virus, adenovirus or parvovirus,**Stephen Wilson, Elisabeth Siedek, Anne Thomas, Vickie King, Catrina Stirling, Edita Plevová, Jeremy Salt, Gordon Sture,Trials in Vaccinology,Volume 3,2014,Pages 107-113,https://doi.org/10.1016/j.trivac.2014.06.001.

Abstract:

The results from three studies determining the efficacy of a canine multivalent vaccine in the presence of maternal antibodies are reported. Each study used 15 six week old dogs; five dogs were sero-negative; the remaining 10 had maternally derived antibodies to CDV, CAV and CPV. The five MDA-negative dogs and five of the MDA-positive dogs were vaccinated twice with the vaccine while the remaining 5 MDA-positive dogs were administered sterile water. According to EU guidelines for MDA studies dogs were challenged when maternally-derived antibodies in non-vaccinated dogs had greatly diminished or disappeared (3–5weeks after second vaccination); clinical observations and rectal temperatures were recorded, and sera and faecal samples (CPV study only) were collected throughout the study. After challenge, non-vaccinated dogs showed clinical signs of infection while none of the vaccinated dogs did. MDA-negative vaccinated dogs sero-converted with increases in titre observed after each vaccination, and further increases observed after challenge. MDA-positive vaccinated dogs showed declining antibody titres following the first vaccination, but increases after the second vaccination and further increases after challenge. In all vaccinated dogs the immune responses generated were protective, irrespective of the presence of maternal antibodies, as demonstrated by heterologous viral challenge. In conclusion, two doses of the DHPPi/L4R vaccine administered to dogs from six weeks of age in the presence of maternal antibodies aided in the protection against virulent challenge with CDV, CAV-1 or CPV.

Keywords: Maternal antibodies; Canine; Parvovirus; Adenovirus; Distemper virus

44. **Canthin-6-one analogs block Newcastle disease virus proliferation via suppressing the Akt and ERK pathways,**Chongyang Wang, Ting Wang, Jiangkun Dai, Yu Han, Ruochen Hu, Na Li, Zengqi Yang, Junru Wang,Poultry Science,Volume 103, Issue 9,2024,103944,https://doi.org/10.1016/j.psj.2024.103944.

Abstract:

Newcastle disease virus, a member of the Paramyxoviridae family, causes significant economic losses in poultry worldwide. To identify novel antiviral agents against NDV, 36 canthin-6-one analogs were evaluated in this study. Our data showed that 8 compounds exhibited excellent inhibitory effects on NDV replication with IC50 values in the range of 5.26 to 11.76 μM. Besides, these analogs inhibited multiple NDV strains with IC50 values within 12 μM and exerted antiviral activity against peste des petits ruminants virus (PPRV) and canine distemper virus (CDV). Among these analogs, 16 presented the strongest anti-NDV activity (IC50 = 5.26 μM) and minimum cytotoxicity (CC50 > 200 μM) in DF-1 cells. Furthermore, 16 displayed antiviral activity in different cell lines. Our results showed that 16 did not affect the viral adsorption while it can inhibit the entry of NDV by suppressing the Akt pathway. Further study found that 16-treatment inhibited the NDV-activated ERK pathway, thereby promoting the expression of interferon-related genes. Our findings reveal an antiviral mechanism of canthin-6-one analogs through inhibition of the Akt and ERK signaling pathways. These results point to the potential value of canthin-6-one analogs to serve as candidate antiviral agents for NDV.

Keywords: antiviral; canthin-6-one; Newcastle disease virus; viral entry

45. **A novel and highly divergent Canine Distemper Virus lineage causing distemper in ferrets in Australia**,Ankita M. George, Michelle Wille, Jianning Wang, Keith Anderson, Shari Cohen, Jean Moselen, Leo Y.Y. Lee, Willy W. Suen, John Bingham, Antonia E. Dalziel, Paul Whitney, Harry Stannard, Aeron C. Hurt, David T. Williams, Yi-Mo Deng, Ian G. Barr,Virology,Volume 576,2022,Pages 117-126,https://doi.org/10.1016/j.virol.2022.09.001.

