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Dipartimento di Biomedicina Comparata e Alimentazione
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in Animal Care

Canine distemper virus in the European badger (*Meles meles*): literature review and a specific case encountered at the wildlife rescue centre (CRAS) in Treviso

Supervisor:

Prof. Laura Cavicchioli

Submitted by

Daniele Yared

Student n.

1227037

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Abstract

Canine distemper virus (CDV) is a Morbillivirus causing a disease mainly found in the canid population, and with high mortality rate. This disease can affect many wild carnivore species such as wild canids, felids, mustelids, ursids, procyonids and hyaenids, and even non-carnivore species. CDV transmission occurs via direct contact, but it is not a zoonotic disease, thus it does not affect humans. A specific case of a badger rescued at the CRAS in Treviso will be analysed, focusing on the symptoms of the specimen, and comparing them with known CDV symptoms.

1 Introduction

Canine distemper virus (CDV) is a Morbillivirus, of the Paramyxoviridae family, along with phocid distemper virus (PDV), measles virus (MV), rinderpest virus (RPV), small ruminant virus (*pest des petits* ruminant virus, PPRV), feline virus (FeMV), and cetacean Morbilliviruses (CeMV).

Morbilliviruses are characterized by a non-segmented, linear, negative-stranded RNA genome, which consists of six genes, which are nucleocapsid protein N, P/C/V protein (phosphoprotein), single-enveloped-associated protein M (matrix protein), fusion protein F, haemagglutinin H, and large protein L (Martella et al., 2008). The H gene is the most variable among the different types of Morbilliviruses, and it encodes for the protein involved in binding to cellular receptors, and subsequent fusion of the target cell membrane with the viral envelope, action carried out with the help of the F protein (Rijks et al., 2012). The two cellular receptors targeted by wild-type morbilliviruses are the signalling lymphocytic activation molecule (SLAM, also known as CD150) and Nectin-4 (also known as poliovirus receptor-like protein-4 or PVRL4) (De Vries et al., 2015). The signalling lymphocytic activation molecule (SLAM) is mainly expressed in immune cells such as activated T and B cells, monocytes, and dendritic cells, while Nectin-4 is expressed in epithelial cells (Carvalho et al., 2012). Additional receptors, contributing to the spread of the infection, may be present since these viruses infect cells of the central nervous system (De Vries et al., 2015), and immunocytochemistry studies have shown limited SLAM expression in the CNS when compared to the lymphoid tissues (Vandevelde et al., 2005).

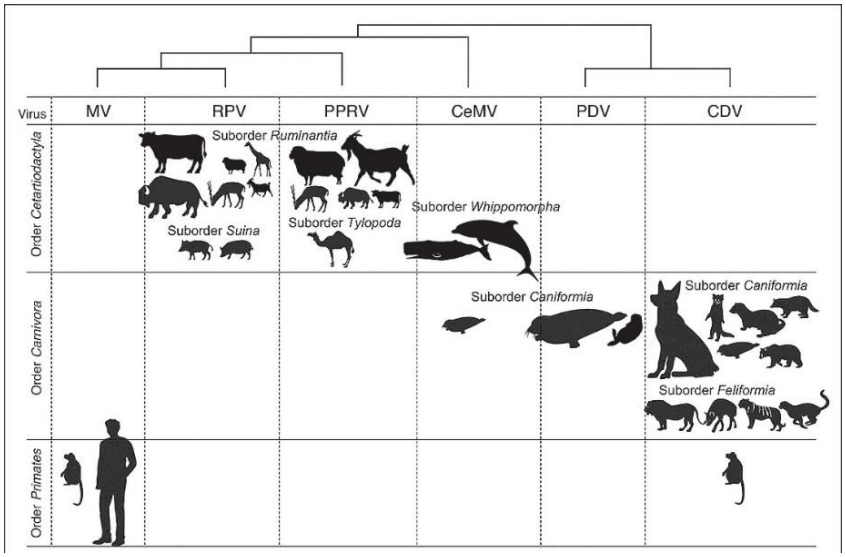


Figure 1. Phylogenetic relationship of morbilliviruses and their host animals (the main host animals have larger silhouette images) (Takeda et al., 2020)

The variation in H protein expression and ability to bind to receptors of specific hosts gives rise to different Morbillivirus species (Figure 1). The common phylogenetic origin of the Morbilliviruses may explain why sometimes they can cross-react in serological tests and additionally, it could happen that the presence of antibodies for one morbillivirus can also protect from another morbillivirus infection (Rijks et al., 2012).

Morbilliviruses can affect multiple animal species, sometimes of different orders (Figure 1), but usually only one or two of these species may be important for preservation of the infection over time, such as cattle for rinderpest (Rijks et al., 2012) (this virus was declared eradicated in 2011 after years of preventive measures and use of vaccines (FAO & OIE, 2011)).

Transmission occurs via direct contact and morbilliviruses can spread rapidly inside populations causing relevant disease outbreaks with high morbidity and mortality, especially in morbillivirus-naïve populations (i.e., where precedent MV infections haven't occurred) (Takeda et al., 2020).

The stability of Morbilliviruses in nature is influenced by environmental conditions. They are inactivated when exposed to sunlight or to high temperatures, and they are stable within a broad pH range and at temperatures of about 0 °C or lower (Rijks et al., 2012).

At the beginning of an infection process, a Morbillivirus is lymphotropic (binding to SLAM receptor), spreading all over the lymphatic system and causing lymphopenia and immunosuppression. Reduced immune activity results in increased host susceptibility to opportunistic infections, which are usually the main cause of deaths related to Morbillivirus (Carvalho et al., 2012). Later in the infection, the virus can become neurotropic, resulting in disruption of normal central nervous system activity. It also becomes epitheliotropic (binding to PVRL4 receptor), causing formation of aggregates, visible as intranuclear and intracytoplasmic inclusion bodies, inside the nucleus and the cytoplasm of infected cells (Rijks et al., 2012).

