INTRODUCTION
From the perspective of complexity and variability of pathogenic mechanisms, few ocular diseases are more fascinating than those initiated by viral infections. Because most is currently known about ocular diseases due to feline herpesvirus type 1 (FHV-1) and because it utilizes so many of these pathogenic mechanisms, most attention will be paid to this agent and its disease manifestations. A brief discussion of some historic details, general principles of viral pathogenesis, and specific mechanisms of tissue injury known to induce ocular lesions in animals is provided initially.

A little History
Dorland describes a virus as “a minute infectious agent not resolved in a light microscope, characterized by a lack of independent metabolism and the ability to replicate only within living host cells.” This apparently simple sentence belittles the centuries of scientific endeavor that led up to proving the existence of viruses and establishing those defining characteristics. A brief review of those critical discoveries, especially in context of the understanding (and undoubtedly some ongoing misunderstanding!) we have of viruses today, is fascinating…

“A minute infectious agent not resolved in a light microscope, characterized by a lack of independent metabolism and the ability to replicate only within living host cells”
Dorland’s Medical Dictionary

The relatively small size of viral particles meant that they were not truly “seen” until 1939. It took William Henle - an avante garde scientist and, by the way, the person after whom the Loop of Henle is named - to propose in 1840 that infectious particles too small to see with the light microscope may not only exist but could answer some of the dilemmas with which he was presented. Of course, these were seen as heretical thoughts and not well received! However, not long after this, people with names now familiar to us, such as Pasteur, Koch, and Lister added irrefutable evidence to Henle’s theorem – but for much larger infectious particles - bacteria. Illnesses we now know as caused by viruses remained an enigma for at least another half a century.

Around the turn of the 20th century, using what became known as tobacco mosaic virus, a number of researchers proposed the existence of a “filterable infectious agent” that caused discoloration of the commercially important tobacco plant. The finding that this agent could reproduce only in the presence of living tissue as opposed to bacteria, which could replicate in cell free media was a subsequent and equally critical discovery. Still these proposed agents could not be observed and so were considered to be fluids. Hence the first name for viruses – “contagium vivium fluidum” and the current name “virus” - derived from the Latin word for “slimy liquid or poison”. In 1898, the first animal virus – foot and mouth disease (FMD) virus was discovered. The first human virus – yellow fever virus – was not identified until 1901. However, it was another 40 years before viruses were actually
“seen” for the first time - using electron microscopy (1939). And so some of the early mysteries surrounding the existence and character of viruses began to be unraveled…

It is tempting to believe that these were all experiments conducted in an earlier time when instrumentation and techniques were “primitive” and that these sorts of misunderstandings were to be expected. That couldn’t happen now, could it?

“PCR made it easier to see that certain people are infected with HIV, and some of those people came down with symptoms of AIDS. But that doesn’t begin even to answer the question, “Does HIV cause AIDS?” The mystery of that damn virus has been generated by the $2 billion a year they spend on it. You take any other virus, and you spend $2 billion, and you can make up some great mysteries about it too.”

Kary Mullis [1993 Nobel Laureate and inventor of the polymerase chain reaction (PCR)]

“It’s the virus – stupid!”

David Ho (Scientific Director, Aaron Diamond AIDS Research Center, NY and Time Magazine’s 1996 Man of the Year)

Pathogenesis of viral infections
Pathogenesis is the means or mechanism by which a virus causes disruption of function and sometimes death within a cell, (and sometimes a tissue or the whole organism) that it infects. It is very important that we realize this can often not cause signs of disease in animals or sometimes even symptoms in human beings. This will become critical when we discuss feline herpesvirus type 1 (FHV-1) pathogenesis in cats. In other words, there is an extremely delicate balance that exists between health and disease and between viral virulence and host defenses. Disease results when the virus “wins”. However, with many viruses, we must also be open to the fact that excessive host defense mechanisms may not always be an advantage and can cause immunopathology or proliferative disease. Think of feline infectious peritonitis or infectious canine hepatitis (more on them later).

