

Review Article

Prevalence and Clinical Impacts of Canine Herpesvirus-1 (CHV-1) in Dogs: Reproductive Effects and Ocular Lesions

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Abstract

Background and Aims: Canine herpesvirus type 1 (CHV-1), belongs to the *Varicellovirus* genus within the *Alphaherpesvirinae* subfamily of the *Herpesviridae* family. It causes severe disease in neonatal canines, often resulting in death, but can also lead to reproductive issues in adult canines. The virus is primarily transmitted through oronasal exposure during birth, but can also spread through venereal and transplacental routes. CHV-1 establishes a latent carrier state after infection, predominantly found in ganglia, salivary glands, tonsils, and hepatic tissue. Reactivation can occur due to stress or immunosuppression. The virus induces a weak immune response, resulting in variable antibody levels. Canine herpesvirus type 1 has a worldwide distribution and is prevalent in large-scale breeding kennels with a history of neonatal infections. In adult canines, CHV-1 can cause ocular manifestations, ranging from eyelid and conjunctival inflammation to corneal conditions. The disease can manifest bilaterally or asymmetrically between eyes, with severity influenced by age and immune status. While primary ocular infections are subclinical or mild in healthy dogs, they can worsen in immunosuppressed individuals. Symptoms include blepharospasm, photophobia, ocular discharge, and corneal lesions like keratitis. Studies have shown varying seroprevalence rates of CHV-1 among canine populations across different regions, with factors like age, gender, and housing conditions influencing antibody titers. In neonatal canines, CHV-1 can lead to fatal systemic disease with clinical signs like rapid respiration and abdominal discomfort. Maternal infection during gestation can result in fetal loss or other adverse outcomes. Maternal antibodies from seropositive dams provide protective immunity to puppies. Various diagnostic methods like PCR, serology, and histopathology are used to confirm CHV-1 infection, with some cases showing active viremia in blood and other samples. Reactivation of latent infection can occur in adult dogs under certain conditions, leading to clinical disease. These findings highlight the importance of monitoring and managing CHV-1 infections in canine populations to prevent disease spread and adverse outcomes.

Keywords: Canine herpesvirus-1, CHV-1, Reproductive effects, Ocular lesions, Prevalence, Latent infection

Introduction

Canine herpesvirus type 1 (CHV-1) is taxonomically classified as a member of the genus *Varicellovirus* within the subfamily *Alphaherpesvirinae* of the family *Herpesviridae*. The etiological agent, subsequently classified as canine herpesvirus type 1, was initially

isolated and characterized in 1965 by three independent research groups through the examination of fatal disease cases in neonatal canines and primary cultures derived from canine renal tissue (1-3). Canine herpesvirus type 1 infection manifests as a severe, systemic necrotizing, and hemorrhagic disease with a fatal outcome in susceptible neonatal canines. However, in post-neonatal individuals, the infection often proceeds sub-clinically (1, 4). Canine herpesvirus type 1 is implicated in the pathogenesis of reproductive disorders in adult canines, including embryonic loss through mechanisms such as resorption, abortion, and fetal demise.

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Canine herpesvirus type 1 infection can induce the formation of vesicular eruptions on the mucosal surfaces of the vestibule and vagina in female canines, often accompanied by acute vaginitis. Similarly, males may exhibit vesicular lesions on the penis and prepuce. Canine herpesvirus type 1 has been sporadically linked to the development of the upper respiratory tract and ocular pathologies in adult canines (5). Primary infection in susceptible neonatal canines predominantly occurs through oronasal exposure to the virus during the parturition process, acquired from the maternal birth canal (6). Additional modes of transmission for canine herpesvirus type 1 include both venereal and transplacental routes (4). Following either an acute clinical presentation or a subclinical infection, canines transition into a latent viral carrier state. In this phase, canine herpesvirus type 1 can be detected within the trigeminal and lumbosacral ganglia, salivary glands, tonsils, and hepatic tissue, despite the absence of overt clinical manifestations (7). Reactivation of latent canine herpesvirus type 1 can be precipitated by physiological stressors or experimentally induced immunosuppression through the administration of immunosuppressive pharmacotherapeutics or anti-lymphocyte antibodies (8).

Canine herpesvirus type 1 is characterized by a weak immunogenicity, resulting in a variable and transient humoral immune response. Neutralizing antibody titers exhibit fluctuations within a 4-8 week period post-exposure, with a limited persistence typically lasting only a few months (9, 10). A multitude of epidemiological factors can influence the seroprevalence of canine herpesvirus type 1 within a given population.

Canine herpesvirus type 1 seroprevalence is significantly higher within large-scale and breeding kennel environments with a documented history of neonatal infections. Epidemiological studies have demonstrated a positive correlation between increasing age, mating status in males, and advanced reproductive age in females with elevated anti-CHV-1 antibody titers (11). Canine herpesvirus type 1 is believed to have a global distribution. Canine herpesvirus type 1 has been documented in numerous co-

untries globally. While its prevalence is ubiquitous, seroprevalence studies have revealed significant geographical disparities in antibody titers among canine populations.

