Advances in canine distemper virus pathogenesis research: a wildlife perspective

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Abstract
Canine distemper virus (CDV) has emerged as a significant disease of wildlife, which is highly contagious and readily transmitted between susceptible hosts. Initially described as an infectious disease of domestic dogs, it is now recognized as a global multi-host pathogen, infecting and causing mass mortalities in a wide range of carnivore species. The last decade has seen the effect of numerous CDV outbreaks in various wildlife populations. Prevention of CDV requires a clear understanding of the potential hosts in danger of infection as well as the dynamic pathways CDV uses to gain entry to its host cells and its ability to initiate viral shedding and disease transmission. We review recent research conducted on CDV infections in wildlife, including the latest findings on the causes of host specificity and cellular receptors involved in distemper pathogenesis.

INTRODUCTION
Accurate identification and understanding of the impact of infectious diseases on the morbidity and mortality of wildlife populations is vital not only as a cautionary measure in the treatment of diseases but also for surveillance and risk assessments of disease outbreaks. Sufficient epidemiological information is rarely available to determine the level of threat a disease poses to the viability of many wildlife populations [1, 2], with rapid identification of disease agents often not being an available option. In many cases, treatment relies on tentative and inaccurate diagnoses [3–5]. This becomes even more important when considering the conservation of endangered species.

Despite the fact that viruses have been associated with several major declines in carnivore populations [6, 7], detailed or long-term investigations of virus–carnivore interactions in wildlife are limited [8, 9]. One such virus infecting carnivores is the canine distemper virus (CDV). This highly contagious pathogen is the cause of canine distemper (CD), a severe systemic disease affecting carnivores worldwide. Initially diagnosed as a life-threatening disease in domestic dogs (Canis familiaris), it has subsequently been recognized in a wide range of hosts including some non-human primates, posing a conservation risk to several free-ranging and captive non-domestic carnivores [10, 11]. The ability of CDV to switch hosts has raised concerns about the extinction threat it poses to several endangered wildlife species [12–14].

The aim of this review is to compile literature from the past decade (since the last comprehensive review in 2001) on CDV infections in wildlife, including the latest findings on the causes of host specificity and cellular receptors involved in distemper viral pathogenesis.

VIRAL PROPERTIES
CDV is a large (100–250 nm) ssRNA virus (Fig. 1a) belonging to the genus Morbillivirus of the family Paramyxoviridae. Examples of diseases caused by members of Morbillivirus are measles in primates, rinderpest in artiodactyls, peste des petits ruminants in small ruminants and phocine and porpoise distemper in marine mammals [15–18]. CDV has a lipoprotein envelope, containing a 15 690-nt-long, non-segmented negative-stranded RNA genome (Fig. 1b) consisting of six genes that code for a single envelope-associated protein [matrix (M)], two glycoproteins [the haemagglutinin (H) and fusion (F) proteins], two transcriptase-associated proteins [phosphoprotein (P) and large protein (L)] and the nucleocapsid (N) protein, which encapsulates the viral RNA [19–21]. The organization of the major gene codes in the CDV genome is 3’-N-P-M-F-H-L-5’, each separated by UTRs [22–25]. Flanking the
six genes are two control regions essential for transcription and replication known as the leader, a 3′ extracistronic region of approximately 52 nt, and the trailer, a 5′ extracistronic region of approximately 38 nt [24–26].

Only one serotype of CDV is recognized with several co-circulating genotypes based on variation in the H-protein [27, 28]. Sequence analyses indicate that the H-protein undergoes genetic drift related to geographical regions, clustering into America 1 (including almost all commercially available vaccine strains), America 2, Asia 1 and 2, Europe/South America 1, Europe wildlife, South America 2 and 3, Arctic, Rockborn-like, Africa and Africa 2 [28–30]. Genotypes are defined on the basis of strains falling within the same clade sharing >95% amino acid similarity in their H-protein [31]. Infection of CDV may be prevented by an adequate host immune response against the H-protein [21], making the H-protein a suitable target for investigating polymorphism of CDV isolates and for molecular epidemiological studies [28, 31–34].