The prevention of Morbillivirus infections is conducted via vaccination projects and monitoring activities, and animals who survive infection develop viral immunity, which can cross-protect from similar morbilliviruses.

2 Canine distemper

Canine distemper (CD) is a disease mainly found in the canid population, but also spread in other wild carnivore species and with high mortality rate. The wide host range includes species in Canid families (wolves, foxes), Felids (tigers, lions) Mustelids (badgers, ferrets, beech martens, also known as stone martens), Procyonids, Ursids (bears, giant pandas), Ailurids (red pandas), Hyaenids, as well as Phocids (seals) and non-human primates (japanese and rhesus macaques) (Martella et al., 2008). Canine distemper virus transmission occurs, as other Morbilliviruses, via direct contact, but it is not a zoonotic disease, as studies have shown that this virus doesn't bind successfully to human-SLAM receptors (Quintero-Gil et al., 2019).

The first report regarding CDV was written by Antonio de Ulloa in 1746, describing a disease that affected dogs in Ecuador and other parts of South America. Later, in the 1760s there were reports of a similar disease in Europe, firstly in Spain (1761), where in 1763 a CDV outbreak caused 900 deaths in Madrid in only one day. From 1764 there were reports of this disease also in Italy and Great Britain (Quintero-Gil, 2019; Blancou, 2004), and in 1809 Edward Jenner wrote about CDV infection describing its symptoms (Jenner, 1809). The causal agent of the disease was discovered in 1905 when the French veterinarian Henri Carré isolated the virus, in fact CDV is also known as *maladie de Carré* (Quintero-Gil et al., 2019).

2.1 Aetiology

Canine distemper is caused by the canine distemper virus (CDV), which is an enveloped single-stranded RNA virus, belonging to the family Paramyxoviridae. The main protein involved in the first virus-host interaction and responsible of initial ligand-receptor bond is hemagglutinin (H protein) (Figure 2.a) . Compared to other Morbilliviruses, CDV's hemagglutinin has shown advanced species-specific mutations, which allows this virus to have the widest infection range of animal species (Takeda et al., 2020). Molecular evolutionary studies have proved that these mutations were caused by positive selection of substitutions at the 530 and 549 binding sites of the H gene to adapt to the SLAM receptor of novel species (Liao et al., 2015).

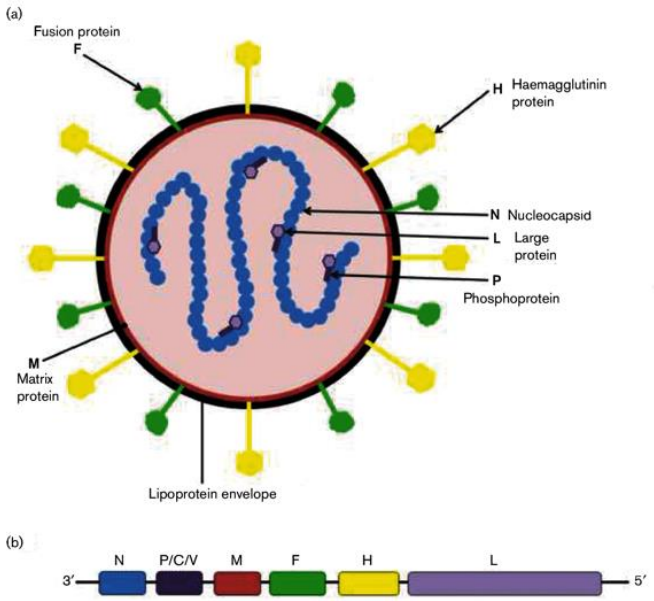


Figure 2. (a) Schematic diagram of the components of a cross-section of canine distemper virus and (b) map of genomic RNA. (https://doi.org/10.1099/jgv.0.000666)

The high genetic variability among the different strains of CDV lead to categorisation in lineages, based on similarities among H gene expression. The lineages that have been classified based on H gene sequences are America-1 to America-5, South America-1 to South America-3, Europe-1, Europe-Wildlife, Artic-like, Africa-1 and Africa-2, Asia-1 to Asia-4 and Asia-5/Indian and Rockborn-like (Bi, 2022).

Like other enveloped viruses, canine distemper virus is relatively fragile and quickly inactivated in the environment by heat and drying. Transmission occurs via direct animal-to-animal contact or by exposure to infectious aerosol (Rijks et al., 2012). High concentration of viral particles can be detected in secretions and excretions, even in urine (Martella et al., 2008). Domestic dogs have been considered as the main reservoirs of the pathogen for the wild carnivores (Rijks et al., 2012).

Morbidity and mortality rates of CDV vary among species and age groups, in which younger animals being susceptible to develop a chronic infection (correlated to decline of maternal derived immunity) (Martella et al., 2008).

2.2 Pathogenesis and clinical signs

When CDV enters a new host by nasal or oral route, it starts replication in cells of the lymphatic tissues in the upper respiratory tract, leading to a leukocyte depletion. Initial incubation period may range from 1 to 4 weeks or even more. After replication, the virus spreads to secondary lymphoid organs, such as tonsils and regional lymph nodes, and within a week the virus may be

found also in the tissues of the digestive system (Peyer's patches, stomach cells and Kupffer cells) [1, 2]. T cells are more affected that B cells and $CD4^+$ lymphocytes (T helper cells) are rapidly depleted, whereas $CD8^+$ cells (cytotoxic T cells) are less affected and can rapidly recover (Vandevelde et al., 2005). Within a few days from virus entry, symptoms such as anorexia (which can lead to poor body condition), light depression, fever, coughing, ocular and nasal discharge, and tonsillitis may be observed. After 1 or 2 weeks after infection the development of subsequent clinical signs depends on various factors, such as the strain virulence, the age of the host, and its immune response. If there is a strong immune response, the virus is cleared from the tissues and the animal has a full recovery from the infection. If the immune response is weak the host might develop a more severe form of the disease, and the virus spreads in epithelial tissues and the central nervous system (CNS) (Carvalho et al., 2012). After virus epithelial localization, clinical signs at the respiratory, intestinal, and dermatologic level can observed. These symptoms are often intensified by secondary bacterial infections and include dyspnea, pneumonia, diarrhea, and dermal pustules (Martella et al., 2008). Animals that survive subclinical or subacute infections may show symptoms such as teeth enamel hypoplasia and hyperkeratosis of foot pads, nose, lips, ears, and eyelids (Figures 3-4) (Rijks et al., 2012).