Heterogeneity in serological methodologies and sampling populations contributes significantly to the observed variability in canine herpesvirus type 1 (CHV-1) seroprevalence across geographic regions. Discrepancies in diagnostic techniques and the specific demographics of study populations can substantially impact the estimation of CHV-1 antibody prevalence.

Ocular Lesions

Canine herpesvirus-1 (CHV-1) has been established as a primary etiological agent in severe systemic neonatal infections and reproductive pathology, including infertility and abortion, for several decades. While the association between CHV-1 and these conditions is well-documented, the ocular manifestations of the virus in adult canines have only recently been definitively characterized.

Clinically, CHV-1 infection in adult canines can manifest as a spectrum of ocular and adnexal pathologies. These can range from eyelid inflammation (blepharitis) and conjunctival inflammation (conjunctivitis) to more severe corneal conditions, including ulcerative and non-ulcerative keratitis. Ocular and adnexal pathologies associated with CHV-1 can arise during both initial and subsequent infections (5). Both individually owned domestic dogs and dogs maintained in communal environments, such as research facilities or kennels, are susceptible to CHV-1 infection. Initial ocular infections caused by Canine Herpesvirus-1 (CHV-1) typically manifest bilaterally. However, the severity and specific clinical features of the disease can exhibit asymmetrical presentation between the affected eyes of individual dogs.

Subsequent ocular infections caused by Canine Herpesvirus-1 (CHV-1) can manifest as either unilateral or bilateral ocular disease. The clinical presentation of ocular CHV-1 infections, both initial and recurrent, is modulated, at least in part, by the age and immunological status of the affected canine. In healthy, immunocompetent adult dogs, primary CHV-1 ocular infec-

tions typically manifest as subclinical or mild ocular disease that resolves spontaneously without intervention. Ocular manifestations of CHV-1 infection are often exacerbated in immunosuppressed adult dogs, characterized by increased severity and prolonged disease duration (5). Ocular manifestations of Canine Herpesvirus-1 (CHV-1) infection in adult dogs exhibit a diverse clinical spectrum, encompassing a range of signs and lesions (12-16). Unlike the systemic and often fatal ocular manifestations observed in fetal and neonatal canines, CHV-1 infections in adult dogs primarily affect the ocular surface and adnexal structures. The clinical presentation of CHV-1 ocular disease in adult dogs is heterogeneous, often characterized by a combination of blepharitis, conjunctivitis, ulcerative keratitis, and non-ulcerative keratitis (5).

Common clinical signs associated with canine herpesvirus-1 (CHV-1) ocular infection include blepharospasm, photophobia, ocular rubbing, elevation of the third eyelid, and ocular discharge (12-14, 16). Canine herpesvirus-1-induced blepharitis is characterized by eyelid inflammation manifested as redness, swelling, exudate formation, crusting, ulceration, and hair loss. Conjunctivitis is the most prevalent ocular manifestation of CHV-1 infection in adult canines, often presenting as an isolated lesion but frequently co-occurring with other eyelid or corneal pathologies (13-15).

Ulcerative keratitis is a commonly observed corneal lesion associated with CHV-1 ocular disease in adult canines (12, 14, 16). The detection and quantification of ocular viral shedding during both primary and recurrent CHV-1 infections is crucial for understanding the epidemiology of the disease and for confirming a clinical diagnosis. This process involves the release of infectious viral particles from infected ocular tissues, facilitating transmission to susceptible canine hosts (5).

Diagnosis and Treatment

A definitive diagnosis of active ocular CHV-1 infection can be established through the analysis of corneal or conjunctival samples using various diagnostic techniques. These include polymerase chain reaction (PCR) for genetic

detection, virus isolation through cell culture, electron microscopy for direct viral visualization, or immunofluorescent antibody assay for antigen detection (4, 12, 17, 18). Prophylactic topical ocular antimicrobial therapy is recommended for all dogs with CHV-1 ocular disease to prevent secondary bacterial infections, which can exacerbate disease progression, leading to severe ocular sequelae, including corneal perforation. Topical ocular atropine administered to induce mydriasis (pupillary dilation) can provide symptomatic relief in dogs with corneal disease by reducing ocular discomfort. Clinical reports have documented the apparent efficacy of topical antiviral therapy in managing CHV-1 ocular infections in dogs.

Antiviral agents such as idoxuridine 0.1% ophthalmic solution, trifluridine 1% ophthalmic solution, and cidofovir 0.5% ophthalmic solution have been reported to produce positive outcomes in some cases (12, 15, 16).