Recall that, by definition, for a virus to replicate, it must enter a cell and co-opt some of the host cell’s synthetic machinery and energy sources. This makes specific drug targets and organism clearance more difficult. At least a superficial understanding of virus-cell interactions is therefore critical. However, for clinicians, it can sometimes be difficult to understand various cellular and sub-cellular events in virus-cell interactions. One scheme that may assist the clinician involves drawing analogies between similar cellular and “whole host” events (Table 1). Each step in the complex series of events that describes viral entry into, replication within, and release from a cell (or from the host organism) can then be considered a barrier to disease production. When the virus successfully overcomes all barriers or if the host has deficiencies in some barriers, then infection (but not always disease) results. Likewise, each step at the host or cellular level can be seen as a potential target for control or therapeutic measures. Therefore a basic understanding of these steps will assist the clinician in suspecting and diagnosing viral etiologies in their patients, implementing therapeutic and disease control methods, and offering clients an accurate prognosis.

Table 1. Comparison of the stages in viral pathogenesis at a “whole animal” and a cellular (and subcellular) level

<table>
<thead>
<tr>
<th>Whole Animal</th>
<th>Cellular/Subcellular</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entry into host</td>
<td>Adsorption</td>
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<tr>
<td>Primary replication</td>
<td>Penetration</td>
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<tr>
<td>Spread throughout host</td>
<td>Uncoating</td>
</tr>
<tr>
<td>Cell and tissue tropism</td>
<td>Transcription</td>
</tr>
<tr>
<td>Host immune responses</td>
<td>Translation</td>
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<tr>
<td>Secondary replication</td>
<td>Replication</td>
</tr>
<tr>
<td>Cell injury</td>
<td>Assembly</td>
</tr>
<tr>
<td>Persistence/Latency/Genomic integration</td>
<td>Release</td>
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</tbody>
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At the individual patient-specific and population-specific (think “feline shelter” or “herd health”) levels, there are a number of distinct but very closely related clinical considerations:
At a cellular level, infection occurs when the viral particle (virion) finds and attaches (adsorbs) to an appropriate cell membrane receptor, penetrates into that cell, exposes (uncoats) its nucleic acids for transcription and protein production, replicates itself, and then assembles new virus particles ready for release from that cell and potential infection of another cell either nearby or at a secondary location. An essential consideration for the clinician is what injurious events occur intracellularly during this reproductive process?

Stated in these ways, it becomes more clear that “successful” infection of the whole animal (rather than just a “few” cells) with disease production can be considered uncommon and, when it occurs, a reflection of how well evolved that virus is for infection of that host or how susceptible that host is to infection by that virus.

The critical steps for clinicians are cell entry, viral transcription, and virally mediated pathology because these are what define host susceptibility, viral virulence, nature of the disease produced, sites of action for potential treatments, and means of control/prevention. Therefore a little time will be spent on these.

Cell Entry
Viral entry, beginning with virus-cell receptor binding and continuing with cell membrane penetration, is essential if the virus is to gain access to host cell synthetic machinery. Enveloped viruses uncoat (lose their envelope) during penetration due to fusion of the viral envelope and the cell membrane. It is assumed that viruses have evolved to use cell receptors that are present for other reasons and that this, in part, also determines species specificity and cell tropism. Like all interactions with cell membrane receptors, this is undoubtedly a complex series of physical, electrical, and chemical interactions, and may involve multiple receptors and/or permissive “cofactors”. The old “key and lock” analogy is undoubtedly useful but represents a gross over-simplification of this process. Various host defense mechanisms and potential therapeutic endeavors could target this aspect of host-virus interaction. For example, lactoferrin (present in tears) exerts an antiviral effect against FHV-1 and many other viruses, which is quite distinct from its iron-binding antibacterial effect. Rather, it appears to be due to inhibition of viral adsorption to and subsequent entry into the host cell.

