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Canine Reproductive, Respiratory, and Ocular Diseases due to Canine Herpesvirus

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KEYWORDS
- Canine Herpesvirus
- Reproductive
- Respiratory
- Ocular
- Diseases
- Detection

Although canine herpesvirus (CHV) (also referred to as canine herpesvirus 1, canid herpesvirus 1, neonatal herpes, genital herpes, ocular herpes, and CHV-1) infections and related diseases have been recognized since the early 1960s, there has been a resurgence of interest in the various clinical manifestations of the virus, which makes this review very timely. The various forms of CHV-associated infections are listed in Table 1. In some cases these infections were directly related to clinical symptoms, such as acute neonatal viremia resulting in puppy mortality; systemic viremia in naive pregnant females resulting in fetal death, abortion, and mummification; and ocular-respiratory disease in dogs of various age ranges.

What has changed within the past decade has been the ability to detect the virus in its subclinical state, which allows for a much clearer understanding of the importance of 2 subpopulations of dogs: carrier-shedder adult dogs, and CHV-latently infected dogs in the animal populations with which we work. The increased sensitivity of both antibody-based serology assays and nucleic acid-based polymerase chain reaction (PCR) assays have increased our level of clinical inquiry regarding CHV, as well as the other canine infectious microorganisms.

In addition to recognizing CHV adult carriers in the general population, this new momentum has allowed for clinicians to screen dogs that are undergoing...
immunosuppressive regimens of therapy for various dermatologic conditions, as well as dogs being treated for various cancers. This review will provide a brief overview of the reproductive aspects of CHV disease and will then bring together the current literature, documenting the involvement of CHV in adult dog respiratory and ocular diseases.

**CONTEMPORARY CLINICAL OBSERVATIONS**

**Reproductive Disorders**

Consistent with the other alpha herpesviruses, CHV has a predilection for pregnant dogs and neonatal puppies.\(^{23–26}\) Early reports focused on the effects of CHV on various reproductive parameters in the dog, in part due to the severity of the clinical symptoms and the profound pathologic effects. In the review by Anvik,\(^{12}\) acute neonatal viremia and systemic infection of naïve pregnant females were regarded as 2 of the most important disease outcomes of CHV infection. The emphasis at that time was on the recognition of clinical symptoms for a rapid diagnosis. Since there are no commercial vaccines currently available for the prevention of CHV-induced disease, it has become paramount to understand the clinical features of CHV infections (see **Table 1** and to incorporate this knowledge with sound management practices to minimize the effects on reproductive efficiency and puppy survival.\(^{27}\)

As was mentioned previously, this has been the primary focus of the earlier literature on CHV infections. Infection may occur during pregnancy or may be acquired by puppies during the first few weeks of life. The key feature during both of these phases is that the pregnant female and puppies are **immunologically naïve** to CHV and therefore highly susceptible to disease. Puppies may acquire the infection in utero, from passage through the birth canal, from contact with oronasal secretions of the dam, or contact shedders. Humans may serve as fomites of the virus if attending to an adult carrier-shedder dog, and then proceeding to a nursery setting without proper disinfection. Naïve neonatal puppies, younger than 1 week, are at highest risk of fatal systemic disease, while naïve dogs older than 3 weeks are

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**Table 1**

| Infection/Disease | Age Groups at Risk | Outcome/Comments |
|-------------------|--------------------|-----------------|
| 1. Mucosal form (respiratory/vaginal) | Older puppies (>3 weeks) and adults | Mild, often inapparent infection, active shedding, with establishment of latency |
| 2. Latent infection | Older puppies, adults, and survivors of neonatal and mucosal forms | Lifelong infection, may show recrudescence at pregnancy and stress |
| 3. Acute neonatal viremia | Puppies from birth to 3 weeks | Fatal systemic disease, active shedding, poor prognosis |
| 4. Systemic infection of naïve pregnant females | Breeding dams | Fetal death, abortion, mummification, source of virus for acute neonatal viremia |
| 5. Ocular form | Older puppies (> 3 wks) and adults | Mild conjunctivitis to severe ocular disease, with active shedding |

*Data from* Anvik JO. Clinical considerations of canine herpesvirus infection. Vet Med 1991;82:394–403.
relatively resistant to disease but can still become infected.\textsuperscript{27–29} Virus infection in naïve older dogs is generally acquired via aerosol, so that replication occurs in the nasopharynx tonsils and retropharyngeal and bronchial lymph nodes.\textsuperscript{2,30–32} This respiratory site will become an important aspect of the ecology of the virus when both respiratory and ocular clinical outcomes are covered in subsequent sections.

Although neonatal infections are regarded as the most common, in utero infection with CHV may occur. Infertility and abortion of stillborn or of weak pups has been reported. While the mortality rate usually approaches 100\% for the fetal puppy, there may be no further clinical manifestations reported in the dam.\textsuperscript{1,5,26}

Passive immunity acquired from the dam appears to be of primary biological importance in the survival of infected pups.\textsuperscript{12,27,33–35} Puppies that are nursing from CHV-seronegative dams usually develop the fatal multisystemic disease, while puppies that suckle from CHV-seropositive dams remain asymptomatic but still become infected. The CHV is usually recovered from the oropharyngeal region in these disease-resistant pups. It is generally accepted that maternal antibody and/or immune lymphocytes acquired through the milk explain why naturally infected dams that have a diseased litter will usually give birth to normal litters on subsequent pregnancies.

Since CHV is one of the few canine viral infections that can proceed to fatal disease and there is no commercial vaccine routinely available, it has become necessary for infection management to prevent reproductive disease. The literature has focused on 3 aspects of the virus and its relationship with host immunity and its carrier-spread dynamics within a population of susceptible dogs.

\textit{Infection management—understanding the risk factors}

The risk factors associated with CHV infection and reproductive disease has been intensively studied over the past 5 years.\textsuperscript{36,37} The studies have used various diagnostic assays including serology, virus isolation, and polymerase chain reaction (PCR). These studies have provided valuable information on controlling CHV-associated reproductive diseases (ie, infertility, abortion, stillbirths, and neonatal mortality). Table 2 lists the 12 risk factors that were studied and whether there was an association with reproductive disease. Of the 12 factors, 8 were identified as having a positive correlation with disease: breeding kennel, age, mating experience, cycle (stage), concurrent kennel cough, kennel size, breeding management, and hygiene.