Abstract:

Canine distemper virus (CDV) causes a highly contagious systemic infection in an array of animal species. In this study we report an outbreak of distemper in ferrets in two research facilities in Australia, caused by a novel lineage of CDV. While the CDV strain caused mainly mild symptoms in ferrets, histopathology results presented a typical profile of distemper pathology, with multi-system virus replication. Through the development of a discriminatory PCR, paired with full genome sequencing, we revealed that the outbreak was caused by a novel lineage of CDV. The novel CDV lineage was highly divergent, with less than 93% similarity across the H gene to other described lineages, including the vaccine strain, and diverged approximately 140–400 years ago. Enhanced surveillance to determine the prevalence of CDV in ferrets, dogs and other at-risk species is critical to better understand the presence and diversity of CDV in Australia currently.

Keywords: Canine distemper virus; Canine morbillivirus; Paramyxoviridae; CDV; Ferrets; Australia

46. **Pathogenesis of Demyelinating Encephalopathy in Dogs with Spontaneous Acute Canine Distemper**,Yao-qian PAN, Xing-you LIU, Li-ping MENG, Guang-rui ZHU, Yin-ke XIA, Jin-shan CHEN, Yoshikawa Takashi,Journal of Integrative Agriculture,Volume 12, Issue 2,2013,Pages 334-343,https://doi.org/10.1016/S2095-3119(13)60233-6.

Abstract:

So far, the pathogenesis of demyelination caused by canine distemper virus (CDV) in the central nervous system has remained unclear, although a lot of studies have been done extensively. To further investigate the relation of variety cells in brain to demyelination, this study was performed on 15 dogs with spontaneous acute canine distemper and 2 controls. According to anatomical relation, the brain was divided into cerebrum, cerebral stem and cerebellum. The sections with no, mild, moderate, or severe demyelinating lesions were selected respectively and stained by HE and immunohistochemistry. Immuno-localisation of CDV antigen was used to confirm CDV infection. The brain was examined for co-localisation of the CDV antigen with either an astrocyte-specific marker, glial fibrillary acidic protein (GFAP), or an oligodendrocyte-specific marker, galactocerebroside (GalC). Apoptotic cell was detected by TdT-mediated nick end-labeling assay (TUNEL). The results demonstrated that the local disturbance of blood circulation mainly included congestion, edema, thrombosis, and disseminated intravascular coagulation (DIC). The CDV neucleocapsid protein positive reaction, metabolic disorder and apoptosis of oligodendrocytes were observed in demyelinating areas. Lots of astrocytes displayed CDV antigen-positive, especially in their process. Some of them became apoptotic cell confirmed by TUNEL staining. Fibrous astrocytes showed more intense GFAP-positive in mild and moderate demyelinating area. Some of nervous cells located in pyramidal cell layers and nucleus nervi were in degeneration, necrosis. Satellitosis, neuronophagia and apoptotic neurons were examined by hematoxylin and eosin (HE) and TUNEL staining. The results suggested that

the demyelinating changes in brain tissues infected with CDV mainly related to the metabolic disorder and apoptosis of ogliodendrocytes and astrocytes; also involved with the local disturbance of blood circulation and some neuron lost.

Keywords: acute canine distemper; demyelinating encephalopathy; oligodendrocyte; astrocyte; apoptosis

47. **Antiviral activity of nitazoxanide against Morbillivirus infections,**Debora Stelitano, Simone La Frazia, Annalisa Ambrosino, Carla Zannella, Daniel Tay, Valentina Iovane, Serena Montagnaro, Anna De Filippis, Maria Gabriella Santoro, Matteo Porotto, Massimiliano Galdiero,Journal of Virus Eradication,Volume 9, Issue 4,2023,100353,https://doi.org/10.1016/j.jve.2023.100353.