Viral transcription
Transcription of viral DNA utilizes host enzymes (“machinery”) and nucleic acids (“building blocks”). This is why viruses are incapable of independent existence and are obligate intracellular pathogens. Some viruses simultaneously down-regulate host cell transcription to ensure better access to this “machinery” and the “building blocks”. Undoubtedly a multitude of complex and very strictly controlled steps are responsible for the success of this process. Although only a few of these are well defined or understood, some have been useful targets for pharmacological intervention. This is discussed more fully in the later section on antiviral therapies. This intimate and intricate relationship between viral and host cell functions during viral transcription results in antiviral drugs being generally more toxic than most antibacterial drugs because they tend to have “overlapping” negative effects on host machinery as well as the viral machinery they are designed to target.

Virally-mediated pathology
Perhaps of most interest to the clinician are the mechanisms by which viruses cause cell injury or dysfunction (and ultimately illness within a patient). Figure 1 is intended to summarize the most frequently encountered of these
mechanisms. In summary, once a virus enters a cell, one of three initial outcomes is possible. The infection may result in cell lysis (and death). This occurs as a result of successful viral replication and coincides with the release of viral progeny from the host cell and therefore is also referred to as a “productive” infection. This is typical of a primary infection with one of the alphaherpesviruses such as FHV-1 or canine herpes virus (CHV) and it is the cell lysis that causes the corneal and conjunctival ulceration visible clinically. Alternatively, viral replication may occur at an extremely low rate, with no or undetectable release of mature virus particles. In this case cell lysis is unlikely however viral antigen production may continue and “drive” an immunological reaction that may be equally or more devastating than cytolysis. This is believed to be responsible for some of the more chronic outcomes of alphaherpesvirus infections such as stromal keratitis in cats (and human beings), and is discussed more fully in that section of these notes. The final alternative is that viral replication is stopped completely (i.e., a non-productive infection results). In a non-productive infection, 3 further outcomes are possible: (1) firstly, the cell may eliminate the virus and continue as before, (2) alternatively the cell may eliminate the virus but subsequently die, (3) the third outcome is of most interest pathologically and results when viral DNA is “sequestered” within the cell with either subsequent oncogenic transformation (e.g., FeLV), or reactivation (e.g., FHV-1).

![Fig 1. Virus-cell interactions and outcomes](image-url)

With that brief introduction to how viruses infect (and sometimes injure) host cells (and sometimes tissues and organs), let’s discuss some specific examples of ophthalmic importance. Unashamedly, the majority of these notes are spent discussing FHV-1, the disease syndromes it causes, and antiviral therapies directed at it.
FELINE HERPESVIRUS TYPE 1 (FHV-1, Feline Rhinotracheitis Virus)

VIRAL FEATURES AND EPIDEMIOLOGY
Feline herpesvirus-1 is a DNA virus taxonomically classified in the Herpesviridae family; subfamily Alphaherpesvirinae. The hallmark biological features of the alphaherpesviruses are:-

- Relatively "tight" species specificity
- A short reproductive cycle
- Rapid spread in culture
- Efficient destruction (lysis) of infected cells
- Capacity to establish latency (especially in sensory ganglia)

While FHV-1 persists for life in latently infected cats, it is extremely labile in the environment. It survives approximately 12 hours in dry environments and approximately 18 hours in more moist conditions and is susceptible to most disinfectants and to desiccation. With respect to multidose vials in your clinic, it is rapidly killed by fluorescein stain and proparacaine; however FHV-1 could be recovered from eyewash for 5 days. Because of this short environmental survival, the major route of infection is via direct transfer of virus-containing macrodroplets between mucosal surfaces (oral, nasal, conjunctival). Of importance to those of us needing to isolate such patients within our hospitals, is the observation that macrodroplets can be sneezed up to 1.3 meters. However it is believed that cats do not form aerosols of any of the respiratory viruses during normal respiratory movements. Likewise, due to the environmental instability of the virus and its susceptibility to most disinfectants, the importance of fomites (most notably our own hands!) can be limited by practicing adequate hygiene between patients.