The underlying risks in the aforementioned factors are CHV infection and an immune susceptible dog. This has led to strategies to naturally immunize (via contact with adult dogs) susceptible female dogs prebreeding, to screen female dogs for CHV infection (by serology and/or PCR) prior to breeding, and to use a defined quarantine period for pregnant dogs with an unknown CHV infection status. An age-risk, immunologically naïve-risk strategy has been used by clinicians and clients to focus on the most susceptible time periods for disease. This time encompasses the pregnant female during the last 3 weeks prior to whelping, and her puppies up to 3 weeks post whelping.\textsuperscript{12,27,33} This understanding has constituted the rationale for the “6-week danger period.”\textsuperscript{12,27}

The primary contributing risk factors that allow for CHV infection and disease are kennel size, hygiene, and kennel cough. All 3 of these are important in the spread and retention of CHV in high-risk dog populations. While the controversy over CHV being a significant contributor to the kennel cough syndrome has been an ongoing debate (see subsequent section on respiratory–ocular infections), it should be noted that CHV was initially reported as a respiratory pathogen as early as it was a reproductive pathogen.\textsuperscript{2} The data from Ronsse and coworkers\textsuperscript{22} support the contention that CHV...
Respiratory Disorders

The disease outcomes of CHV infections are age dependent. In naive puppies that are less than 1 month of age, natural and experimental infection with CHV may be highly fatal. Natural exposure of pups occurs by ingestion or inhalation of virus containing material. The primary replication sites are nasal mucosa, pharynx, and tonsil. Systemic spread of the virus is enhanced by a cell-associated viremia.\textsuperscript{29–32} The pathology induced by CHV in the lungs of newborn pups is depicted in Figs. 1 and 2.

Experimental infection of older dogs (3 months or older) with CHV has resulted in a mild rhinitis and pharyngitis. Symptoms of tracheobronchitis were produced following experimental inoculation with CHV isolated from naturally infected dogs.\textsuperscript{38,39} Experimental infection of 5- to 12-week-old pups induced mild rhinitis and pharyngitis and virus replication was demonstrated in the upper respiratory tract. Although CHV has been isolated from dogs with upper respiratory disease, reproduction of “kennel cough” has only been rarely reported. Thompson and coworkers\textsuperscript{32} reported that aerosol exposure of 12-week-old dogs caused a necrotizing rhinitis, bronchointerstitial pneumonia, and multifocal alveolar necrosis. More severe disease can occur when CHV infects dogs that are immunosuppressed.\textsuperscript{39} A case of generalized CHV infection in a 9-year-old dog with a normal immune system was documented recently (Gadsden BJ, Langohr IM, Maes R. Fatal herpesviral infection in an adult dog. Submitted for publication, 2011). The most severe lesions were seen in the liver. The histologic lesions observed in the lung of this dog are presented in Fig. 3.

Infection rates, based on serologic studies, are high enough to explain entry of CHV into multidog environments, either as an active infection or as the result of reactivation of latent virus in environments associated with natural, or pharmacologically induced immunosuppression. In Belgium the seroprevalence in adult dogs was found to be

| Risk Factors          | Risk Criteria                  | Disease Correlation |
|-----------------------|--------------------------------|---------------------|
| Breeding Kennel       | 77 kennels sampled             | Yes                 |
| Sex                   | Male (n = 137); female (n = 409) | No                  |
| Shows                 | Attended or not                | No                  |
| Breed                 | 41 different breeds            | No                  |
| Age                   | 14 different age ranges        | Yes                 |
| Mating experience     | Males mated or not             | Yes                 |
| Cycle (stages)        | Five different stages          | Yes                 |
| Number of litters     | Zero to >1                     | No                  |
| Kennel cough          | History of respiratory disease | Yes                 |
| Kennel size           | Ranged from <6 to >20 dogs     | Yes                 |
| Breeding management   | Use of nonresident males       | Yes                 |
| Hygiene–biosecurity   | Ranged from very good to insufficient | Yes              |

Data from Evermann JF. Canine herpesvirus infection: Update on risk factors and control measures. Vet Forum 2005;69:32–7; and Ronsse V, Verstegen J, Onclin K, et al. Risk factors and reproductive disorders associated with canine herpesvirus-1 (CHV-1). Theriogenology 2004;61:619–36.
45.8%.\textsuperscript{22} Rijsewijk and colleagues\textsuperscript{21} reported a seroprevalence of 39% in the Netherlands. Reading and Field,\textsuperscript{20} using an antibody detection ELISA, found a seroprevalence of 88% in the United Kingdom. In Japan, the seroprevalence was recently reported to be 21.7%.\textsuperscript{6} Since CHV is regarded as a weak immunogen, these antibody-based surveys are probably an underrepresentation of the true infection rate in the dog populations.\textsuperscript{33}

Canine infectious respiratory disease (CIRD) is most commonly seen in rescue centers, boarding kennels, and veterinary hospitals. Most of the affected dogs have a dry cough of limited duration. In complicated cases, bronchopneumonia is seen and can be fatal. Multiple infectious agents can play a role in the induction of CIRD. Canine parainfluenza virus and \textit{Bordetella bronchiseptica} are frequently involved. Canine distemper and canine adenovirus type 2 (CAV-2) have been associated with CIRD but are not routinely detected due in part to effective vaccines, and the population immunity is fairly high. Canine influenza, canine respiratory coronavirus, and, most recently, canine pneumovirus, are emerging components of CIRD, which have added to the complexity of this disease syndrome.\textsuperscript{18,19,40}