EPIDEMIOLOGY

Host range and prevalence

Although CDV was initially described as an infectious disease of domestic dogs, it has increasingly become known as a worldwide multi-host pathogen, infecting a wide range of carnivores [10]. Its ability to infect multiple species has led to mass mortalities in a range of carnivore species from wild canids to felids, hyaenids, procyonids, ailurids, ursids, mustelids and viverrids. Distemper outbreaks have also been reported in marine mammals, including Baikal and Caspian seals [35, 36], with the viral strains likely originating from terrestrial carnivores [37]. More recently, CDV was reported in non-human primates [rhesus monkey (Macaca mulatta) and cynomolgus macaques (Macaca fascicularis)] with high mortality rates [38, 39]. Infections in these primates have raised several concerns of a potential zoonotic risk of CDV in humans. There are, however, no known reports of CDV infecting humans. Speculations regarding the potential adaptation of CDV to infect humans are
outside the scope of this review and readers are referred to a review by Cosby [40].

Reports of CDV outbreaks in large felids such as lions (Panthera leo), leopards (Panthera pardus) and tigers (Panthera tigris) have challenged the belief that the Felidae group of animals is resistant to CDV infection [41–45]. When experimentally exposed to a highly virulent strain of CDV [46] or inoculated with homogenized tissues from a dead leopard infected with CDV [43], domestic cats (Felis catus) were seropositive with no signs of clinical disease or viral shedding [47]. Recent studies on the seroprevalence of captive and free-ranging cheetah (Acinonyx jubatus) from Namibia to several viral pathogens have shown that cheetah are able to be infected by CDV (seropositive) but, similar to domestic cats, do not show clinical signs [48, 49].

The last decade has seen numerous CDV outbreaks in various wildlife species worldwide. Outbreaks were confirmed in critically endangered species such as the Ethiopian wolf (Canis simensis) and Amur tiger (Panthera tigris altaica) [45, 50]. Concern for the conservation efforts of the giant panda (Ailuropoda melanoleuca) in China has also been raised due to several recent reports of CDV-induced mortality in captive populations [51, 52]. These outbreaks have highlighted the lack of knowledge on the extent of CDV susceptibility in wildlife species. This is even more evident for African wildlife with most studies originating from Tanzania, Kenya and Botswana. The CDV epidemic of 1994 that spread through the Serengeti National Park, Tanzania, is probably the best known of all, killing one-third of the lion (P. leo) population and causing deaths in several other species such as bat-eared fox (Otocyon megalotis), African wild dog (Lycaon pictus), silver-backed jackal (Canis mesomelas) and spotted hyena (Crocuta crocuta) [44]. More recently, CDV outbreaks occurred in several reserves within South Africa. CD in a lion population on a privately owned nature reserve in the Waterberg in December 2015 resulted in 95% mortality. This outbreak also infected other carnivore species, resulting in the first reported case of CDV mortality in an endangered brown hyena (Hyaena brunnea). Four months later, the devastating effect of CDV was also observed in African wild dog populations of Kruger National Park and Tswalu Kalahari Reserve, South Africa, with the total eradication of two packs (26 animals in total).

**Transmission and stability**

CD is highly contagious and is readily transmitted between susceptible hosts through contact or aerosolized oral, respiratory and ocular fluids and exudates containing the pathogen. During the acute phase of infection, other body excretions and secretions (e.g. urine, faeces, skin) can also contain the virus [53, 54]. Viral shedding may follow infection for up to 90 days and occurs even if the animal was subclinically infected [53, 55].

CDV is extremely sensitive to UV radiation, heat, desiccation, oxidizing agents, detergents and lipid solvents [56]. At room temperature, the virus is short lived, surviving between 20 min and 3 h in tissues and exudates. Although the virus is able to survive for several days at temperatures below zero if protected by organic material [57], transmission of CDV is largely dependent on the close association between affected and susceptible animals. To sustain an epidemic of CD, dense populations of susceptible individuals and the continued presence of a biological reservoir are required [54, 58]. Owing to their wide distribution, domestic dogs (C. familiaris) are key reservoirs for a variety of diseases and are considered the primary reservoir for CDV infection [58–62]. Domestic dogs, from communities surrounding protected wildlife areas, are often unvaccinated and occur in high densities with a rapid population turnover. These and wildlife come into contact as both may wander several kilometres in and out of the protected areas [63], increasing the risk of disease transmission, especially if these areas are unfenced. This risk of disease transmission between domestic dogs and wildlife is further augmented by a general lack of vaccination programmes, particularly in rural areas. Pathogen maintenance in the system is further increased through interspecies transmission of CDV in a wide variety of hosts [58]. Interactions among potential vectors of CDV, such as jackal, hyenas and lions, at kills provide a potential mechanism for subsequent cross-species transmission [60].