The structure of FHV-1 is similar to that of other alphaherpesviruses. It consists of 136 kilobase pairs with a long component (104 kb) and short component (30 kb). The nucleocapsid is 1200 Å in diameter and surrounded by a protein coat ( tegument) that is enveloped by glycoproteins. The best studied of all FHV-1-encoded enzymes is thymidine kinase (TK). The FHV-1 TK gene is located approximately 40% along the long unique component of the FHV-1 genome. The location (but not the sequence) of the TK gene is reasonably conserved among alphaherpesviruses. Restriction endonuclease analysis reveals whole-genome cleavage patterns to be well conserved among FHV-1 strains, suggesting that there is little diversity in the FHV-1 genome on a worldwide basis. On the basis of restriction analysis, 3 major FHV-1 genotypes appear to exist (C7301, F2 and C7805). The C7301 appears to be the most common type; represented by 64 of 78 isolates in one study. Little variability is seen in pathogenesis of various viral strains and some of this variability may actually represent different host immune responses to viruses of similar pathogenicity.

The natural host range of FHV-1 appears restricted to domestic and wild felids. This is likely a function of virus entry to the cell and mediated by surface receptor interactions. An estimated 75-97% of the world’s cat population is seropositive, and the virus is considered to be responsible for 45% of all upper respiratory infections.

Viral Replication
Viral replication occurs primarily within epithelium of the upper respiratory tract and eye; principally the conjunctiva, nasal turbinates, and nasopharynx; with more limited replication in corneal epithelium. Like species-specificity, tissue-specificity is likely mediated by virus-host cell surface receptor interactions. The initiation of clinical disease is independent of the presence of conjunctival or upper respiratory flora as shown by infection of germfree cats; and a study of anaerobic bacterial involvement in ulcerative keratitis failed to show coincident presence of FHV-1 DNA [as assessed by the polymerase chain reaction (PCR)] or cultivable virus [as assessed using virus isolation (VI)]. However, the role of secondary bacteria in the severity and progression of ulcerative corneal disease should not be under-estimated.

At the cellular level, adsorption occurs between viral surface glycoproteins and specific cell receptor/s. Evidence shows that cell surface receptors containing heparan sulfate are critical for adsorption of FHV-1 and other related alphaherpesviruses. Subsequent events include uncoating, penetration of the viral capsid into the infected cell, release of viral DNA from the capsid, viral gene expression, transcription, synthesis of viral proteins, and either establishment of latency or assembly of viral progeny, and egress from the infected cell. It is during the process of leaving the cell that the viral progeny obtain their envelope and induce cytolysis.
At least 17 FHV-1-specific peptides and 7 glycoproteins have been identified.\textsuperscript{18} Gene expression during FHV-1 replication occurs in 3 phases; immediate early (IE), early, and late. Products of IE genes are important for the regulation of viral reproduction and latency, and are capable of transactivating heterologous viral and cellular proteins. FHV-1 has 3 major immunogenic glycoproteins (gp) that are present predominantly in the viral envelope.\textsuperscript{27,28} Of these, gD (originally called gp60) is the viral hemagglutinin that elicits HI antibodies.\textsuperscript{29} In addition to being major targets for neutralizing antibodies, viral glycoproteins, particularly gC, are essential for attachment to cells and subsequent adsorption.\textsuperscript{30-32} Use of small interfering RNAs targeting gD reveal how critical it is for infectivity.\textsuperscript{33-35} The article by Maeda et al\textsuperscript{18} provides a more thorough review of properties and functions of FHV-1 glycoproteins.