Although CHV infections have been documented in multidog environments, its etiologic role in CIRD is still being assessed. During a 2-year longitudinal study of viruses associated with CIRD at a rescue center in the United Kingdom, CHV was found in 12.8% of the tracheal samples examined and in 9.6% of the lung samples. Infections with CHV were seen 3 to 4 weeks after entry and were associated with more severe respiratory signs.\textsuperscript{18} The delay in detection of the virus by PCR was corroborated by the serologic data, which also indicated that CHV infections occurred at a later time point. A possible explanation offered for its detection in more severe cases was the possibility that latent CHV could have been reactivated as a result of the stress induced by a primary CIRD episode that was triggered by other viral or bacterial agents. The virus source was not determined. The authors speculated that genetically different CHV strains would have been detected if the source of virus was the result of reactivation of latent virus from different dogs. It has been reported, however, that CHV strains show very low sequence variability.\textsuperscript{41}
Erles and Brownlie monitored dogs in 2 training centers in the United Kingdom for 1 year. All dogs were vaccinated against CAV-2, CPV-2, and Leptospira interrogans. Tonsillar swabs and serum samples were collected at entry and every 3 months thereafter. Blood samples were collected at entry and every 4 weeks thereafter. Most CIRD cases were observed in autumn and winter. Most dogs were healthy at arrival and were in the kennel for at least 2 weeks before developing clinical signs. Seroconversion to CHV was detected throughout the year. The most logical explanation for the seroconversion pattern would be continuous introduction in the kennel by acutely infected dogs or reactivation of latent virus in the resident population. The authors concluded that while CHV contributed to the CIRD, it was not an obligate pathogen in that environment, since some asymptomatic dogs also seroconverted.
Kawakami and colleagues described an outbreak of infectious tracheobronchitis in Japan accompanied by death in adult dogs. The only pathogen identified during the outbreak was CHV. Molecular testing led to the conclusion that a single strain was involved, with virulence characteristics that were only slightly higher than those of previously tested CHV strains. As was the case in the study reported by Erles and colleagues, it was not clear whether the virus was introduced into the center in the form of acute infections or was the result of reactivation of latent infections in the resident population. Regardless, the authors emphasized that there was sufficient amounts of immunosuppression in shelter populations to allow for CHV to be a significant primary pathogen in that environment.

Ocular Disorders

Ocular manifestations of CHV infection may develop during both primary and recurrent infection and are dependent upon host age and immune status. In fetal and neonatal dogs with primary CHV infection, severe intraocular lesions are frequently present concurrent with systemic viral disease. Subclinical or mild recurrent ocular surface disease is typically observed in immunocompetent mature dogs. In immunosuppressed mature dogs, ocular lesions associated with CHV infected are often more severe, persist for a longer duration, and may be refractory to treatment.

Primary CHV infection in fetal and neonatal dogs

Primary CHV infection occurring after in utero or early neonatal CHV transmission (ie, first 2 to 3 weeks of life) is associated with a cell-associated viremia. Hematogeneous dissemination of virus results in CHV infection of intraocular tissues with severe clinical ocular manifestations. Ocular disease is typically bilateral and becomes evident within a short period after the development of systemic disease in many, but not all, dogs. Panuveitis, retinitis, and optic neuritis with extensive monocular and neutrophilic infiltrates, edema, hemorrhage, and necrosis are observed histopathologically within the iris, ciliary body, choroid, retina, and optic nerve. Intranuclear viral inclusions are frequently detected during the acute inflammatory phase in uveal and retinal tissues. As the palpebral fissures do not open until 10 to 14 days postpartum in dogs, ocular changes may not be externally visible in young animals. In dogs with open eyelids, most clinically detectable ocular lesions are sequelae to panuveitis and include keratitis, corneal edema, aqueous flare, anterior synechiae, cataracts, and chorioretinitis (Fig. 4). Reduced vision or blindness may result from various combinations of the ocular lesions.

Following the acute inflammatory stage of infection, developmentally mature tissues (eg, cornea, uvea) undergo varying degrees of necrosis, fibrosis, gliosis, and atrophy. The canine retina is incompletely developed at birth and responds by a combination of necrosis, disorganization, and reorganization. Retinal dysplasia, characterized by formation of retinal folds with rosette-like structures, and retinal degeneration are the final result. In dogs surviving neonatal CHV infection, blindness, cataracts, optic nerve atrophy, retinal degeneration, and retinal dysplasia are frequent residual sequelae.

Primary and recurrent ocular CHV infections in mature dogs

In contrast to fetal and neonatal dogs, ocular lesions associated with CHV infection in mature dogs are typically restricted to the ocular surface with a variety of corneal, conjunctival, and eyelid lesions. In immunocompetent dogs these lesions are frequently mild and self-limiting; however, they are a source of discomfort and their recurrent nature may be frustrating to clients. Nonspecific clinical signs associated
with CHV ocular infection in mature dogs include blepharospasm, photophobia, and ocular discharge. Blepharospasm and ocular pain are often disproportionately severe compared to that expected from the extent of ocular lesions. Ocular discharge is initially restricted to epiphora, but becomes mucoid, mucopurulent, or serosanguineous with progression of infection.\(^7,44\)

Primary and recurrent ocular CHV infection may be subclinical or associated with various combinations of blepharitis, conjunctivitis, keratitis, and corneal ulceration.\(^7,44–46\) In all published descriptions of naturally-acquired primary ocular CHV infection, clinical lesions were bilateral; however, the severity and specific manifestations of CHV infection were not always symmetrical between eyes of individual dogs. In most cases, primary ocular CHV infection resolves spontaneously and without permanent ocular lesions; however, recovered dogs are at risk for developing recrudescence of ocular disease associated with reactivation of latent CHV. Recrudescence of CHV ocular disease may present with either unilateral or bilateral lesions. Recurrent CHV ocular infection may occur in dogs with no identifiable risk factors; however, an immunocompromise state is present in most dogs.\(^7,44\) Naturally acquired recurrent CHV ocular infection is reported in dogs with a variety of immunomodulating Fig. 3. (A) Dog, adult dog (9 years of age), canine herpesvirus 1 infection. The pulmonary architecture is focally mildly disrupted by fibrin, cell debris, and hemorrhage (*). Vessels are acutely congested and alveoli are flooded with macrophages and proteinaceous material indicative of diffuse pulmonary edema (hematoxylin-eosin, original magnification \(\times20\)). (Courtesy of Dr Ingeborg Langohr, Michigan State University, East Lansing, MI.) (B) Liver; dog. Canid herpesviral 1 protein is detected within areas of hepatic necrosis (immunohistochemistry). (Courtesy of Dr Matti Kiupel, Michigan State University, East Lansing, MI.) (C) Liver; dog. Canid herpesviral 1 nucleic acid is present in the areas of hepatic necrosis. In situ hybridization. (Courtesy of Dr Matti Kiupel, Michigan State University, East Lansing, MI.)
systemic conditions and receiving a variety of immunosuppressive therapeutics. Systemic conditions included diabetes mellitus, immune-mediated thrombocytopenia, and lymphoma. Immunosuppressive therapeutics included topical ocular corticosteroids, topical ocular cyclosporine, systemic corticosteroids, and a variety of antineoplastic chemotherapeutics (eg, cyclophosphamide, doxorubicin, vincristine). In many reported dogs, potentially immunosuppressive conditions were concurrently present with the administration of multiple topical and systemic immunosuppressive medications.