Reproductive Effects

CHV1 is a cytoadherence pathogen disseminated via either cellular coalescence or passive vector-mediated dispersal. Following epithelial progeny production at the inoculation site, leukocyte-associated viremic dissemination ensues, resulting in tropism for a broad spectrum of neural and lymphoid tissues. Disseminated maternal infection during gestation can induce placental inflammation, facilitating transplacental viral transfer to the offspring (19). Irrespective of the inoculation route, all canines exhibit nasal mucosal viral shedding during the acute phase of infection (20). Simultaneous viral excretion and concomitant genital and respiratory tract infections can manifest despite the presence of circulating humoral immune factors. Horizontal transmission from infected males to susceptible females via venereal contact is considered an insignificant mode of viral propagation.

Conversely, genital viral localization in females represents a primary conduit for perinatal transmission to offspring (21). Within the adult host population, infection is prevalent without overt clinical manifestations. However, during the initial acute phase, mucosal viral entry can

induce localized inflammatory responses characterized by lymphoid follicular hyperplasia, submucosal bleeding, and vascular congestion (22). Subclinical or mildly symptomatic upper respiratory tract disease can manifest in both juvenile and adult canines (23). Epithelial vesicle formation has been observed to coincide with the initiation of proestrus in females, subsequently undergoing spontaneous resolution during the anestrus phase (21).

The gestational timing of CHV1 inoculation in nulliparous pregnant canines can lead to a spectrum of fetal outcomes, including subclinical fetal loss, fetal desiccation, fetal expulsion, or preterm birth of live offspring. Canine dams with a history of CHV-1 exposure and subsequent seroconversion typically deliver uncomplicated litter. Passive transfer of maternal humoral or cellular immune components via colostrum from seropositive dams confers protective immunity in puppies against the clinical manifestations of CHV1 infection (21, 24). The temporal window of peak susceptibility to CHV1-induced pathology has been precisely defined as the six weeks encompassing three weeks antepartum and three weeks postpartum (25). Postnatal viral exposure is more prevalent. In litters exposed peripartum or postnatally, clinical disease primarily manifests between the first and third weeks of life. The interval between viral exposure and disease onset, or incubation period, spans three to seven days. Clinical presentation is nonspecific, characterized by rapid, shallow respiration, abdominal discomfort, anorexia, and emesis (21).

Diagnosis and Treatment

Serological assays have historically been employed for CHV1 diagnosis; however, the virus' limited immunogenicity can compromise the accuracy of such diagnostic approaches. Following CHV-1 exposure and potential abortion in canines, antibody titers exhibit a rapid and transient elevation, typically peaking and declining within a four- to eight-week period, with an estimated duration of no more than 60 days post-exposure (25). Necrotic tissue regions containing characteristic intracellular viral inclusions are pathognomonic of CHV1 infection

and are disseminated throughout multiple organ systems (26). Therapeutic interventions for CHV1-infected puppies are typically ineffective once clinical signs manifest due to the rapidly progressive and severe nature of the disease. Augmentation of environmental temperature for affected individual puppies fails to modify the disease progression. Case fatality rates within outbreak settings may be mitigated through intraperitoneal administration of serum derived from previously infected bitches. A singular dosage ranging from one to two milliliters is deemed adequate (21).

Risk Factors

In vitro investigations have been conducted to characterize CHV-1 and elucidate its pathogenic mechanisms (19, 27). In vitro studies have been conducted to elucidate CHV-1 characteristics and pathogenesis, including viral latency and the potential for reactivation following prednisolone administration (28, 29). Seo et al. (30) and Van Gucht et al. (31) independently observed a positive correlation between canine age and seroprevalence, with a statistically significant increase in antibody titers detected in dogs older than six and twelve months, respectively. Lacheretz and Cognard (32) documented a progressive acquisition of antibodies in dogs up to six years of age, with a markedly lower antibody prevalence of 0.7% observed in the under-two-year-old cohort. Seo et al. (30) reported a modest gender disparity in CHV-1 seroprevalence, with males exhibiting a higher antibody positivity rate (42%) compared to females (33%). In contrast to other species with established evidence of herpesvirus recurrence during gestation and parturition (33, 34), the influence of sex steroids on CHV-1 reactivation and subsequent seroconversion in canines remains largely unexplored.

Clinical manifestations of CHV-1 reactivation have been documented with increased frequency in female dogs during estrus and parturition (22, 35). Historically, CHV-1 has been frequently isolated as an etiological agent in canine infectious respiratory disease complex cases. However, its role as a primary pathogen in the respiratory tract is considered to be of second-

dary importance (23, 36, 37). Environmental variables, including farm dimensions and animal population density, have been shown to facilitate the transmission of herpesvirus and the development of subsequent immune responses (38-40).