**Clinical signs**

Reported clinical signs due to CDV infection in wildlife species largely resemble those in domestic dogs. However, the severity and the outcome of the infection may vary greatly among species and depend on several factors, such as strain virulence, host age and host immune status. Initial signs of CDV infection are often subtle and rarely observed [54]. If an animal develops a strong immune response, no clinical illness ensues. An estimated 50–70% of CDV infections in domestic dogs are thought to be subclinical [57]. A weak immune response results in non-specific signs such as listlessness, appetite loss and fever. Despite a strong immune response that promotes recovery of the infected animal, CDV can persist for extended periods in the neurons, uvea, urothelium and skin (causing hyperkeratosis most dominantly seen in domestic dogs) [55, 64, 65]. CDV infection during early developmental stages, before the eruption of permanent dentition, can also infect tooth buds and ameloblasts causing clear enamel hypoplasia [66, 67] (Fig. 2).

Two clinical forms of CDV can be distinguished in animals with minimal or no immune response: an acute systemic form and a chronic nervous form [68, 69]. Acute systemic disease occurs 2–3 weeks post-infection [54]. The virus continues to replicate and spread throughout the body causing severe clinical signs, which include fever, mucopurulent ocular nasal discharge, coughing, dyspnoea, depression, anorexia, vomiting and diarrhoea (which may be bloody) [70, 71] (Fig. 3). During this stage of infection, the virus is found in every secretion and excretion of the body [69]. Neurological signs may be concurrent or follow systemic
disease within 2–3 weeks. Signs are progressive and varied depending on the area of the brain affected but commonly include abnormal behaviour, convulsions or seizures, chewing-gum movements of the mouth, blindness, cerebellar and vestibular signs, paresis or paralysis, incoordination and circling [54, 72]. Infection in the central nervous system results in acute demyelination, and most animals die 2–4 weeks after infection [71, 73]. Due to the immune compromising nature of CDV, clinical signs are often exacerbated by secondary bacterial infections of the skin and respiratory tract [53].

**PATHOGENESIS**

Prevention of CDV requires knowledge of the potential hosts susceptible to infection as well as the dynamic pathways CDV uses to gain entry to host cells and its ability to initiate viral shedding. In domestic dogs, CDV may infect a new host by the nasal or oral route, coming into contact with the upper respiratory tract epithelium [64]. There it multiplies in tissue macrophages, spreading, within 24 h, via the lymphatics to the tonsils and respiratory lymph nodes, resulting in severe immunosuppression [71, 74, 75]. Within 2 to 4 days, other lymphoid tissues become infected, and by day 6, the gastrointestinal mucosa, hepatic Kupffer cells and spleen are infected, resulting in a systemic reaction characterized by fever and leukopenia [54, 64]. Further spread of CDV occurs by cell-associated viraemia to other epithelial cells and the central nervous system [71, 73]. Viral shedding from various host excretions and secretions begins approximately 1 week after infection [55].

**Host range specificity**

Host range specificity of a virus is determined by various mechanisms including the means by which viruses gain entry to host cells via cellular receptors and the ability of the host to respond to these viral infections through their innate and/or adaptive immune response [76–79].

**Cellular receptors**

Two major host cellular receptors have been identified that play a critical role in CDV pathogenesis: the signalling lymphocyte activation molecule (SLAM, CD150) and nectin-4 (poliovirus-receptor-like-4) [80–82]. Both of these receptors possess an immunoglobulin-like variable domain (V) that provides a binding surface for morbilliviruses [80, 83]. SLAM serves as an immune cell receptor and is expressed on the surface of activated T and B lymphocytes, dendritic cells and macrophages [82, 84]. The second cellular receptor, nectin-4, has only recently been recognized as the epithelial cell receptor for CDV [80, 81, 85]. Nectin-4 is involved in the cell adhesion, participating in the organization of epithelial and endothelial junctions of host cells [86]. It is thought to be an exit receptor, functioning in the later stages of infection when the virus is amplified and released from epithelial cells [85].