Blepharitis is occasionally present with ocular CHV and may appear as focal or generalized eyelid erythema, edema, exudates, and crusting. Regions of alopecia may be present. The blepharitis may represent self-trauma resulting from discomfort associated with conjunctival or corneal disease, or active viral infection of eyelid cutaneous epithelium as described for other dermal regions in dogs with CHV infection.46 Conjunctivitis is the most frequently reported ocular lesion associated with both primary and recurrent CHV infection44,47 and can be presented with conjunctival hyperemia, chemosis, and ocular discharge. Ulceration of the conjunctival epithelium may occur and appears as flat, irregular, pale or pink regions on the conjunctival surface surrounded by regions of hyperemia. Conjunctival ulcerations are readily detected with application of sodium fluorescein, rose Bengal, or lissamine green stains. Although the clinical features of CHV conjunctivitis are often indistinguishable from other etiologies, conjunctival petechiae are frequently reported in dogs with CHV infection (Fig. 5).9,44,47 Although not specific to CHV infection, this clinical finding is uncommon with most other etiologies of conjunctivitis and should be considered suggestive of CHV.

Ulcerative keratitis and nonulcerative keratitis are frequent lesions associated with primary and recurrent ocular CHV infection.7,8,47 A variety of clinical manifestations are observed in the cornea associated with CHV infection and these likely represent
a continuum along the progression of active corneal epithelial infection. Punctate keratitis is the earliest detectable CHV corneal ulceration and appears clinically as a fine stippling of epithelial loss. This subtle lesion is often clinically overlooked when examination is performed without the aid of magnification, but application of corneal vital stains (particularly rose Bengal or lissamine green) facilitate detection.47 As punctate ulcerations progress, they form the classic alphaherpesvirus corneal lesion of dendritic corneal ulcers. Dendritic corneal ulcerations are strongly suggestive of CHV infection in the dog. These linear, branching ulcers stain brightly with sodium fluorescein, rose Bengal, and lissamine green (Fig. 6).7,47 Prominent terminal end bulbs are a consistent feature of CHV dendritic ulcers in the dog and can be used to differentiate CHV corneal lesions from other potential causes of linear corneal ulcers that might appear clinically similar (eg, external trauma, cilia abnormalities, entropion). Terminal end bulbs are club-shaped, rounded ends to the CHV dendritic ulcer branches, and are not seen with other causes of linear corneal ulcers. Coalescence of dendritic ulcerations may result in the formation of geographic corneal ulcers.47 These appear as larger, irregular-shaped areas of corneal epithelial loss. In dogs with CHV ulcerative keratitis, corneal ulcers are commonly located in discrete groups or linear arrangements on the corneal surface. Unless complicated by secondary bacterial infection, CHV corneal ulcers remain superficial and corneal stromal loss is not appreciable. Nonulcerative keratitis is a less frequent lesion reported with CHV ocular infection.47 Clinically, nonulcerative keratitis appears as a circumferential ring of cornea stromal neovascularization with epithelial and subepithelial leukocyte infiltrates in the peripheral cornea. Nonulcerative keratitis may represent a resolution stage of active corneal epithelial disease.

The largest published case series of primary CHV ocular disease described an outbreak of CHV infection a closed colony of young adult laboratory beagles.47 In this group of 27 dogs, conjunctivitis was detected in 100% of dogs, ulcerative keratitis in
26% of dogs, and nonulcerative keratitis in 19% of dogs. Corneal ulcerations were further subclassified by clinical appearance as punctate (7% of dogs), dendritic (19% of dogs), and geographic (4% of dogs). This report confirmed CHV-associated ocular disease in group housed susceptible dogs, and provides an overview of the spectrum and relative frequency of ocular lesions associated with primary ocular CHV infection in dogs.

Under experimental conditions, acquisition of primary CHV infection by ocular surface inoculation consistently produces self-limiting conjunctivitis in immunocompetent mature dogs. This route of infection likely occurs frequently under natural conditions and has direct clinical relevance. Viral inoculation by other anatomic routes, such as the genital tract, is associated with inconsistent development of ocular disease. Clinical signs were manifested in both eyes, even when viral inoculation was unilateral, but the magnitude of conjunctivitis may not be symmetric between eyes. The clinical severity of ocular lesions peak approximately 7 to 10 days after infection and lesions slowly resolve over the following 2 weeks. Histopathologic findings in dogs with acute experimental CHV conjunctivitis include conjunctival epithelial necrosis, subepithelial lymphocyte and macrophage infiltration, and edema of the substantia propria.

Experimental induction of recurrent ocular CHV infection was demonstrated by administering immunosuppressive dosages of systemic corticosteroids to latently infected dogs recovered from primary CHV ocular infection. Recrudescence CHV ocular disease was detected in 83% of immunosuppressed dogs in one study. Bilateral conjunctivitis or linear corneal ulcers developed as early as 3 days after initiating corticosteroid administration. The mean duration of detectable ocular disease was 8.6 days and was shorter than the experimental primary ocular CHV infection in the dogs. Cellular lesions observed by in vivo confocal microscopy in the dogs included conjunctival leukocyte infiltrates, corneal leukocyte infiltrates, abnormal corneal epithelial cell morphologies, and corneal Langerhans cell infiltrates. Subsequent research determined topical ocular corticosteroid administration does not result in recurrent CHV ocular disease in latently infected dogs under experimental conditions. In this study, topical ophthalmic prednisolone acetate (1.0% suspension) was administered 4 times daily for 28 days to both eyes of dogs with experimentally induced latent CHV infection. Viral shedding and recurrent CHV ocular disease were not detected; however, crystalline corneal opacities developed in some dogs. These bilateral corneal lesions appeared clinically as subepithelial and anterior stromal punctate, white, refractile opacities within the central cornea. It was unclear if the crystalline corneal opacities were a nonspecific result of corticosteroid administration or influenced by prior CHV corneal disease.