Prevalence

Anette Krogenæs *et al.* (41) found CHV-1 infection prevalent among breeding bitches in eastern Norway. While traditional risk factors showed no association, seasonal variations, prior litters, and participation accounted for a substantial portion (67-78%) of antibody titer differences. Serological studies in domestic dogs have yielded highly variable prevalence rates, ranging from zero to complete seropositivity across different geographical regions (42). Ronsse *et al.* determined a 45.75% prevalence of antibodies against Canine Herpesvirus-1 (CHV-1) within the Belgian canine population (43). A subsequent study conducted in 2008 within the Gauteng Province of South Africa revealed a 22% seroprevalence of CHV-1 antibodies among canine populations, as determined by both serum neutralization test (SNT) and enzyme-linked immunosorbent assay (ELISA) methodologies (10). A separate study conducted by Dahlbom *et al.* in 2009 revealed a significantly higher CHV-1 seroprevalence of 81.5% within the Finnish canine population (44). A high seroprevalence rate of 85.5% for CHV-1 infection was established within the Norwegian canine population through the application of immunoperoxidase monolayer assay (IPMA) (41). A concurrent study conducted in Turkey employed both virus neutralization test (VNT) and enzyme-linked immunosorbent assay (ELISA) to assess CHV-1 seroprevalence. ELISA demonstrated a higher antibody detection rate of 39.3% compared to the 29.4% identified through VNT (45). A study conducted by Babaei *et al.* in 2010 determined an overall CHV-1 seroprevalence of 20.7% within the canine population through the application of an indirect immunofluorescence antibody (IFA) assay (46). Real-time PCR analysis detected Canine Herpesvirus-1 (CHV-1) DNA in 15% (21/140) of examined

canine samples (47). Losurdo *et al.*, employing real-time PCR, demonstrated that CHV-1 infection can manifest as a fatal systemic disease with characteristic clinical and histopathological findings. Furthermore, the study revealed the potential for prolonged viral shedding via nasal, ocular, and fecal routes (48). Cargnelutti *et al.* (2015) documented a systemic fatal disease outbreak characterized by neonatal mortality. The study employed histopathology, virus neutralization test (VNT), and polymerase chain reaction (PCR) for diagnostic confirmation (49). A study by Larsen *et al.* (2015) in Denmark revealed a 22.8% prevalence of CHV-1 infection among 57 deceased puppies as determined by qPCR. Histopathological and *in situ* hybridization findings exhibited inconsistent correlation with the PCR results (50).

Historically, CHV-1 has been primarily classified as a respiratory pathogen, with its presence detected in nasal discharge samples through PCR analysis (51, 52). Ledbetter *et al.* (2009) demonstrated the capacity of systemic prednisolone to induce viral reactivation of latent Canine Herpesvirus-1 (CHV-1) infection in adult dogs through immunosuppression (53). Malone *et al.* (2010) reported a case of widespread CHV-1 infection in an adult canine with compromised immune function (15). The detection of CHV-1 DNA in both blood and vaginal swab samples via PCR confirmed active viremia in the reported case (15). Gadsden *et al.* (2012) documented a fatal case of CHV-1 infection in a nine-year-old spayed female Bichon Frise. The diagnosis was confirmed through PCR, immunohistochemistry, and *in situ* hybridization (54). A Mexican study confirmed CHV-1 infection in deceased puppies through a comprehensive diagnostic approach encompassing histopathology, direct immunofluorescence, electron microscopy, and PCR (47).

Pratelli *et al.* (2014) reported seroprevalence rates of 14.6% and 18.6% for CHV-1 in an Italian population, as determined by serum neutralization test (SNT) and *in-house* immunofluorescence (IF), respectively. However, no CHV-1 DNA was detected in any of the vaginal swab samples analyzed (55). A separate Italian study yielded negative results for

CHV-1 antibodies in all serum samples analyzed using both serum neutralization test (SNT) and nested polymerase chain reaction (PCR) assays (56). Li et al. (2016) demonstrated through experimental models that Canine Herpesvirus-1 (CHV-1) exhibits superior replication and invasion capabilities within the vaginal mucosa compared to the respiratory mucosa, as evidenced by the greater extent and depth of viral antigen-positive cell clusters (57). Despite the absence of statistically significant breed-related differences reported by Yeşilbağ et al., a higher prevalence of CHV-1 was observed in Golden Retrievers (56.2%) compared to Terriers (50%) within the Turkish canine population (45). Previous research has indicated that housing conditions do not significantly influence the prevalence of Canine Herpesvirus-1 (CHV-1) (43, 46, 55).