Abstract:

The measles virus (MeV) and canine distemper virus (CDV) belong to the genus Morbillivirus of the Paramyxoviridae family. They are enveloped viruses harboring a non-segmented negative-sense RNA. Morbilliviruses are extremely contagious and transmitted through infectious aerosol droplets. Both MeV and CDV may cause respiratory infections and fatal encephalitis, although a high incidence of brain infections is unique to CDV. Despite the availability of a safe and effective vaccine against these viruses, in recent years we are witnessing a strong resurgence of Morbillivirus infection. Measles still kills more than 100,000 people each year, and CDV causes widespread outbreaks, especially among wild animals, including non-human primates. No drugs are currently approved for MeV and CDV. Therefore, the identification of effective antiviral agents represents an unmet medical need. Here, we have investigated the potential antiviral properties of nitazoxanide (NTZ) against MeV and CDV. Antiviral activity was explored with live virus and cell-based assays. NTZ is a thiazolide that is approved by the FDA as an antiprotozoal agent for the treatment of Giardia intestinalis and Cryptosporidium parvum. Further, nitazoxanide and its metabolite tizoxanide have recently emerged as broad-spectrum antiviral agents. We found that NTZ blocks the MeV and CDV replication, acting at the post-entry level. Moreover, we showed that NTZ affects the function of the viral fusion protein (F), impairing viral spread. Our results indicate that NTZ should be further explored as a therapeutic option in measles and canine distemper virus treatment.

Keywords: Nitazoxanide; Thiazolide; Measles; Morbillivirus; Canine distemper virus; Antiviral

48. **Molecular and pathological screening of canine distemper virus in Asiatic lions, tigers, leopards, snow leopards, clouded leopards, leopard cats, jungle cats, civet cats, fishing cat, and jaguar of different states, India,**Rahul Ganpatrao Kadam, M. Karikalan, Chandra Mohan Siddappa, K. Mahendran, Gaurav Srivastava, K.K. Rajak, Yogesh Bhardwaj, Rajat Varshney, Zahoor Ahemad War, Rahul Singh, Mayukh Ghosh, V. Beena, Abhijit M. Pawde, K.P. Singh, A.K. Sharma,Infection, Genetics and Evolution,Volume 98,2022,105211,https://doi.org/10.1016/j.meegid.2022.105211.

Abstract:

The present investigation was conducted to rule out canine distemper (CD) diseases in Indian wild felids (Asiatic lions, tigers, leopards, snow leopards, clouded leopards, leopard cats, jungle cats, civet cats, fishing cat, and jaguar). The collected samples were screened for CD virus (CDV) by

histopathology (HP), immunohistochemistry (IHC) and reverse transcriptase-polymerase chain reaction (RT-PCR) targeting H gene and N gene. The HP and IHC of suspected samples portrayed that 22 [11 leopards, 6 lions, 3 tigers, 1 snow leopard and 1 civet cat] out of 129 (17.05%) wild felids were positive for CD. The major pathological consequences were observed in spleen, lung, kidney and brain. The syncytia and intranuclear as well as intracytoplasmic eosinophilic inclusion bodies were seen in CDV infected cells. Although the histopathological lesions in spleen were more specific and consistent, however, the severe demyelinated leukoencephalitis (usually expected in CD infected dog) was not observed in the brain of any Indian wild felids. Conversely, the CDV antigen has been portrayed via IHC in pancreatic islets of Langerhans of tiger species for the first time in this study. Moreover, the concurrent CD and babesiosis has also been observed in a lioness without a usual coffee-coloured urine. The N gene and H gene of CDV isolates were amplified, sequenced and subsequently constructed the phylogenetic tree. The phylogenetic analysis of H gene revealed that the CDV isolates from Indian lion formed separate clade with CDV isolates from Indian dog and Indian palm civet cat. Furthermore, two CDV isolates from Indian tigers formed clade with Onderstepoort vaccine strain and CDV isolates from dogs of Uttar Pradesh, USA and UK. Evidently, CDV is circulating in Indian wild felids and causing diseases in them.

Keywords: Canine distemper virus; Wild felids; Immunohistochemistry; RT-PCR; Phylogenetic analysis

49. **Membrane-bound SIV envelope trimers are immunogenic in ferrets after intranasal vaccination with a replication-competent canine distemper virus vector,**Xinsheng Zhang, Olivia Wallace, Kevin J. Wright, Martin Backer, John W. Coleman, Rebecca Koehnke, Esther Frenk, Arban Domi, Maria J. Chiuchiolo, Joanne DeStefano, Sandeep Narpala, Rebecca Powell, Gavin Morrow, Cesar Boggiano, Timothy J. Zamb, C. Richter King, Christopher L. Parks,Virology,Volume 446, Issues 1–2,2013,Pages 25-36,https://doi.org/10.1016/j.virol.2013.07.012.