In immunocompromised dogs, such as lymphoma who are receiving chemotherapy or dogs with autoimmune systemic disorders receiving long-term immunosuppressive therapy, relatively severe ocular lesions may develop during recurrent CHV infection. These lesions include severe ulcerative conjunctivitis and extensive corneal ulceration that is refractory to treatment. Development of viremia, systemic CHV dissemination, and visceral hemorrhagic necrosis, similar to what is typically observed in fetal and neonatal dogs, has been reported in a mature dog with ocular CHV infection while receiving chemotherapy for lymphoma. In the reported dog, it was speculated that viremia and systemic CHV disease developed secondary to localized ocular CHV reactivation with an insufficient immune response to contain virus to the anatomic site of recurrent disease.

Recent evidence suggests CHV ocular diseases in mature dogs are clinically underappreciated. A survey of dogs with idiopathic conjunctivitis determined CHV
was the most common viral etiology of conjunctivitis in mature, vaccinated dogs and was detected in ocular samples from approximately 17% of study dogs.\textsuperscript{44} Conjunctivitis is among the most common ocular diseases in dogs presented to veterinarians and, if these results are extrapolated to the general canine population, it implies CHV ocular diseases occur commonly.\textsuperscript{49}

**VIRUS DETECTION**

**Latency Sites**

To determine the sites of latency of CHV, Miyoshi and colleagues\textsuperscript{16} experimentally inoculated adult seronegative dogs via the intranasal (\(n = 2\)), intranasal and intravenous (\(n = 3\)), or intravaginal (\(n = 3\)) routes with a strain of CHV. Although clinical signs were not observed, infectious virus was isolated from swabs until 4 to 6 days postinoculation. Tissues were collected 2 to 4 months postinoculation and examined for the presence of latent viral DNA. It was determined that the trigeminal ganglion (TG) was an important latency site for CHV, regardless of the inoculation route. Latency was detected also in lumbosacral ganglia of 2 of 3 dogs inoculated intravaginally, 1 of 2 dogs inoculated intranasally, and 1 of 3 dogs inoculated both intranasally and intravenously. Abortion and stillbirths could also be associated with reactivation of latent CHV, but the mechanism by which this takes place has not been investigated. Retropharyngeal lymph nodes were another important latency site, since latency was detected in this tissue in 7 of 8 dogs. Conversely, all attempts to demonstrate latency in peripheral blood lymphoid cells were negative.

In humans, herpesviruses have been detected in the inner ear and are considered to play a role in vestibular dysfunction. Parzefall and colleagues\textsuperscript{50} reported on the prevalence of canine herpesvirus DNA in the vestibular ganglia (VG) and vestibular labyrinth (VL) of 52 dogs that were included in their study. CHV DNA was detected in the VL of 17% of the dogs and in the VG of 19% of the dogs. Although no attempt was made to differentiate between acute and latent infection, it is very likely that the PCR was detecting latent virus. Interestingly, infection of the VG or VL was not always associated with infection of TG. Since the VG, in contrast to the trigeminal and geniculate ganglia, do not have direct connection with sensory nerve endings on body surfaces, it remains most probable that there was primary infection of the TG or geniculate ganglia, with subsequent spread to the VG.

**Spontaneous Reactivation**

Burr and colleagues\textsuperscript{13} examined tissues from 12 adult dogs that had been euthanized for various reasons. From each dog 12 tissues that have been associated with latency in other herpesvirus infections were examined. Viral DNA was detected in the organs of 9 of the 12 dogs. The tissues most commonly found to be positive were lumbosacral ganglia, tonsil, parotid salivary gland, and liver. Based on the data, lumbosacral ganglia are an important site of latency and potential source of reactivated virus for venereal infections and infection of pups as they pass through the birth canal. Finding of latent virus in tonsils and salivary glands points to the role of oronasal spread in the transmission of CHV. It was noted that viral DNA was detected in the trigeminal ganglia extracts of only 2 of the dogs. None of the 12 blood samples tested were found to be positive, indicting a lack of detectable viremia. The authors commented that CHV is either totally absent from peripheral blood or that the level of infection is limited to 1 genomic copy per 2000 mononuclear cells. They also pointed out that basing the incidence of CHV infection on serology only may lead to an underestimation of the true infection rate.
The difficulty in detecting circulating CHV in a kennel situation is highlighted in a study by Ronsse and colleagues. Dogs in a breeding facility were followed for the duration of 1 reproductive cycle. A number of dogs seroconverted (negative to positive) to CHV during this period. Conversely, antibody-positive dogs became seronegative. The serologic data clearly indicate that CHV was circulating in this kennel in the form of acute and/or reactivated form, primary infections. However, despite the fact that samples were taken at regular intervals, the results of PCR testing with a previously validated assay were uniformly negative both on all nasal and vaginal swabs and buffy coat samples. A possible explanation is that the shedding interval after reactivation is very short. Even during acute infection, shedding of CHV is limited to 2 to 6 days.

Reactivation Following Corticosteroid Administration

Latent CHV has been reactivated by treatment with corticosteroids. Okuda and colleagues treated dams with a history of CHV infection with 600 mg of prednisolone for 5 consecutive days. Reactivation of latent CHV infection was confirmed in 4 of 5 dams. Infectious CHV was recovered from nasal, oral, vaginal, and ocular secretions on the 5th to 21st days after initiation of treatment and also from nasal mucosa and tonsil tissues. These results indicate that latent CHV infections develop frequently and that the latent virus may be reactivated, without clinical signs, in dogs with a history of CHV infection.

Ledbetter and colleagues investigated whether systemic administration of an immunosuppressive regimen of corticosteroids (3 mg/kg/day for 7 consecutive days) to experimental adult dogs would lead to reactivation and recrudescence. Group 1 dogs were latently infected and received corticosteroid treatment. Group 2 dogs were latently infected and received a placebo. Group 3 dogs were control dogs and received corticosteroid treatment. Bilateral ocular disease, consisting of conjunctivitis and keratitis, was seen in 83% of the group 1 dogs between days 3 and 18 of the experiment. Ocular shedding was detected in 50% of the group 1 dogs, and a 4-fold rise in antibody titer was detected in all dogs in group 1. None of the dogs in the control groups showed ocular disease, shed virus, or seroconverted. Corticosteroid-induced reactivation is likely the result of enhanced expression of both viral and cellular genes. Corticosteroid also lead to host immune response suppression, As discussed by the authors, the immunosuppression could be involved directly in the reactivation event, or indirectly in facilitating the spread of reactivated virus to peripheral tissues, leading to renewed replication at peripheral mucosal sites and potential transmission to susceptible animals that are in contact with the animal in which reactivation takes place.