Conversely, numerous studies have documented a higher prevalence of CHV-1 infection within kennel or shelter environments compared to individually owned dogs (45). Dahlbom et al. (2009) identified a correlation between elevated antibody titers and reproductive complications (44); However, conflicting evidence exists, with other studies demonstrating no significant correlation between CHV-1 infection and reproductive disorders (55, 58). Ronsse's study revealed no significant correlation between reproductive status and CHV-1 antibody titers, despite a trend towards increased abortion rates in seropositive breeding kennels (11, 59). Conversely, other research studies have failed to establish a definitive link between CHV-1 antibody titers and reproductive outcomes (56, 58). Ström Holst et al. (2012) postulated that the reactivation of latent Canine Herpesvirus-1 (CHV-1) infection during pregnancy is unlikely under optimal sanitary and management conditions (60). The prevalence of CHV-1 infection exhibits geographical variability and is significantly influenced by factors such as sampling methodology and the specific characteristics of study populations. The diagnostic accuracy of different methodologies employed to detect CHV-1 can vary significantly, influencing the overall seroprevalence estimates. Canine Herpesvirus-1 (CHV-1) is characterized by its poor immunogenicity, re-

sulting in a transient antibody response that typically wanes within a few months post-infection (11, 56). Standardization of serological assays for CHV-1 is lacking, resulting in inconsistent results across different laboratories and hindering comparative analysis of seroprevalence data. Consequently, serological methods may underestimate the true prevalence of CHV-1 infection due to limitations in assay standardization and the transient nature of antibody responses. Polymerase chain reaction (PCR) is widely recognized as a gold standard diagnostic technique for detecting both active CHV-1 infections in puppies and latent viral reservoirs in adult dogs (18, 42).

The study by Ada Rota et al. revealed a high prevalence of Canine Herpesvirus-1 (CHV-1) infection within the Piedmont breeding dog population, although a notable degree of heterogeneity existed among kennels, with some exhibiting complete absence of the virus. Within kennels where CHV-1 infection was detected, seroprevalence rates were notably high, with a substantial proportion of dogs exhibiting elevated antibody titers. Canine females exhibit a post-pubertal increase in antibody titers, suggesting the development of protective immunity during the reproductive period, potentially conferring benefits to both dam and offspring. Despite the potential for naturally acquired immunity, vaccination of seronegative pregnant bitches is recommended to mitigate the risk of CHV-1 infection in kennels with endemic viral circulation.

Prophylactic vaccination is advisable for kennels with a previously negative CHV-1 status to mitigate the risk of disease introduction through latent carriers, thereby safeguarding reproductive health and preventing potential severe consequences for seronegative pregnant bitches (61). Blood specimens were collected from canine populations exhibiting clinical disease manifestations within dog shelters and collection centers located in Konya and Antalya. A total of 141 canine blood samples were subjected to commercial enzyme-linked immunosorbent assay (ELISA) to detect anti-CHV antibodies. Concurrently, leukocyte samples derived from the same blood specimens were analyzed via immunofluorescence test (IFT)

for the isolation of CHV. Madin-Darby Canine Kidney (MDCK) cell cultures were employed as a host system for virus isolation. Serological analysis revealed a 68.8% prevalence of antibodies against Canine Herpesvirus (CHV) among the 141 samples tested. Conversely, virus isolation attempts using an immunofluorescence test (IFT) on blood leukocytes yielded no positive results. The high seroprevalence rate observed in this study suggests a substantial level of Canine Herpesvirus (CHV) exposure within the canine population of Konya and Antalya shelters. To mitigate the potential spread of the virus and associated disease, identification and isolation of infected individuals are crucial for effective disease management (62).

A comparative analysis of seroprevalence was conducted using a commercial ELISA and virus neutralization test on blood samples collected from dogs housed in Konya and Antalya shelters. The ELISA kit employed utilized intact viral particles as the immunogenic component. Given the limited cytopathic effect induced by CHV-1, a plaque reduction neutralization test coupled with immunoperoxidase staining was employed to enhance the detection of viral plaques.

Neutralizing antibodies were identified in a randomly selected subset of twenty samples. The ELISA-based serological analysis revealed a high prevalence of Canine Herpesvirus-1 (CHV-1) antibodies, with 87% of the study population testing positive. The ELISA, despite its high sensitivity, is not entirely reliable for confirming CHV-1 infection due to its low positive predictive value. Therefore, a confirmatory test like the SN test is crucial for accurate diagnosis. However, a negative ELISA result is a strong indicator of the absence of CHV-1 infection. This information is essential for effective disease management and control strategies in canine populations. A statistically significant positive correlation was observed between ELISA and serum neutralization (SN) titer values, as determined with a 95% confidence interval. No statistically significant associations were identified between clinical presentation factors and the outcome variable. The high seroprevalence rates observed in the Mexican

canine population suggest a prevalent state of latent herpesvirus infection (63).

Conclusions

Canine herpesvirus type 1 (CHV-1) is a virus that affects neonatal and adult dogs, causing severe disease and reproductive issues. It is primarily transmitted through birth but can also spread venereally and transplacentally. CHV-1 establishes a latent carrier state in the body and can reactivate due to stress or immunosuppression. In adults, it can cause ocular symptoms like inflammation and corneal conditions. Variations in seroprevalence rates are seen in different regions, with maternal antibodies protecting puppies. CHV-1 diagnosis is done using methods like PCR and histopathology. Effective monitoring and management of CHV-1 infections are crucial to prevent disease transmission and complications in canine populations.