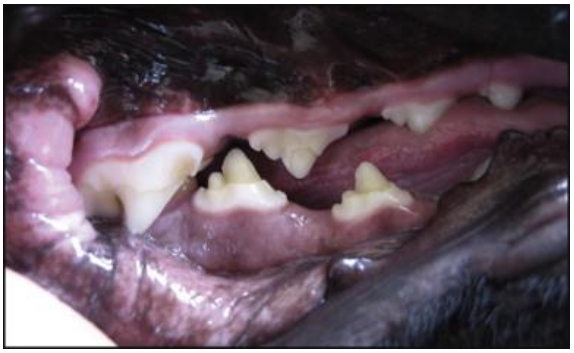


Figure 3. Enamel hypoplasia due to CDV infection

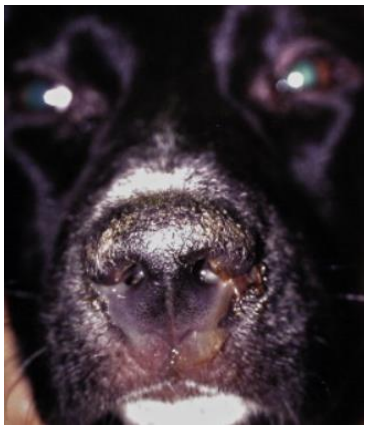


Figure 4. Nasal planum hyperkeratosis with mucopurulent nasal discharge in a 4-months old Border Collie with canine distemper

The onset of clinical signs observed in CDV-infected wild animals are similar to those shown in infected domestic dogs, which means that the virus spreading pathways are similar in different species.

Canine distemper virus spreading to the CNS occurs via the cerebrospinal fluid (CSF) and neurological signs, which depend on the affected area of the brain, can be observed about 2-3

weeks after infection, and include abnormal behaviour, involuntary twitching or contraction of muscles, convulsions, incoordination and partial or complete paralysis (Martella et al., 2008).

At microscopical level, demyelination is the main lesion observed in the brains of CDV-infected animals. Noteworthy, there are two types of demyelination lesions. In the acute stage of canine distemper, myelin lesions are associated with severe immunodepression due to CDV-induced leukopenia, thus they are not related to inflammation, because of lack of perivascular cuffs. Acute demyelination is caused by virus-induced activation of microglial cells (to which is attributed the early presence of cytotoxic T cells in the CNS) and decreased myelin synthesis in infected oligodendrocytes. The initially observed lesions are ballooning of myelin sheets with vacuolation of the white matter, and astrocytic hypertrophy (Vandevelde et al., 2005). In chronic forms of canine distemper, the inflammatory reaction in the demyelinating lesions can lead to developed tissues damage due to CDV-specific immune response and endurance of CDV infection. In the areas of demyelination there's formation of perivascular cuffing with monocytes, plasma cells and lymphocytes (Martella et al., 2008).

2.3 Diagnosis

Clinical diagnosis of CD is difficult due to the many signs that may be mistaken with other respiratory and enteric diseases of dogs and the other species affected by CDV.

Histology is a useful tool to detect CDV infection in necropsy specimens or skin biopsies because an important diagnostic feature of CD is the presence of intracytoplasmic and intranuclear eosinophilic inclusion bodies in epithelia, neurons, and astrocytes. Inclusion bodies are also often present in gastric mucosa, enterocytes, and epithelium of the respiratory and urogenital tract (Rijks et al., 2012).

Immunohistochemistry is useful for CDV-antigen detection in formalin-fixed, paraffin-embedded tissues.

The use of serological methods, such as enzyme-linked immunosorbent (ELISA) and serum virus neutralization (SVN) assays, have little diagnostic value because high concentrations of antibodies to CDV may be detected for several months after vaccination or after subclinical/clinical infection (Elia et al., 2006). For example, an ELISA assay could be used to assess recent CDV infection in an animal by recognition of virus-specific IgM (immunoglobulin M) which persists for about 3 months

post-infection. In dogs, diagnosis of CD by virus isolation on canine cells is fastidious and time consuming (Martella et al., 2008).

The use of direct and fast viral nucleic acid detection methods such as reverse transcriptase-polymerase chain reaction (RT-PCR) is an extremely sensitive and efficient assay to detect CDV in various tissues and it can also be used in phylogenetic analyses (for strain characterization). Viral RNA presence can be demonstrated in brain, lungs, spleen, liver, kidney, urinary bladder, colon samples and buffy coat cells (Rijks et al., 2012).

2.4 Treatment

Prevention of CDV infection in domestic dogs consists in vaccination of the animals with attenuated live or recombinant vaccines (e.g, attenuated Onderstepoort-strain vaccine, or canarypox recombinant CDV vaccine), and it's particularly important for animals that can wander in areas where they may encounter wild species. Vaccine against canine distemper is mandatory for dogs, as well as canine Parvovirus, hepatitis B, leptospirosis, and rabies vaccines. There aren't any CDV vaccines available for wild animals as monitoring after administration can be difficult, and some vaccines may retain their pathogenicity when used in wildlife animals, but there are studies trying to assess the safety of these vaccines in captive wild species (Sadler et al., 2016). Scientists have aimed at reducing the risk of CDV spread to wildlife by focusing on vaccination of domesticated species.