Ledbetter and colleagues also administered topical ocular prednisolone acetate or a placebo to mature dogs experimentally inoculated with CHV via the ocular route and previously tested for reactivatable latency by systemic administration of an immunosuppressive dose of corticosteroids. The dogs were treated 4 times daily for a total of 28 days. The results of this study showed that topical ocular prednisolone at the concentration and treatment regimen used did not result in detectable reactivation of CHV latency, based on a combination of recrudescent clinical signs, confocal microscopy findings, ocular infectious virus shedding, real-time PCR findings, and serologic response. A potential explanation for the data is that the concentration of topically administered corticosteroid that is absorbed systemically is insufficient to induce reactivation.

Malone and colleagues described a disseminated CHV infection, which led to euthanasia, in an adult dog. The dog had undergone chemotherapy for the
treatment of generalized lymphoma. It was not clear whether generalized infection in this case was the result of enhanced susceptibility to CHV as a result of immunosuppression or whether it was due to reactivation of a preexisting latent CHV infection in this dog.

**Molecular Methods to Detect CHV**

Amplification of target sequences by PCR method is currently the most common and most sensitive molecular diagnostic approach to the detection of CHV in natural or experimentally infected animals. The PCR assays described initially were gel based, implying that the amplified products are visualized by UV illumination of ethidium bromide–stained agarose gels. Miyoshi and colleagues\(^{16}\) combined a nested PCR with Southern blotting and showed that the detection limit of this combination was equivalent to 1 TCID\(_{50}\).

Schultze and Baumgärtner\(^{17}\) described nested gel-based PCR and in situ hybridization assays to diagnose acute CHV infection in formalin-fixed paraffin-embedded tissues of 1- to 3-week-old puppies that were naturally infected. The specificity of the PCR products was confirmed by restriction endonuclease digestion. Viral DNA was detected in a variety of cell types, such as bronchiolar and alveolar epithelial cells, hepatocytes, renal tubular epithelial cells, neurons, fibrocytes, cardiac myocytes, and endothelial cells. This is in accordance with the previously described “pantropism” of CHV. When paraffin-embedded tissues are used for PCR, it has to be kept in mind that the quality of the DNA can be affected by several factors, such as the length of time between tissue removal and fixation, the presence of nucleases in the tissue, and the length of storage of the paraffin blocks.

Burr and colleagues\(^{13}\) developed a gel-based PCR for CHV and used it in conjunction with Southern blotting to confirm the authenticity of the amplicons. They also assessed the PCR compatibility of each sample for CHV PCR by first verifying that primers specific for a portion of the canine pancreatic lipase gene-amplified their target in each of the tissue extracts. The assay was capable of detecting approximately 14 genomic copies spiked into 1 \(\mu\)g of placental DNA and approximately 3500 copies when spiked into 0.2 ml of blood.

Erles and colleagues\(^{18}\) described a gel-based PCR targeting a 494–base pair region of a gene homologous to HSV-1 UL 37. Reubel and colleagues\(^{52}\) described a nested PCR that had a sensitivity that was 100 times higher than virus isolation. Ronsse and colleagues\(^{22}\) described the use of 2 PCR assays for CHV. One of these assays had a sensitivity of 0.01 CCID\(_{50}\).

The most sensitive and specific method currently available to detect CHV DNA is probe-based real-time PCR. A fluorogenic real-time PCR assay was described by Reubel and colleagues\(^{52}\) and reported to have a detection limit of 10 copies of viral DNA. The first probe-based multiplex real-time PCR for CHV was reported by Ledbetter and colleagues.\(^{8}\) Very recently, Decaro and colleagues\(^{14}\) reported the development and complete validation of a probe-based real-time quantitative PCR for the detection and quantitation of CHV DNA in clinical samples. The assay was found to be very sensitive, since it could detect as few as 10 copies of the target per sample. In comparison with the gel-based PCR assay described by Schulze and Baumgärtner,\(^{17}\) which was used in parallel, this assay has a 10-fold lower detection limit. Specificity for CHV was very high, as determined by lack of amplification of other canine viruses. The dynamic range was validated by successful amplification of a number of CHV-positive samples from different geographic locations. Reproducibility of the assay was determined by determining both intra-assay and interassay variability between the results obtained with samples containing variable amounts of...
target DNA. Both intra-assay and interassay variability, expressed as a coefficient of variation, were fairly low, were dependent on the target concentration, and were found to increase with decreasing target copy numbers. A potential pitfall of PCR assays is that the sample contains substances that are inhibiting the reaction, thus potentially leading to false-negative results. To control for this possibility, an internal control construct was spiked into each sample at known quantity and co-amplified. This way, any inhibition would be readily detectable from a decrease in the expected signal resulting from the amplification of this internal control. A relatively simple way to avoid inhibition was to prepare a 10-fold dilution of the sample.

Since it allows absolute quantitation, the assay was used to determine viral loads in tissues of pups that had died from acute infection and a vaginal swab collected from the dam. The viral load in the vaginal swab was $1.57 \times 10^3$ copies/10$^-3$ l. The highest viral load in tissues was $5.76 \times 10^9$ copies/10$^-9$ l, present in kidney homogenates. The authors concluded that, since it quantitates copy numbers over a wide range, this assay will be very useful not only for diagnostic purposes, but also for future pathogenesis studies and for the testing of the effect of antivirals on the replication of CHV.

CARRIER STATES AND SHEDDING PATTERNS

Carriers

The phrase “carrier animal” has been used extensively to describe an animal that harbors an infectious agent beyond the usual time allowed for the incubation phase of the infection and the acute and convalescent phases of clinical disease.\(^{53}\) When it comes to the herpesviruses this is problematic since there are at least 2 phases that exceed those previously mentioned and are characterized by latency and exacerbation of clinical symptoms from latency. According to Povey, a carrier animal may or may not shed virus in excretions or secretions, and shedding may occur continuously or intermittently.\(^{53}\) As was noted in the preceding section, latency in its strict definition is the lack of viral transcription and translation, so no mature virus is being produced. A latently infected dog with CHV would be defined as a carrier dog that is not shedding virus and would not be contagious to in-contact, susceptible dogs. Exacerbation of the latent state to a replicative state would result in virus replication and shedding. The dog may have mild to severe clinical symptoms during this exacerbation phase.