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Conflict of Interest

No conflict of interest is declared.

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All data generated or analyzed during this study are included in this article.

References

1. Carmichael LE, Squire RA, Krook L. Clinical and pathologic features of a fatal viral disease of newborn pups. *Am J Vet Res.* 1965;26(113):803-14.
2. Spertzel RO, Huxsoll DL, McConnell SJ, Binn LN, Yager RH. Recovery and characterization of a herpes-like virus from dog kidney cell culture. *Proc Soc Exp Biol Med.* 1965;120(3):651-5.
3. Stewart SE, David-Ferreira J, Lovelace E, Landon J, Stock N. Herpes-like virus isolated from neonatal and fetal dogs. *Science.* 1965;148(3675):1341-3.
4. Evermann JF, Ledbetter EC, Maes RK. Canine reproductive, respiratory, and ocular diseases due to canine herpesvirus. *Vet Clin North Am Small Anim Pract.* 2011;41(6):1097-120.
5. Ledbetter EC. Canine herpesvirus-1 ocular diseases of mature dogs. *N Z Vet J.* 2013;61(4):193-201.
6. Appel M. Canine distemper virus in virus infections of carnivores. Elsevier, 1987.
7. Burr PD, Campbell ME, Nicolson L, Onions DE. Detection of canine herpesvirus 1 in a wide range of tissues using the polymerase chain reaction. *Vet Microbiol.* 1996;53(3-4):227-37.
8. Ledbetter EC, da Silva EC, Kim SG, Dubovi EJ, Schward WS. Frequency of spontaneous canine herpesvirus-1 reactivation and ocular viral shedding in latently infected dogs and canine herpesvirus-1 reactivation and ocular viral shedding induced by topical administration of cyclosporine and systemic administration of corticosteroids. *Am J Vet Res.* 2012;73(7):1079-84.
9. Greene CE, Carmichael LE. Canine herpesvirus infection. *Infect Dis Dog Cat.* 2006;4:48-54.
10. Nöthling JO, Hüsey D, Steckler D, Ackermann M. Seroprevalence of canine herpesvirus in breeding kennels in the Gauteng Province of South Africa. *Theriogenology.* 2008;69(3):276-82.
11. Ronse V, Verstegen J, Onclin K, Farnir F, Poulet H. Risk factors and reproductive disorders associated with canine herpesvirus-1 (CHV-1). *Theriogenology.* 2004;61(4):619-36.
12. Ledbetter EC, Riis RC, Kern TJ, Haley NJ, Schatzberg SJ. Corneal ulceration associated with naturally occurring canine herpesvirus-1 infection in two adult dogs. *J Am Vet Med Assoc.* 2006;229(3):376-84.
13. Ledbetter EC, Hornbuckle WE, Dubovi EJ. Virologic survey of dogs with naturally acquired idiopathic conjunctivitis. *J Am Vet Med Assoc.* 2009;235(8):954-9.
14. Ledbetter EC, Kim SG, Dubovi EJ. Outbreak of ocular disease associated with naturally-acquired canine herpesvirus-1 infection in a closed domestic dog colony. *Vet Ophthalmol.* 2009;12(4):242-7.
15. Malone EK, Ledbetter EC, Rassnick KM, Kim SG, Russell D. Disseminated canine herpesvirus-1 infection in an immunocompromised adult dog. *J Vet Intern Med.* 2010;24(4):965-8.
16. Gervais KJ, Pirie CG, Ledbetter EC, Pizzirani S. Acute primary canine herpesvirus-1 dendritic ulcerative keratitis in an adult dog. *Vet Ophthalmol.* 2012;15(2):133-8.
17. Schulze C, Baumgärtner W. Nested polymerase chain reaction and in situ hybridization for diagnosis of canine herpesvirus infection in puppies. *Vet Pathol.* 1998;35(3):209-17.
18. Decaro N, Amorisco F, Desario C, Lorusso E, Camero M, Bellacicco AL, et al. Development and validation of a real-time PCR assay for specific and sensitive detection of canid herpesvirus 1. *J Virol Methods.* 2010;169(1):176-80.
19. Hashimoto A, Hirai K, Yamaguchi T, Fujimoto Y. Experimental transplacental infection of pregnant dogs with canine herpesvirus. *Am J Vet Res.* 1982;43(5):844-50.
20. Miyoshi M, Ishii Y, Takiguchi M, Takada A, Yasuda J, Hashimoto A, et al. Detection of canine herpesvirus DNA in the ganglionic neurons and the lymph node lymphocytes of latently infected dogs. *J Vet Med Sci.* 1999;61(4):375-9.
21. Greene CE, editor. Infectious diseases of the dog and cat. 3rd ed. Elsevier; 2006.
22. Poste G, King N. Isolation of a herpesvirus from the canine genital tract: association with infertility, abortion and stillbirths. *Vet Rec.* 1971;88(9):229-33.
23. Appel MJ, Menegus M, Parsomon IM, Carmichael LE. Pathogenesis of canine herpesvirus in specific-pathogen-free dogs: 5-to 12-week-old pups. *Am J Vet Res.* 1969;30(12):2067-73.
24. Kraft S, Evermann J, McKeirnan A, Riggs M. The role of neonatal canine herpesvirus infection in mixed infections in older dogs. *Compend Contin Educ Vet.* 1986;8(10):688-94.
25. Evermann J. Diagnosis of canine herpetic infections. In Kirk RW. *Current Veterinary Therapy X: Small Animal Practice.* WB Saunders. 1989; p. 1313-6.
26. Hashimoto A, Hirai K, Okada K, Fujimoto Y. Pathology of the placenta and newborn pups with suspected intrauterine infection of canine herpesvirus. *Am J Vet Res.* 1979;40(9):1236-40.
27. Aurelian L. Effect of environmental conditions on formation and release of canine herpesvirus in infected canine kidney cells. *Am J Vet Res.* 1968;29(10):1945-52.
28. Okuda Y, Hashimoto A, Yamaguchi T, Fukushi H, Mori S, Tani M, et al. Repeated canine herpesvirus (CHV) reactivation in dogs by an immunosuppressive drug. *Cornell Vet.* 1993;83(4):291-302.
29. Okuda Y, Ishida K, Hashimoto A, Yamaguchi T, Fukushi H, Hirai K, et al. Virus reactivation in bitches with a medical history of herpesvirus infection. *Am J Vet Res.* 1993;54(4):551-4.
30. Seo IIB, Seong WW, Lim CH. Survey on the seroepidemiology of canine herpesvirus infection in Korea. *Korean J Vet Res.* 1994;34(3):647-52.
31. Gucht SV, Nauwynck H, Pensaert MB. Prevalence of canine herpesvirus in kennels and the possible association with fertility problems and neonatal death. *Vlaams Diergeneeskund Tijdschr.* 2001;70(3):204-211.