Treatment of infected animals consists of parenteral therapies when gastrointestinal signs are present (e.g., vomiting or diarrhoea), and supportive care, such as antibiotics administration to avoid secondary bacterial infections (which are usually lethal due to CDV-induced immunosuppression), use of antiemetics, and it's important to isolate sick individuals from other animals to avoid spreading of the virus (Sykes, 2014).

CNS signs can appear weeks or months after apparent recovery of the infected animals, and due to the poor prognosis of neurological symptoms progression, euthanasia is recommended when the suffering of the sick individuals has been ethically evaluated and assessed as unbearable for the animals (Sykes, 2014).

3 CDV-infection in wild species

3.1 Canids

Domestic dogs (*Canis lupus familiaris*) are the main host of CDV, but many other animals of the same family are affected from this virus. CD infection has been reported in animals belonging to the Canini tribe (dog-like) such as golden jackals (*Canis aureus*), Australian dingos (*Canis dingo*), coyotes (*Canis latrans*) and wolves (*Canis lupus*).

As for Italy, in the Abruzzo region, during the winter of 2013 in 20 out of 30 carcasses of Apennine wolves CDV was detected with RT-PCR and immunohistochemistry application in lung and CNS samples. Six of the wolves were rescued alive and showed typical distemper symptoms (mucopurulent ocular and nasal discharge, high body temperature, involuntary muscle twitching), and died within a few days. Sequencing of sampled H gene and phylogenetic analysis showed that the virus belonged to the Arctic lineage, which was unusual as studies reported that strains of this lineage in Europe were detected only in domestic dogs. Infection might have occurred from contact of wolves with shepherd dogs in villages or farm areas, in fact during the CDV epizootic in wolves there were reports of unvaccinated, shepherd dogs with clinical signs of distemper (Di Sabatino et al., 2014). This outbreak was a major threat to the other Carnivores susceptible to CDV at the Abruzzo, Lazio, and Molise National Park (ALMNP), especially to the Marsican brown bear (*Ursus arctos marsicanus*), a critically endangered subspecies of the European brown bear (Di Francesco et al., 2022).

American grey wolves were reintroduced in Yellowstone National Park (YNP) in 1995, and after monitored population showed short-term declines due to disease outbreaks, serological surveys of infectious diseases were performed on the carnivore species living in the YNP, and CDV antibodies were found in many grey wolves, coyotes and red foxes (*Vulpes vulpes*) (Almberg et al., 2009).

In the Bale Mountains National Park (PMNP), in south-eastern Ethiopia, there is the world's rarest canid species and the most threatened carnivore in Africa, the Ethiopian wolf (*Canis simensis*). With a population of less than 500 individuals, this species is considered "endangered" according to the International Union for Conservation of Nature's (IUCN) Red List of Threatened Species, and the

intense social behaviour of the wolf packs, inside and among distinct packs of different territories, enhances the chances of spreading transmissible diseases (main threats being rabies and CD). Evidence of CDV presence in Ethiopian wolf population was firstly reported by serological surveys, performed in the last years of the 1980s, where 9 out of 30 samples resulted seropositive to CDV. Since 2001 the populations were intensely monitored, and the investigated wolf packs in the BMNP were grouped in 3 major subpopulations, all linked by narrow geographic corridors: the Morebawa, the Web Valley, and the Sanetti Plateau. Via this prolonged monitoring, two main CD outbreaks were observed in 2005-2006 and in 2010. In the 2005 outbreak, initial spreading caused the death of about 60 domestic dogs in a village; a few months later, distemper symptoms were observed in wolves belonging to packs whose territory border was close to the village (near the Sanetti Plateu). CDV spread continued for 12 months and then died out, and by the end of 2006 population numbers of packs in the Sanetti region had strongly decreased, with higher death rate among subadults than adults. In the 2010 outbreak, infection started in wolf packs from the Web Valley and Sanetti regions and reached wolves in Morebawa region. Packs in the Web Valley region were the most damaged because populations were still recovering from the rabies outbreak of 2008-2009, and 4 of the 7 packs in that region went eradicated. By 2012 two new packs formed in the Web Valley, due to migration of wolves from Morebawa region. Like in the 2005-2006 outbreak, 2010 epizootic mortality rate was higher in subadults animals, also because some of the adults had partial immunity gained from previous exposure to the disease. By the year 2015, growth in number of Ethiopian wolves resulted in population densities similar to those previous to the decline caused by the 2010 CDV epizootic (Gordon et al., 2015).

Many carnivore species in Tanzania, in particular in the Serengeti National Park ecosystem, are susceptible to CDV infection, as it has been reported from 1994 disease outbreak which affected lions (*Panthera leo*), and previous outbreaks which affected spotted hyenas (*Crocuta crocuta*) and bat-eared foxes (*Otocyon megalotis*). Among these susceptible species, there are the endangered African wild dogs (*Lycaon pictus*), whose population decline over the years has been attributed to various factors combined, such as habitat loss, human persecution, prey availability and infectious diseases (mainly rabies and CD). In 1995 at Mkomazi Game Reserve, a captive breeding program for the African wild dog was established, and the founder members were vaccinated against CD. At the end of 2000 a disease quickly spread among the dog packs, and serological and histopathological analyses identified CDV as the aetiological agent, and by February 2001, 49 of the 52 animals died, showing that the vaccine used wasn't effective (van de Bildt et al., 2002). In

2007, fatal CDV infection in six members of a pack of African wild dogs occurred near the Serengeti National Park. Molecular tests demonstrated that concurrent infections in the animals contributed to mortality of the CDV infection, and phylogenetic analysis of a sampled P gene fragment revealed high homology among this CDV variant and the variants from previously reported epizootics (the CDV outbreak in the Serengeti in 1994 and in Mkomazi Game Reserve in 2000) (Goller et al., 2010).