**SLAM (CD150)**

Of the six structural proteins described for CDV, the H-protein has the greatest genetic variation and is a key protein in the attachment of the virion to receptors on the host cell surface [31]. The specificity with which CDV-H interacts with SLAM and its potential as a determinant of host range have been investigated [87–90]. Amino acid residues Y525, D526 and R529 of CDV-H have been
identified by site-directed mutagenesis to interact with SLAM [91, 92]. Two other residues at amino acid sites 530 and 549 have also been studied, and it is thought that these are important determinants of infectivity in carnivores. Both 530 and 549 fall into the receptor-binding domain located on propeller β-sheet 5 of CDV-H protein [88]. Previously suggested to be an adaptation of CDV to non-domestic dog hosts [88], residues at site 530 have subsequently been shown as generally conserved within CDV lineages regardless of host species [89]. Positive selection at site 549 of CDV-H and the specific substitution of tyrosine (Y) by histidine (H) is thought to have contributed to the spread of CDV from dog to non-dog host species [88]. The majority of CDV strains isolated from Canidae have Y at site 549, whereas CDV strains from other carnivore families mostly have H [93]. Studies on the impact of specific amino acid substitutions within the H protein are, however, speculative and several other factors could also have contributed to the spread of CDV. Conversely, when comparing the amino acid sequences of the entire H binding site in SLAM among various carnivores, a high similarity among residues from Canidae species was found, suggesting a similar sensitivity to CDV among animals in this group [89]. In contrast, comparing Felidae to Canidae, several residue differences were identified that ultimately led to electric charge differences in the SLAM interface of felids [90]. CDV strains that are well adapted to bind to dog SLAM receptors may thus be less adapted to bind to SLAM receptors from another non-canid host.

Nectin-4

The role of the epithelial receptor, nectin-4, in CDV pathogenesis in the domestic dog has only very recently been investigated. Six to nine days after infection with CDV, the virus enters the epithelial cells of the respiratory, gastro-intestinal, urinary and endocrine system via an epithelial receptor [94, 95] now known as nectin-4 [85, 96]. CDV amplification within the cells is promoted, after which the virus is released causing extensive respiratory, intestinal and dermatological symptoms [95, 97]. In a host with a weakened immune response, CDV will move into the central nervous system, producing neurological symptoms [98]. Nectin-4 has also been suggested to play a role in the neurovirulence of CDV [81]; however, other, thus far uncharacterized, receptors might also be involved [99]. Two protein variants of nectin-4 have been identified, acting equally well for both viral entry and cell-to-cell spread [85, 96, 100].

Fig. 3. African wild dog (Lycaon pictus) afflicted by CDV showing clinical signs of mucopurulent oculonasal discharge (a, b) and weight loss (c) (photos: A. K. Loots).
**DIAGNOSIS**

Ante-mortem diagnosis of CDV is preferred due to the disease’s high infectious potential, combined with a high mortality rate and fast progression. Initial diagnosis of CDV is mostly reliant on identifying the clinical signs associated with an infection. However, this form of diagnosis remains problematic and difficult due to the many varied clinical presentations of the disease. Differentiation from other diseases with respiratory, neurological and/or gastrointestinal signs, such as rabies, feline panleukopenia, coronavirus, toxoplasmosis, bacterial enteritides and parvovirus, should be conducted. Several serological and immunological diagnostic tests have been developed for the detection of CDV in domestic animals. Diagnosis of CDV infection in wildlife is more difficult due to the challenges associated with acquiring and cold storage of samples in the field for further testing in the laboratory. Diagnosis is mostly confirmed post mortem using histopathology and immunological tissue stains although the specificity and sensitivity for the latter are not known for most wildlife species.

**Molecular assays**

The advent of molecular techniques brings diagnostic tools that are excellent with regards to sensitivity and specificity [101, 102]. One of several techniques that have been developed for the detection of CDV is the reverse-transcription (RT) PCR assay [103–106], which has been widely used predominantly targeting the highly conserved N gene. While RT-PCR methods are more sensitive, specific and rapid compared to conventional culturing methods, they are still technically demanding and require several hours with additional post-PCR analyses [107]. Sensitivity also varies depending on the sample source, extraction method and choice of primers [105].