32. Lacheretz A, Cognard S. Epidemiologie et diagnostique serologique de l'herpevirose canine. *Rev Med Vet (France)*. 1998;149(8):1477-88.
33. Bujko M, Sulović V, Zivanović V, Lako B, Dotlić R. Effect of progesterone and pregnancy on the replication of herpes simplex virus type 2 in vivo. *Clin Exp Obstet Gynecol*. 1988;15(1-2):34-7.
34. Thiry E, Saliki J, Schwers A, Pastoret PP. Parturition as a stimulus of IBR virus reactivation. *Vet Rec*. 1985;116(22):599-600.
35. Anvik JO. Clinical considerations of canine herpesvirus infection. *Vet Med*. 1991;86(4):394-403.
36. Karpas A, Garcia FG, Calvo F, Cross RE. Experimental production of canine tracheobronchitis (kennel cough) with canine herpesvirus isolated from naturally infected dogs. *Am J Vet Res*. 1968;29(6):1251-7.
37. Binn LN, Eddy GA, Lazar EC, Helms J, Murnane T. Viruses recovered from laboratory dogs with respiratory disease. *Proceedings of the Society for Experimental Biology and Medicine*. *Proc Soc Exp Biol Med*. 1967;126(1):140-5.
38. Leontides L, Ewald C, Willeberg P. Herd risk factors for serological evidence of Aujeszky's disease virus infection of breeding sows in Northern Germany (1990–1991). *Zentralbl Veterinarmed B*. 1994;41(7-8):554-60.
39. Maes D, Deluyker H, Verdonck M, Castryck F, Miry C, Vrijens B, et al. Herd factors associated with the seroprevalences of four major respiratory pathogens in slaughter pigs from farrow-to-finish pig herds. *Vet Res*. 2000;31(3):313-27.
40. McDermott JJ, Kadohira M, O'Callaghan CJ, Shoukri MM. A comparison of different models for assessing variations in the sero-prevalence of infectious bovine rhinotracheitis by farm, area and district in Kenya. *Prev Vet Med*. 1997;32(3-4):219-34.
41. Krogenæs A, Rootwelt V, Larsen S, Renström L, Farstad W, Lund A. A serological study of canine herpesvirus-1 infection in a population of breeding bitches in Norway. *Acta Vet Scand*. 2014;56(1):19.
42. Sykes JE, Greene CE, editors. *Infectious diseases of the dog and cat*. (Saunders, 4th ed.). Elsevier; 2011.
43. Ronsse V, Verstegen J, Onclin K, Guiot AL, Aeberlé C, Nauwynck HJ, et al. Seroprevalence of canine herpesvirus-1 in the Belgian dog population in 2000. *Reprod Domest Anim*. 2002;37(5):299-304.
44. Dahlbom M, Johnsson M, Myllys V, Taponen J, Andersson M. Seroprevalence of canine herpesvirus-1 and *Brucella canis* in Finnish breeding kennels with and without reproductive problems. *Reprod Domest Anim*. 2009;44(1):128-31.
45. Yeşilbağ K, Yalçın E, Tuncer P, Yılmaz Z. Seroprevalence of canine herpesvirus-1 in Turkish dog population. *Res Vet Sci*. 2012;92(1):36-9.
46. Babaei H, Akhtardanesh B, Ghanbarpour R, Namjoo A. Serological evidence of canine herpesvirus-1 in dogs of Kerman city, south-east of Iran. *Transbound Emerg Dis*. 2010;57(5):348-51.
47. Rezaei M, Jajarmi M, Alizadeh R, Khalili M, Babaei H. First molecular study of Canine herpesvirus-1 in reproductive specimens of adult dogs in southeast of Iran. *Comp Immunol Microbiol Infect Dis*. 2020;71:101487.