All genera belonging to the Vulpini tribe (i.e., *Vulpes*, *Urocyon* and *Otocyon*) are susceptible to CDV infections and can develop clinical signs. As previously mentioned, in the Serengeti-Mara ecosystem bat-eared foxes (*Otocyon megalotis*) have been affected by CDV, and the virus strain was used for comparison with the CDV-strain that caused the 1994-outbreak in lions for phylogenetic analyses, and they resulted to belong to the same cluster (Alexander et al., 1995; Roelke-Parker et al., 1996).

CDV infections in red foxes (*Vulpes Vulpes*) have been reported in many European countries, such as Germany, Switzerland, Spain, Portugal, and Italy. In particular, there have been recent outbreaks of a CDV variant, belonging to the Europe-1 and Europe wildlife lineages, among the Alpine carnivore populations (red foxes, badgers and beech martens), which caused mortality in northern Italy regions such as Lombardia, Veneto, Trentino Alto Adige and Friuli-Venezia Giulia, in the years 2009-2010 and later from 2018 to 2020 (Martella et al., 2010; Trogu et al., 2021).

In the USA, in California, in 1999 there was a strong decline in island foxes (*Urocyon littoralis catalinae*) population of the Santa Catalina Island (SCI), a relative of the mainland grey fox (*Urocyon cinereoargenteus*). The decline was detected by demographic surveys conducted in the years 1999 and 2000, which compared to population estimations from 1991 showed a decrease in number of individuals by 85%, and serological analyses of recovered carcasses showed either sign of previous CDV exposure (i.e., detection of high titers of CDV antibodies) or co-infection of CDV with other secondary diseases, such as Toxoplasmosis. Sequence analyses of the P gene from a lung sample revealed high similarity with a strain of CDV isolated from a mainland USA raccoon (*Procyon lotor*). Because of the confined territory of island foxes leads to increased availability of infectious disease to spread among individuals, close monitoring plans, captive breeding programs and vaccination plans for dogs inhabiting the Catalina Island resulted in recovery of the Santa Catalina Island fox populations (Timm et al., 2009).

3.2 Felids

In the Felids family, mainly wild species animals have been seen develop fatal canine distemper disease. There aren't reports of naturally occurring systemic CDV infections in domestic cats (*Felis silvestris catus*), although CDV antibodies have been detected in individuals of this species, e.g., during a virological survey of wild cats (*Felis silvestris*) in Portugal (Duarte et al., 2012). Experimental studies conducted on domestic cats consisting of infection with highly virulent CDV strains, resulted in either asymptomatic infections or transient leukopenia. On the contrary, species of wild felids are more susceptible to CDV infection, and over the last decades there have been some reports of CD in animals belonging to the Pantherinae subfamily, such as the Serengeti ecosystem lions (*Panthera leo*) whose population was drastically reduced by one-third after the 1994-epidemic, due to virus spreading from South African domestic dogs. Histopathological tests, performed after some lions were noted to be disoriented, ataxic and profoundly depressed, showed presence of multinucleated syncytia, intranuclear and intracytoplasmic viral inclusions characteristic of CDV infection, and some monitored lions, showed CNS signs such as recurrent twitching. Death was mainly caused by non-suppurative encephalitis and pneumonia. In 2001 a second high mortality CDV-epidemic affected the lion population located in the nearby Ngorongoro Crater. Serological analyses in the different populations, indicated that CDV was not necessarily fatal for these large felids, in fact investigated lions had titers of CDV antibodies demonstrating at least five "silent" CDV epidemics occurred in the two populations between 1976 and 2006, without clinical signs or measurable mortality. The higher mortality rates of 1994 and 2001 epidemics have been attributed to concurrent adverse climatic conditions, such as drought events, prey die-offs, and unusual higher number of parasitic infections (Roelke-Parker et al., 1996; Goller et al., 2010). Even in wild Siberian (or Amur) tigers' populations (*Panthera tigris altaica*) CDV represents a threat. The tiger species are listed as "endangered" by the IUCN Red List, and for the Siberian tiger subspecies, with less than 500 individuals, located mainly in Far East Russia and northern China, a highly contagious disease such as canine distemper could extremely reduce these animals' chance for survival. Emergence of this disease among tiger populations occurred at the beginning of the 21st Century, with first reports of abnormal behaviour of Siberian tigers in 2001, and later, reports of neurological signs in some individuals in 2004 and unexpected population decline in the years 2009 and 2010. Histology, immunohistochemistry, and RT-PCR were performed on tissues from affected tigers that died in these three periods, and results indicated CDV as cause of death. Furthermore, phylogenetic analyses with H and P genes obtained from brain tissues,

revealed homology between the Siberian tiger CDV and the Artic-like strains isolated in Russian Baikal seals (*Phoca sibirica*) and domestic dogs (Seimon et al., 2013). CDV infections similar to those of wildlife populations occur even in captive large felids, including tigers, lions, leopards (*Panthera pardus*) and jaguars (*Panther onca*), e.g., in 2015 a Siberian tiger died for CD in a zoo of the Guangdong province (China). Affected animals exhibited respiratory and gastrointestinal signs, followed by neurological signs. Some studies demonstrated that possible sources of CDV in zoo outbreaks were small carnivores, such as wild Asian raccoon dogs (Zhang et al., 2017).