Shedding Patterns

Primary, systemic neonatal CHV infection is associated with extensive viral shedding from numerous anatomic sites. High CHV viral titers are detected in respiratory secretions, ocular discharge, saliva, and urine and on many mucosal surfaces (eg, genital, nasal, ocular, oral, pharyngeal, rectal, tracheal\(^4,28\)). Viral shedding may persist for up to 3 weeks in dogs that survive neonatal infection. Viral shedding from infected neonates may serve to spread CHV, either through direct contact or fomites, to littermates and other dogs.

Primary and recurrent CHV infection in mature dogs is associated with mucosal viral shedding that is detectable by PCR assay or virus isolation. The duration and anatomic site of shedding vary markedly between dogs and infection episodes in individual animals. Canine herpesvirus-1 shedding often occurs from multiple mucosal surfaces simultaneously and may be detected at sites anatomically distant to regions of overt clinical disease. Reports of experimentally induced primary and recurrent CHV infection suggest viral shedding during primary infection is prolonged and associated with higher viral titers than recurrent infection.\(^8,45,51,54\) There is an individual dog susceptibility to CHV reactivation and shedding. Latent CHV infection
can be reactivated, with induction of viral shedding, by short durations of corticosteroid administration in some dogs; however, other dogs are resistant to corticosteroid-induced viral reactivation.\(^8,51,54\)

When naturally infected mature bitches that previously aborted CHV-infected pups where experimentally immunosuppressed by a 5-day course of systemic corticosteroid administration, CHV was shed from the nasal, oral, ocular, and vaginal mucosa.\(^51\) Viral shedding could not be induced in all dogs. Viral shedding was detected by virus isolation as early as 5 days, and as late as 20 days, after initiating corticosteroid administration. The duration of detected CHV shedding ranged from 1 to 7 days in individual dogs. In a similar study\(^54\) using 3-month- and 2-year-old dogs experimentally infected with CHV by nasal and intravenous routes, CHV reactivation and mucosal viral shedding were repeatedly induced by systemic corticosteroid administration. Primary oronasal infection was associated with nasal CHV shedding of approximately 2 weeks’ duration. Following recovery from primary infection, systemic corticosteroid administration induced viral shedding from the nasal, oropharyngeal, and genital mucosa. The onset of detectable shedding was between 5 and 9 days after initiating corticosteroid treatment and persisted for up to 32 days with marked variation between individual dogs. A second round of corticosteroid administration was administered 3 months later and again resulted in viral shedding in some, but not all, dogs. The duration of viral shedding was shorter in all dogs during the second experimental reactivation and was associated with a tendency for lower viral titers.

In studies examining ocular CHV infection, a similar pattern of viral shedding is reported. Experimental primary ocular CHV infection in mature dogs produced by direct ocular surface inoculation resulted in conjunctival viral shedding that persisted for 10 days after inoculation.\(^46\) Virus was detected in conjunctival samples by virus isolation and CHV PCR, and viral titers peaked 5 days postinoculation. CHV was inoculated into a single eye, but viral shedding was detected bilaterally in some dogs. Following recovery from primary ocular infection, viral shedding was not detected over the subsequent 8 months. Experimental recurrent ocular CHV infection induced by systemic corticosteroid administration to dogs recovered from primary ocular infection again resulted in viral shedding.\(^8\) Ocular CHV shedding was detected by PCR assay in 50% of dogs between 10 and 13 days after administering the first dose of corticosteroid. In comparison to primary ocular CHV infection, ocular viral shedding associated with recurrent infection was briefer and viral titers in samples were lower.

Experimental primary CHV genital mucositis in mature dogs, produced by intravaginal and intrapreputial CHV inoculation, resulted in genital viral shedding that was detected by virus isolation for up to 20 days.\(^46\) Several dogs also developed nasal, pharyngeal, and conjunctival viral shedding during this period. Canine herpesvirus tracheobronchitis induced by intranasal viral inoculation was associated with viral shedding for up to 18 days.\(^5\) In the dogs with CHV upper respiratory tract infection, viral shedding from the nasal mucosa was detected by virus isolation in all dogs and a some had concurrent tracheal and rectal viral shedding.

**CLINICAL ECOLOGY AND EPIDEMIOLOGY**

*Five Key Questions*

The clinical ecology and epidemiology of CHV can be summarized in Table 3. It basically starts with a series of questions that inquire into the status of the virus, the host, and the environment with which both are localized.\(^55\) The critical question is whether CHV infection and disease are of economic concern? As was mentioned earlier, the reproductive diseases associated with CHV were the initial driving force
behind the recognition of the economic and emotional effects upon dog owners. While the costs of CHV-associated reproductive diseases have not been reported, it would be conceivable that a dam that loses an entire litter to CHV would result in a loss of $10,000, since multiple puppies are involved. In cases of respiratory disease and ocular disease, the costs of treatment and long-term care of recurrent infections may exceed $1000 per case.

The second question pertains to the zoonotic or public health risks associated with CHV infections. The virus is species specific and there is no evidence to support its involvement in human disease.  

The third question is the key to the persistence of CHV in the canine population—Where is the virus when not causing disease? This has been a key factor in understanding the virus and controlling it. The virus maintains itself in subclinical carrier dogs by way of latency. It may be exacerbated throughout life by stress, which results in mild to severe clinical symptoms that most commonly affect the respiratory and ocular systems. Concurrent with these clinical episodes there is shedding from excretions and secretions to susceptible dogs. The 2 most susceptible age groups are pregnant CHV naïve dogs and puppies of these dams (in utero, postnatal).

The fourth question revolves around the epidemiology of CHV once its infection occurs in the susceptible dog. The course of the infection to disease is variable and has been reviewed earlier under the contemporary clinical observations. One important aspect to reiterate here is the importance of immunity in controlling the infection–disease process in pregnant dams and their offspring during the postnatal period. Early postnatal infection (3 weeks or less) results in high morbidity accompanied by high mortality. Later infection (3 weeks or later) results in low morbidity and very low mortality. However, it is usually the later postnatal infection that establishes the lifelong carrier state via latency.