48. Losurdo M, Dowgier G, Lucente MS, Cinone M, Camero M, Colaianne ML, et al. Long-term shedding of Canine alphaherpesvirus 1 in naturally infected newborn pups. *Res Vet Sci*. 2018;119:244-6.
49. Cargnelutti JF, Masuda EK, Neuls MG, Weiblen R, Flores EF. Outbreaks of canid herpesvirus 1 disease in puppies in southern Brazil. *Pesqui Vet Bras*. 2015;35(06):557-61.
50. Larsen RW, Kiupel M, Balzer HJ, Agerholm JS. Prevalence of canid herpesvirus-1 infection in stillborn and dead neonatal puppies in Denmark. *Acta Vet Scand*. 2015;57(1):1-7.
51. Monteiro FL, Cargnelutti JF, Martins M, Anziliero D, Erhardt MM, Weiblen R, et al. Detection of respiratory viruses in shelter dogs maintained under varying environmental conditions. *Braz J Microbiol*. 2016;47(4):876-81.
52. Piewbang C, Rungsipipat A, Poovorawan Y, Techangamsuwan S. Viral molecular and pathological investigations of 1 infection associated respiratory disease and acute death in dogs. *Acta Vet (Beogr)*. 2017;67(1):11-24.
53. Ledbetter EC, Kim SG, Dubovi EJ, Bicalho RC. Experimental reactivation of latent canine herpesvirus-1 and induction of recurrent ocular disease in adult dogs. *Vet Microbiol*. 2009;138(1-2):98-105.
54. Gadsden BJ, Maes RK, Wise AG, Kiupel M, Langohr IM. Fatal canid herpesvirus 1 infection in an adult dog. *J Vet Diagn Invest*. 2012;24(3):604-7.
55. Pratelli A, Colao V, Losurdo M. Serological and virological detection of canine herpesvirus-1 in adult dogs with and without reproductive disorders. *Vet J*. 2014;200(2):257-60.
56. Bottinelli M, Rampacci E, Stefanetti V, Marenzoni ML, Malmlov AM, Coletti M, et al. Serological and bio-molecular survey on canine herpesvirus-1 infection in a dog breeding kennel. *J Vet Med Sci*. 2016;78(5):797-802.
57. Li Y, Negussie H, Qiu Y, Reddy VRAP, Mateusen B, Nauwynck HJ. Early events of canine herpesvirus 1 infections in canine respiratory and genital mucosae by the use of ex vivo models. *Res Vet Sci*. 2016;105:205-8.
58. Krogenæs A, Rootwelt V, Larsen S, Sjøberg EK, Akselsen B, Skår TM, et al. A serologic study of canine herpes virus-1 infection in the Norwegian adult dog population. *Theriogenology*. 2012;78(1):153-8.
59. Ronsse V, Verstegen J, Thiry E, Onclin K, Aeberlé C, Brunet S, et al. Canine herpesvirus-1 (CHV-1): clinical, serological and virological patterns in breeding colonies. *Theriogenology*. 2005;64(1):61-74.
60. Ström Holst B, Hagberg Gustavsson M, Grapperon-Mathis M, Lilliehöök I, Johannisson A, Isaksson M, et al. Canine herpesvirus during pregnancy and non-pregnant luteal phase. *Reprod Domest Anim*. 2012;47 Suppl 6:362-5.
61. Rota A, Dogliero A, Biossa T, Messina M, Pregel P, Masoero L. Seroprevalence of canine herpesvirus-1 in

breeding dogs with or without vaccination in Northwest Italy. *Animals (Basel)*. 2020;10(7):1116.

62. Yapici O, Avci O, Bulut O, Hasircioglu S, Kale M, Simsek A, et al. Detection of canine herpesvirus infection on dogs. *Microbiol Res J Int*. 2018;24(1):1-6.

63. Lara EGV, Solis JIÁ, Verde CC, Crespo JAM, Marín LC, García JCDR, et al. Canine Herpesvirus Seroprevalence and Associated Factors in Dogs of Mexico. *Open J Vet Med*. 2016;6:149-62.