Members of the genus Lynx are also susceptible to CDV, but usually infections are restricted to one or few individuals, because animals belonging to the Lynx species lead a solitary lifestyle, thus social interactions are minimal, which reduces chances of rapid spreading of the disease. First cases of lynxes affected from CDV in North America, come from reports registered between the years 1993 and 1999, indicating encephalitis in six Canadian lynxes (*Lynx canadensis*) and two bobcats (*Lynx rufus*), and by RT-PCR and nucleotide sequencing CDV was identified as the causative agent (Daoust et al., 2009). In 2005 a carcass of Iberian lynx (*Lynx pardinus*), an endangered species endemic to Spain, resulted positive to CDV, and a serological survey showed presence of CDV antibodies in 15% of the 88 lynxes tested, even though there weren't any previous report of CDV cases. Phylogenetic analyses demonstrated similarity with European dog lineage of CDV, thus infection might have occurred through contact with a sick dog. Nevertheless, there aren't any more recent reports of CDV-induced deaths in the Iberian lynx species, and although its population numbers have increased significantly, from 94 individuals in 2002 to 1100 individuals in 2022, it is still listed as "endangered" according to the IUCN (Meli et al., 2010; Nájera et al., 2021). In Switzerland, in the years 2009 and 2010 a CD outbreak spread rapidly in wild carnivore species, and among the CDV-positive carcasses, such as red foxes, Eurasian badgers and beech martens, there was also one Eurasian lynx (*Lynx lynx*) (Origgi et al., 2012).

CDV presence have been confirmed by serological surveys in Namibian free-ranging and captive cheetahs (*Acinonyx jubatus*), Namibian caracals (*Caracal caracal*), Argentinian Geoffroy's cats (Leopardus *geoffroyi*), Brazilian pumas (Puma concolor), and Californian mountain lions (*Puma concolor*) (Beineke et al., 2015).

3.3 Ursids

The high pathogenic potential of CDV poses a threat to the conservation of rather small populations and endangered species. An example of this type of threat is the infection of giant pandas (*Ailuropoda melanoleuca*) in China, animals considered "vulnerable" in the IUCN Red List. In 1997 there was the first report stating that three giant pandas at the Chongqing Zoo were infected by CDV, as confirmed by recovery and sequencing of the H gene in the infected animals. In the following years serological tests performed on unvaccinated giant pandas kept at the Wolong Nature Reserve showed presence of detectable CDV-antibody titers. In 2014 six giant pandas kept at the Shanxi Rare Wild Animal Rescue and Research Center in China and housed together, tested positive to CDV by RT-PCR performed on nasal swabs, urine, faeces and blood, tests carried out after five out of six of the animals showed typical clinical signs of distemper such as foot pads hyperkeratosis, nasal and ocular discharge, and involuntary twitching. These five animals died within one month, while the surviving individual had been previously vaccinated against CDV in 2012 and had high titers of CDV-antibodies, which emphasizes the importance of vaccination as preventive tool against CDV infection (Feng et al., 2016).

In America, first report of CD infection in a wild black bear (*Ursus americanus*) was registered in 2011, when a bear cub was discovered in Pennsylvania having frequent seizures and hyperkeratosis of footpads, and post-mortem examinations demonstrated presence of non-suppurative encephalitis with eosinophilic intranuclear and intracytoplasmic inclusion bodies in neurons. Partial H gene sequence had high similarity to the Rockborn vaccine strain, which could indicate that transmission occurred from vaccinated domestic animals, but there weren't any samples of domestic dogs or wild raccoons available from that region, thus source of infection of the Pennsylvanian black bear is uncertain (Cottrell et al., 2013).

In 2021 in Italy, four Marsican brown bears (*Ursus arctos marsicanus*) in the Abruzzo, Lazio, and Molise National Park (ALMNP) were monitored and clinically examined to identify main viral pathogens causing infectious diseases, and all the animals resulted negative expect for one bear, which tested positive for CDV. The virus strain detected was similar to the one isolated in symptomatic red foxes and dogs analysed in the same period of the study, and belonged to the Europe wildlife lineage, thus different from the CDV strain belonging to the Artic lineage responsible of the 2013 epizootic in both wild and domestic carnivores (Di Sabatino et al., 2014). The CDV infected brown bear didn't exhibit any symptom, although this might be due to early stage

of infection at time of sampling (virus neutralization assay didn't show any detectable antibody levels). Nevertheless, further monitoring programs are necessary to ensure this Eurasian bear subspecies survival, with an estimated population of only 50 animals (Di Francesco et al., 2022).

3.4 Ailurids

Red pandas are another species susceptible to CDV infection. They live mainly in the forests of the Himalayas and Southern China, and they are classified in the IUCN Red List as "endangered", with fewer than 10 000 individuals in the wild. In 2015 in a zoo in the Guangdong province, in China, there was an outbreak of CDV in captive Siberian tigers. To control the spread of the disease, a live attenuated combination CDV vaccine was used for the other carnivores kept in the zoo, except for red pandas, in which another recombinant combination CDV vaccine was used. A few months later re-emergence of CDV caused deaths among red pandas, and the stray cats who wandered in the zoo were suspected of being the intermediate hosts. Although the use of recombinant CDV vaccine for this species is safe, its effectiveness has to be further investigated (Zhang et al., 2017). Moreover, even though live attenuated vaccines have proved effective in other carnivore species, safety in red pandas must be investigated, as previous reports have shown the ability of live vaccines to induce canine distemper in this species (Bush et al., 1976).

3.5 Procyonids

Animals belonging to the Procyonidae family are generally omnivorous and inhabit a wide range of environments. While most of the Procyonid species, such as kinkajous (*Potos flavus*) and olingos (*Bassaricyon gabbii*), are found in Central and South American regions, the common raccoon (*Procyon lotor*) is native to North America, but can be found also in Central Europe, because they were introduced in Germany in the 1920s for fur trading purposes and were later released in the wild, and in Japan, where in the 1970s they were imported so people could adopt them as pets. Among Procyonids, predominantly raccoons are susceptible to CDV infection, and CD regularly occurs in wild North American populations, with high risk of transmission to sympatric carnivores, such as American grey wolves and red foxes. CDV-induced clinical signs and pathologic lesions in raccoons are similar to those seen in domestic dogs, with diarrhoea in addition to upper respiratory symptoms and hyperkeratosis of foot pads (Ramsay, 2015). Due to the wide habitat range and

tendency to circulate even in urban areas, wild raccoons may act as reservoirs and intermediate hosts for CDV not only to wild carnivore species, but also to domestic dogs (Stope, 2019). In Japan, during the 2007-2008 CDV epizootic that affected wild animals near the city of Tanabe in Wakayama prefecture, raccoons contributed to intensify disease transmission, as demonstrated by serological studies conducted before and after the epidemic (Suzuki et al., 2015).