A more rapid diagnostic technique for the detection of CDV is real-time RT-PCR [107–109]. Real-time RT-PCR is used for both diagnostics and research and is especially useful for pathogen detection. Scagliarini et al. [108] developed a rapid and sensitive real-time RT-PCR assay based on TaqMan technology, which is able to detect and quantify CDV in clinical samples and cell cultures. This assay is based on a highly conserved region of the P gene and is highly sensitive both as one-step and two-step reactions, confirming its suitability for research and diagnostic purposes.

Additionally, nested PCR techniques have been developed for the detection of CDV. Both Shin et al. [110] and Alcalde et al. [111] used a nested PCR with the product of a one-step conventional RT-PCR to detect the virus. Fischer et al. [112] took it one step further by developing a technique of reverse transcription followed by a nested real-time PCR. The technique was performed on several clinical samples and proved to be two orders of magnitude more sensitive than RT-PCR.

**Serological assays**

Serological assays to detect and determine specific titres against CDV are the indirect fluorescent antibody test (IFAT), ELISA and the serum-neutralization test. Both the IFAT and ELISA are used to detect IgM and IgG antibodies against CDV in domestic dogs and various non-dog hosts. The presence of IgM not only confirms current acute distemper infection but is used to retrospectively diagnose distemper by detecting seroconversion in paired serum samples collected during the acute and recovering phase of the disease [113, 114]. However, there are not always suitable conjugated anti-species antibodies for wildlife species available for use with IFAT or ELISA. A systematic literature review of all possible non-dog hosts of CDV showed that ELISA was used 13.8 % of the time as serological test, followed by IFAT (7.7 %) [29]. The highly specific and sensitive serum-neutralization test is more commonly used (75.4 %) for the detection of CDV from serum samples and can be seen as the gold standard for detecting antibodies [29, 59, 115, 116]. Serology as a diagnostic test is, however, not reliable in distinguishing between naturally acquired CDV infection (wild-type CDV strain), infection with attenuated virus vaccine strain [as used in the modified live vaccine (MLV)] or immune response to recombinant, virus-vectored vaccine and should thus if possible be combined with other techniques, such as RT-PCR and viral antigen ELISA [104, 117–119].

**Virus isolation**

Virus isolation is typically conducted in pulmonary alveolar macrophages or by co-cultivation of infected tissues with mitogen-stimulated lymphocytes derived from healthy dogs [120] or with the aid of ferret blood lymphocytes [54, 121, 122]. These methods are demanding and time consuming, taking several days to weeks [104, 107, 118, 119]. In 2003, Vero cells expressing the canine SLAM, the principal receptor for morbilliviruses in vivo, were engineered [82]. These Vero. DogSLAM cells are highly sensitive for virus isolation, with cytopathic effects evident within 24 h of inoculation [84, 122].

**Pathological examination**

Routine post-mortem diagnosis of CDV is by pathological examination of the spleen, lymph nodes, stomach, lung, small intestine, liver, pancreas, urinary bladder, kidney with renal pelvis and brain. Diagnosis is made by demonstration of typical histopathological lesions including the presence of viral inclusion bodies in lymphoid tissue, respiratory, urinary and gastro-intestinal tract epithelium and brain; by the presence of distinctive virions in negatively stained electron-microscopic preparations of faeces; and through the detection of viral antigen in tissue by immunofluorescence or immunohistochemistry [54, 104]. Immunofluorescence has routinely been used as a diagnostic test; however, it is not sensitive and can detect CDV antigens only when the virus is still present in the epithelial cells [55, 107] with false-negative results under certain clinical conditions [112, 123].