Abstract:

We are investigating canine distemper virus (CDV) as a vaccine vector for the delivery of HIV envelope (Env) that closely resembles the native trimeric spike. We selected CDV because it will promote vaccine delivery to lymphoid tissues, and because human exposure is infrequent, reducing potential effects of pre-existing immunity. Using SIV Env as a model, we tested a number of vector and gene insert designs. Vectors containing a gene inserted between the CDV H and L genes, which encoded Env lacking most of its cytoplasmic tail, propagated efficiently in Vero cells, expressed the immunogen on the cell surface, and incorporated the SIV glycoprotein into progeny virus particles. When ferrets were vaccinated intranasally, there were no signs of distress, vector replication was observed in the gut-associated lymphoid tissues, and the animals produced anti-SIV Env antibodies. These data show that live CDV-SIV Env vectors can safely induce anti-Env immune responses following intranasal vaccination.

Keywords: Canine distemper virus; Human immunodeficiency virus; Simian immunodeficiency virus; Envelope; Replicating viral vector; Immunogen; Ferret

50. **Aetiology of Canine Infectious Respiratory Disease Complex and Prevalence of its Pathogens in Europe**,M.J. Day, S. Carey, C. Clercx, B. Kohn, F. Marsillo, E. Thiry, L. Freyburger, B. Schulz, D.J. Walker,Journal of Comparative Pathology,Volume 176,2020,Pages 86-108,https://doi.org/10.1016/j.jcpa.2020.02.005.

Abstract:

The canine infectious respiratory disease complex (CIRDC) is an endemic worldwide syndrome involving multiple viral and bacterial pathogens. Traditionally, Bordetella bronchiseptica (Bb), canine adenovirus type 2 (CAV-2), canine distemper virus (CDV), canine herpesvirus (CHV) and canine parainfluenza virus (CPiV) were considered the major causative agents. Lately, new pathogens have been implicated in the development of CIRDC, namely canine influenza virus (CIV), canine respiratory coronavirus (CRCoV), canine pneumovirus (CnPnV), Mycoplasma cynos and Streptococcus equi subspecies zooepidemicus. To better understand the role of the different pathogens in the development of CIRDC and their epidemiological relevance in Europe, prevalence data were collected from peer-reviewed publications and summarized. Evidence of exposure to Bb is frequently found in healthy and diseased dogs and client-owned dogs are as likely to be infected as kennelled dogs. Co-infections with viral pathogens are common. The findings confirm that Bb is an important cause of CIRDC in Europe. CAV-2 and CDV recovery rates from healthy and diseased dogs are low and the most likely explanation for this is control through vaccination. Seroconversion to CHV can be demonstrated following CIRDC outbreaks and CHV has been detected in the lower respiratory tract of diseased dogs. There is some evidence that CHV is not a primary cause of CIRDC, but opportunistically re-activates at the time of infection and exacerbates the disease. The currently available data suggest that CIV is, at present, neither a prevalent nor a significant pathogen in Europe. CPiV remains an important pathogen in CIRDC and facilitates co-infection with other viral and bacterial pathogens. CnPnV and CRCoV are important new elements in the aetiology of CIRDC and spread particularly well in multi-dog establishments. M. cynos is common in Europe and is more likely to occur in younger and kennelled dogs. This organism is frequently found together with other CIRDC pathogens and is significantly associated with more severe respiratory signs. S. zooepidemicus infection is not common and appears to be a particular problem in kennels. Protective immunity against respiratory diseases is rarely complete, and generally only a reduction in clinical signs and excretion of pathogen can be achieved through vaccination. However, even vaccines that only reduce and do not prevent infection carry epidemiological advantages. They reduce spread, increase herd immunity and decrease usage of antimicrobials. Recommending vaccination of dogs against pathogens of CIRDC will directly provide epidemiological advantages to the population and the individual dog.

Keywords: canine infectious respiratory disease complex; dog; pathogen; vaccination