3.6 Mustelids

Cases of naturally occurring CD diseases among the weasel-like animals have been reported in Eurasian badgers (*Meles meles*), beech martens (*Martes foina*), domestic ferrets (*Mustela furo*), black-footed ferrets (*Mustela nigripes*), American minks (*Neogale vison*), Eurasian otters (*Lutra lutra*), and European polecats (*Mustela putorius*), thus it can be assumed that all members of the Mustelid family are susceptible. Clinical signs exhibited in mustelids indicating CDV infection are high fever, hyperkeratinization of nose and footpads, abnormal behaviour (such as lack of fear and salivation), and CNS signs, including depression, uncoordinated movements and muscle tremors or convulsion. Post-mortem examinations usually demonstrate lymphopenia, interstitial pneumonia, enteritis, and encephalitis (van Moll et al., 1995). Common cause of death in mustelids is co-infection by secondary parasitic or bacterial diseases.

In Denmark, in 2002, eight Eurasian badgers were found with typical CDV symptoms (with exception of hyperkeratosis of footpads or nose) and after death, either natural or via euthanasia, virological, bacteriological, and parasitological analyses demonstrated presence of bacteria and parasites other than CDV evidence (Hammer et al., 2004). Danish carnivore populations may cross-infect each other, as indicated by CDV reports of affected red foxes, ferrets, and minks in same geographical areas and in the same periods. American minks have been imported to Danish fur farms in the early 1930s, and since then free-ranging populations have formed (founders are the animals escaped from these farms). In 2011 three Danish farm minks died because of CDV, and then in 2012 a large distemper outbreak occurred, causing death in 64 minks. At the same time a high number of infected and dead foxes and ferrets were observed, raising the hypothesis of horizontal infection spread among these populations. Phylogenetic analyses of samples derived from animals belonging to the three affected species showed that the CDV strain belonged to the Europe-1 cluster. Moreover, identification of CDV presence in fleas collected from a

mink carcass has led to speculations about investigating about a possible vector-mediated transmission of viruses between mink and other species (Trebbien et al., 2014).

In Switzerland, the 2009-2010 CD outbreak that spread rapidly among wild carnivore species, included among the infected carcasses some Eurasian badgers and beech martens (Origgi et al., 2012). In 2015, in the Abruzzo region in Italy, where the 2013 CDV-outbreak caused death in shepherd and domestic dogs, and Apennine wolves, two Eurasian badgers affected by CD were found, one already dead, the other showing mild neurological signs. After four days the surviving badger died and necropsy conducted on the two animals demonstrated that the virus strain belonged to the Artic-like lineage, which was the same of the previous outbreak, suggesting persistence of the CDV strain among Central Italy wildlife (Di Sabatino et al., 2016). In the Emilia-Romagna region, in 2018, a CD outbreak spread among some beech martens kept at the Monte Adone wildlife rescue centre, in Bologna province. During a 15 months monitoring of the subjects, two out of the six rescued animals died, and among the remaining four only one showed clinical signs typical of CD disease. Phylogenetic and serological analyses revealed similarity of the CDV strain with the Europe wildlife lineage, and CDV-antibodies were detected in the four surviving martens, implying formation of specific immunity in case of future infection (Balboni et al., 2021).

CD susceptibility has also been detected in striped skunks (*Mephitis mephitis*), which belong to the mustelid related Mephitidae family, but in these wild animals usually a coinfection of CDV and rabies can be observed (Beineke et al., 2015).

3.7 Hyaenids

Serological analyses conducted on spotted hyenas (*Crocuta crocuta*) in the Masai Mara National Reserve (MMNR), in Kenya, after the 1990 CD epizootic that affected dogs of nearby areas, demonstrated presence of CDV antibodies in the hyaenid animals, indicating that subclinical infections occurred without fatal consequences. Later, in 1994, in the adjacent Tanzanian Serengeti ecosystem, a CDV strain homologous to the one responsible of CD-outbreak in lion populations, infected a group of spotted hyaenas, who exhibited CD clinical signs (Alexander et al., 1995; Haas et al., 1996).

3.8 Non-carnivore species

Distemper cases have been reported in seal species, and causative agent has been identified in either CDV or the closely related phocine distemper virus (PDV). PDV is phylogenetically more similar to CDV, rather than to the other cetacean morbilliviruses (as indicated in the Figure 1), and PDV-infected animals exhibit clinical signs similar to those observed in CDV-infections, such as ocular or nasal discharge, weight loss, hyperthermia, interstitial pneumonia, enteritis, and with disease progression even CNS signs, such as encephalitis, tremors and behavioural changes.

First epizootic outbreak of aquatic mammals in Europe occurred in 1988, when deaths caused by morbillivirus infections in pinniped and cetacean species, lead to identification of three new morbilliviruses: phocine distemper virus (PDV), dolphin morbillivirus (DMV), and porpoise morbillivirus (PMV). During the same period, CDV strains belonging to the Artic-like lineage caused epidemics among Lake Baikal seals (*Pusa sibirica*), in the years 1987-1988 in Siberia, and similar strains were identified in later years, indicating that CDV continued to circulate among Baikal seals after 1988 (Butina et al., 2010). CDV was isolated also in Caspian seals (*Pusa caspica*), during CD-outbreaks in 1997, 2000 and 2001, which caused drastic declines in the pinniped populations of the Kazakhstan, Azerbaijan, and Turkmenistan coasts. In all the previously mentioned CD-outbreaks involving seal species, source of infection has been identified in domestic dogs, suggesting virus spillover in phocid species from land carnivores (Kennedy et al., 2000; Beineke et al., 2015).