**TREATMENT AND CONTROL**

The treatment and control of infectious viral diseases is often difficult, especially in wildlife populations. Treatment of CDV infection is commonly based on symptomatic and...
supportive therapy as there is no specific antiviral drug available for therapeutic use against CDV infection in any species, including domestic dogs. Studies on the in vitro effect of antiviral compounds in the treatment of CDV are ongoing and several future experiments are still required to determine their safety and efficacy in treating CDV in various species. Krumm et al. [124] evaluated an orally available, shelf-stable pan-mollivirus inhibitor that targets viral polymerase. They found that treatment of CDV-infected ferrets at the onset of viraemia with the inhibitor resulted in ferrets with low-grade viral loads, remaining asymptomatic and subsequently recovering from the infection. Other compounds such as fucoidan, a sulfated polysaccharide found in brown algae, have also been evaluated for their ability to act as antiviral drugs against CDV [125]. In vitro results showed that fucoidan was able to inhibit initial stages of the viral replication cycle, strongly suppressing the formation of syncytia in infected cells. Carvalho and colleagues [126] evaluated the antiviral activity of several flavonoids (quercetin, morin, rutin and hesperidin) and phenolic acids (cinnamic, trans-cinnamic and ferulic acids), concentrating on their in vitro ability to inhibit stages of the CDV replication cycle. All flavonoids and phenolic acids demonstrated antiviral action against CDV infection. Other methods of treating CDV infection that have been explored include mesenchymal stem cell therapy [127] and the use of a veterinary pharmaceutical preparation of silver nanoparticles [128].

An effective intervention strategy against CDV infection includes vaccination. In the 1960s, two MLVs against CDV were introduced. The first, the Onderstepoort vaccine, was developed from a natural isolate, passaged in ferrets (Mus tuta furo) and then adapted to chicken embryos (these were later replaced with chicken cell culture) [129]. The second MLV was generated by adaptation of the CDV Rockborn strain to canine kidney cells [130]. These modified live virus vaccines are sufficient for management of CDV in domestic dogs, but can on rare occasions cause post-vaccination encephalitis and lead to vaccine-induced illness [131]. The susceptibility of various species to vaccination with the MLV vaccine is largely unknown. Species differences in their response to vaccination have been observed, for example the avian cell adapted CDV vaccine can be fatal in European mink (Mustela lutreola) and ferrets [132, 133], but was shown to give protection to the maned wolf (Chrysocyon brachyurus), fennec fox (Vulpes zerda) and both red and grey foxes (Vulpes vulpes) [134, 135]. Concerns with differences in efficacy of MLV vaccines have led to the development of recombinant vaccines [136]. Canarypox-vectored vaccines, developed for use in domestic canines, are incapable of replicating in the host cell, but can elicit an appropriate host immune response [137–139]. The canarypox-vectored vaccine has proven to be effective in challenge studies in various wildlife species including European ferrets (M. putorius furo), giant panda (A. melanoileuca), fennec foxes (V. zerda), meerkats (Suricata suricatta) and Siberian polecats (Mustela eversmanni) [140–143]. A more recent study on vaccine efficiency in tigers (P. tigris) found that both the live attenuated and the recombinant canarypox-vectored vaccine appeared safe for use, although the live attenuated vaccine produced a significantly stronger and more consistent immune response in the tigers [144].

A general lack of quantitative data on the effect of CDV vaccine in wildlife has made it necessary to focus efforts on controlling CDV infection in the domestic dog reservoir surrounding conserved areas. While this approach benefits domestic dogs, vaccine coverage is rarely sufficient to reach the 95% target considered necessary to control CDV [145] and often fails to prevent infection in wildlife species that share their environment. Thus, the question whether endangered wildlife should specifically be targeted for vaccination is raised. Several challenges associated with wildlife vaccination need to be considered including (1) knowledge on the safety and efficacy of the vaccine in the specific species targeted; (2) mode of vaccine delivery either during opportunistic animal handling (when fitting tracking collars, translocation or medical examination), or by hypodermic dart (could cause injury and stress), or orally through laced bait (reduced efficacy if not eaten by target species); (3) the logistics of administering the required booster shots; and finally (4) the cost involved in initiating and implementing a vaccination programme in wildlife [13, 142, 146, 147].

CONCLUSION

CDV is an emerging pathogen posing a serious threat to the conservation of several captive and free-ranging wildlife populations. Its ability to infect multiple hosts considerably hampers disease eradication. Up to recently, CDV had only been studied in domestic dogs, with wildlife research greatly lacking. It is thus of great importance to study the factors influencing host susceptibility and CDV pathogenesis in all known and potential hosts of CDV. Further evaluation of the two known cellular receptors (SLAM and nectin-4) in various wildlife species will aid in determining host specificity of the virus.

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Conflicts of interest
The authors declare that there are no conflict of interest.

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