The fifth question is a natural extension of the sequence of clinical inquiry and addresses the control of CHV, so that infection is minimized during disease-susceptible periods and maximized during disease-resistant periods. As noted previously, shedding states are important in maintaining the virus infection on the population to attain a certain degree of population immunity. Knowing when dogs are potentially contagious, and maintaining the 6-week barrier to infection, allows for

Table 3
Clinical ecology and epidemiology of canine herpesvirus infection and disease

| Question                                                                 | Answer                                                                                           |
|--------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| 1. Is the infection/disease of economic concern?                         | Yes, may result in high mortality of litters, increased respiratory and ocular disease in susceptible dogs. |
| 2. Is the infection/disease a public health risk? (zoonosis)              | No, restricted host range to the canids.                                                          |
| 3. Where is the agent when not causing disease? (ecology)                | Subclinical carrier animals, latency. Readily inactivated outside dog's body.                    |
| 4. What are the key contributing factors to the infection/disease process? (epidemiology) | Naïve susceptible puppies, naïve pregnant dams, and susceptible (stressed) adult dogs.           |
| 5. What factors can we control to minimize or eliminate the infection/disease process? | Shedding to susceptible dogs/puppies during critical 6 week danger period; maintain good kennel biosecurity. No vaccine available. |

Data from Evermann JF, Eriks ES. Diagnostic medicine: The challenge of differentiating infection from disease and making sense for the veterinary clinician. Adv Vet Med 1999;41:25–38.
maximum protection during this susceptible period. Since there is no reliable vaccine available, kennel hygiene and biosecurity are essential.34

THERAPEUTICS

Therapy for neonatal CHV infection is largely supportive and carries a poor prognosis for survival once clinical disease is manifested.23 In instances where dogs survive neonatal CHV infection, cardiac, neurologic, and ocular lesions may be permanent. Elevating the environmental temperature of dogs in a litter after CHV infection is diagnosed may provide some protection to uninfected pups. Viral replication is reduced at elevated body temperatures and there are lower morbidity and mortality rates in dogs that are subsequently infected; however, this is ineffective for individual dogs if implemented after viral infection.23 Intraperitoneal injection of immune sera obtained from CHV-seropositive dogs is described as a method to reduce mortality in an exposed litter, but it must be administered prior to infection to be most effective.26 Lactoferrin possesses in vitro antiviral activity against CHV and inhibits cellular infection.56 Administration of lactoferrin to dogs at risk for infection could theoretically provide protection; however, this is not demonstrated in vivo. Isolated reports of apparently successful therapy of neonatal CHV infection with the antivirals vidarabine and acyclovir are described. Acyclovir was administered orally as a 10-mg total dose per dog at 6-hour intervals until 3.5 weeks of age.26

The pharmacokinetics and tissue distribution of intravenous, subcutaneous, and oral acyclovir were investigated in dogs.57,58 Additionally, a sustained release buccal tablet form of acyclovir was evaluated in the dog.59 Acyclovir is bioavailable when administered orally to dogs and is widely distributed within tissues; however, target plasma concentrations and effective dosages for CHV infection are currently unknown.57–59 Acyclovir toxicosis resultant from accidental ingestion is reported in dogs with dosages as low as 40 mg/kg and the routine clinical use of this, and other systemic antiviral medications, in dogs for CHV infection requires further investigation of safety and efficacy.60 The canine pharmacokinetics of newer-generation anti-herpesviral drugs, including famciclovir, are reported. Similar to acyclovir, safe and effective doses for dogs with CHV infection are undetermined.61

Treatment of respiratory and genital CHV infection is primarily symptomatic. Unless complicated by secondary bacterial infection, these conditions are typically self-limiting and specific antiviral therapy is not reported. In contrast to respiratory and genital infection, there are detailed reports of the successful clinical management of ocular CHV infection. In addition to nonspecific treatments to prevent secondary bacterial infection (topical ocular antimicrobials) and improve comfort (topical ocular atropine), antiviral therapy with 0.1% idoxuridine or 1% trifluridine ophthalmic solution was used. Idoxuridine and trifluridine are nucleoside analogues, possess good anti-herpesvirus activity, and are well tolerated by dogs when applied topically as ocular formulations. Trifluridine is available under the trade name Viroptic, and idoxuridine can be acquired from compounding pharmacies. Both antivirals are administered 6 to 8 times daily for the first 48 hours and then 4 times daily until resolution of clinical signs of active infection. Cidofovir 0.5% ophthalmic solution is an alternative ophthalmic antiviral for CHV ocular disease that is effective with twice daily administration (E.C. Ledbetter, unpublished data, 2011).

SUMMARY

This review has documented well that our level of clinical inquiry expands as our knowledge base about CHV increases. While earlier studies focused on the
reproductive effects of CHV in susceptible pregnant dogs and neonatal puppies, it
has become apparent that in order to control CHV-related diseases that we must
understand the various forms of CHV infection that may occur in the dog population
(Fig. 7). This has prompted the veterinary community to develop more sensitive
diagnostic assays, such as PCR, in order to answer the questions, where is the virus
when not causing disease, and what is its relationship with respiratory and ocular
diseases in adolescent and adult dogs (1 year or older)?

Molecular and serologic studies have clearly demonstrated that we are dealing with
an infection that is more common than we considered a decade ago. Reports have
indicated that up to 70% of some high-risk dog populations have been infected with
and are latent carriers of CHV. This is important for veterinarians to know as we confer
with clients on the best management steps we can take to protect our at-risk
populations. While pregnant CHV-naive dams and neonatal puppies born from a
CHV-naive dam are considered at high risk for disease, we must also take into
consideration dogs in kennels and rescue centers. It is these dogs that are at risk for
either exposure-infection or stress-induced exacerbation of latent CHV, which had
been acquired at an earlier age.

The manifestations of CHV in adolescent and mature dogs may range from
subclinical to severe respiratory and/or ocular diseases. The reports by Malone
and colleagues, Gadsden and colleagues (submitted, 2011), and Ledbetter and

Fig. 7. Schematic of the potential interactions amongst three subpopulations of dogs and
the infection-disease cycles of CHV.
colleagues\textsuperscript{7,47} all indicate that CHV can cause disease in older dogs and that it is not just a “puppy disease.”

Recognition of the various forms of CHV-induced disease, availability of diagnostic assays with increased sensitivity, and the formation of reliable biosecurity measures will allow for better control steps to be taken in dogs at-risk for infection and disease.

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