In 1989, first case of natural CDV infection in a non-human primate was observed in a Japanese macaque (*Macaca fuscata*) that died from an encephalitis, with presence of typical CDV-induced brain lesions assessed via post-mortem investigation. The 22 macaques in the same group as the diseased one, had relatively high titers of CDV-neutralizing antibodies, indicating viral spread of the disease among the animals, without fatal consequences (Yoshikawa et al., 1989). In 2006, other cases of large CDV outbreaks in non-human primates occurred, with reports of about 10000 infected rhesus macaques (*Macaca mulatta*) at a breeding farm in Guangxi, China. The monkeys displayed measles-like signs, which are very similar to those observed in CDV infected animals. Through RT-PCR, measle virus infection was excluded, and it was confirmed that the 4250 deceased macaques, died because of CD (Qiu et al., 2011).

4 Badger rescued case

Since 2018, there has been an increase in CDV case reports in carnivore wildlife species of the northern regions of Italy, especially of red foxes and badgers. In late July 2022, a male specimen of badger (*Meles meles*) with CDV associated symptoms was observed in Cordignano, in the Veneto region, and was brought to the wildlife rescue centre (CRAS) in Treviso. The animal was found in a residential garden, and displayed clinical signs such as copious nasal discharge, reduced fear of humans, uncoordinated movements, and muscle twitching. After being captured and anesthetized (Figure 5), the animal was examined by a veterinarian, and general physical examination resulted in presence of multiple ticks all over the animal's skin, mild hyperthermia, and respiratory tachypnoea (i.e., increased respiratory frequency).

Figure 5. Photo of the anesthetised badger during physical examination in a veterinary clinic.

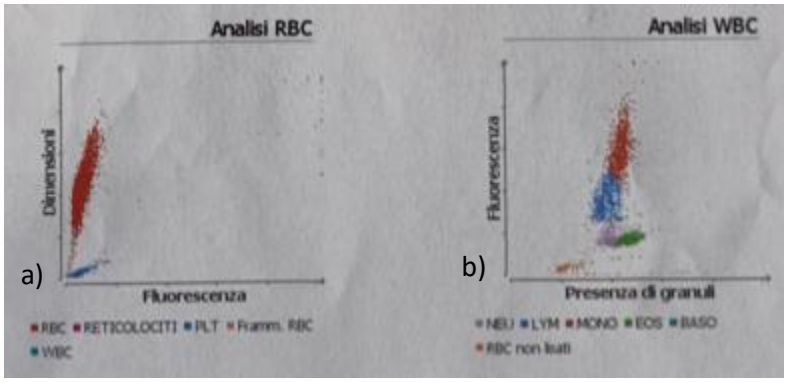
A complete blood count (CBC) test was conducted on a blood sample, and it revealed normal lymphocyte count (even though lymphopenia is one of the typical symptoms observed in other wild animals CDV infection). On the other hand, neutrophil and platelet counts were slightly below average, according to normal values for the badger species (Figure 6 and Figure 7.b). Nevertheless, neutropenia and thrombocytopenia can occur after initial lymphopenia, in more chronic CDV cases, which is compatible with the presence of CNS signs in the investigated badger. An infectious disease was suspected as the causative agent, but because no other tests were performed, the prognosis was reserved.

Test	Risultati	Range di riferimento
ProCyte Dx (27 luglio 2022 16.55)		
RBC	6,72 M/µL	6.93 – 12.88
HCT	27,1 %	30.09 – 53.23
HGB	10,0 g/dL	10.73 – 19.12
MCV	40,3 fL	38.05 – 49.71
MCH	14,9 pg	
MCHC	36,9 g/dL	
RDW	27,6 %	
%RETIC	0,0 %	
RETIC	1,3 K/µL	
Leucociti	3,84 K/µL	2.56 – 10.36
%NEU	* 23,2 %	
%LYM	20,6 %	
%MONO	33,3 %	
%EOS	* 21,1 %	
%BASO	1,8 %	
NEU	* 0,89 K/µL	1.07 – 8.27
LYM	0,79 K/µL	
MONO	1,28 K/µL	
EOS	* 0,81 K/µL	
BASO	0,07 K/µL	
PLT	177 K/µL	279.36 – 817.45
MPV	9,2 fL	
PDW	7,3 fL	
PCT	0,16 %	

* Confermare con dot plot e / o striscio ematico.

Figure 5. (left) Results of complete blood count (CBC) with relative badger-specific values.

Figure 6. (below) Dot plot graphs of a) red blood cells (RBC) and b) white blood cells.



Treatment therapy included daily administration of sodium lactate solution, five-days administration of an antibiotic drug, one dose of an anthelmintic drug, and one dose of an anti-inflammatory drug. The badger was able to eat and drink from the bowls offered by the staff of the rescue centre, and initial recover was observed, with reduction in intensity of respiratory problems but still presence of neurological signs. After less than one week the health status of the animal degenerated, and euthanasia was conducted. There isn't any available report of post-mortem investigation.

The case of this sick badger inspired me to research information about the suspected disease affecting him, in order to give a diagnosis comparing clinical signs in the patient and disease-associated symptoms, and lead me to write this paper, which reviews literature about canine distemper virus and its spread among different wild animal species.

5 Conclusion

Canine distemper virus is the most versatile among all morbilliviruses, and although dogs were considered the main host, CDV susceptibility has been detected in many carnivore species worldwide and, recently, even in non-carnivore species, such as phocids and non-human primates. Due to this virus's wide range of hosts, and its high infectivity and mortality rates, distemper disease outbreaks pose a threat for sympatric CDV-susceptible animal species (both wild and domestic) and reduce chances of survival of small endangered species populations. Prevention of CDV infections is conducted via mass vaccination either with an attenuated live virus or a

recombinant vaccine (method also used to avoid spreading of already ongoing infections among animals of same groups) but further investigation has to be performed on the safety of use of live attenuated vaccines, especially in wild animal species.

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