**Latency**

During acute replication within peripheral epithelial cells, virus particles ascend axons of sensory neurons to the associated ganglia where they establish lifelong latency in the majority (probably about 80\%) of cats.\textsuperscript{36} In the case of ocular, nasal and facial epithelium, the regional sensory ganglion is the trigeminal ganglia.\textsuperscript{37-42} The definition of latency has evolved as new techniques have become available. Originally, viral latency was described as a period of total absence of clinically or histologically detectable inflammation. Virologically, latency describes a period during which virus cannot be cultured; i.e., a non-productive, non-lytic phase. Molecular biology techniques have permitted investigation of viral transcription, which is extremely limited during latency. The major gene expressed during this period is the “latency-associated transcript” (LAT). FHV-1 LAT has been characterized\textsuperscript{43} and identified within trigeminal ganglia but not cornea of cats without signs of ocular disease\textsuperscript{37} suggesting that although virus can be found in normal feline corneas, true corneal latency has not yet been proven. However, the relatively consistent detection of virulent and viable virus in the corneas of normal cats (especially within shelters)\textsuperscript{43} has important clinical implications for corneal transplantation and diagnostic testing in cats.

Periodic reactivation of latent FHV-1 occurs after stress; either physiological (e.g., re-housing, transport, parturition/lactation, etc.), or pharmacological (corticosteroid or epinephrine administration).\textsuperscript{36,44} Corticosteroid administration has been advocated as a method of detecting carriers in endemic populations.\textsuperscript{44} The reactivated virus is believed to descend the same sensory nerve axons to reach the peripheral epithelial tissues again; the so-called “round trip theory”. Clearly this provides a highly adapted and successful means of perpetuating an environmentally labile virus within a host population. This viral reactivation may be associated with varying degrees of recrudescent disease; alternatively viral shedding can occur in the absence of clinical signs. Note that the virus is reactivated while the disease is recrudescent. The molecular aspects of reactivation and recrudescence are reviewed by Maes.\textsuperscript{45}

The so-called “round trip” axonal transport theory led to the assumption that viremia is unimportant in the pathogenesis of FHV-1 in cats. However, in other species infected with herpesviruses (and in cats infected with viruses other than FHV-1), viremia allows dissemination of the virus to all organs, and can cause abortion, encephalitis, disseminated intravascular coagulation, and generalized organ failure.\textsuperscript{46-49} In fact, FHV-1 DNA has been detected in the blood of cats exhibiting clinical signs of herpetic disease\textsuperscript{46-52} as well as apparently normal cats,\textsuperscript{52,53} and in distant sites such as bone or tendon in some cats that underwent mucosal infection.\textsuperscript{54} However, these have not been consistent findings.\textsuperscript{55} We have examined this in cats undergoing experimental primary infection or natural disease presumed to be due to herpetic reactivation.\textsuperscript{56} In that study,\textsuperscript{56} FHV-1 DNA was detected at least once in blood of all cats between 2 and 15 days after inoculation. However, FHV-1 DNA was never detected and live virus was not isolated (cultured) from blood mononuclear cell samples from any adult shelter cat undergoing presumed recrudescent disease. Therefore it appears that a brief period of viremia may be important in the pathogenesis of primary herpetic disease but maybe not during recrudescence.\textsuperscript{56}

**Persistent viral state**

An additional viral state distinct from latency (without disease), and primary or recrudescent cytolytic disease has been proposed for HSV-1 and may be relevant in FHV-1-related disease. This has been called a “persistent” state. The persistent states mimic some aspects of latency – especially the inability to culture viable (viral) virus. However, persistent virus is distinct from latent virus because (a) it is associated with (and likely induces) a chronic inflammatory response by the host and (b) because genes in addition to LATs are expressed. It also differs from recrudescent disease because viral antigens cannot be detected and virus cannot be cultured.\textsuperscript{57} Persistent HSV-1 has been demonstrated in the cornea, conjunctiva, and eyelid skin of mice infected with HSV-1.\textsuperscript{57,58} The role that persistent virus may have in chronic immunopathological diseases associated with FHV-1 such as stromal keratitis, chronic conjunctivitis, herpetic dermatitis, and potentially anterior uveitis requires investigation. However, studies
utilizing PCR in cats known to be infected with FHV-1 but not showing clinical signs have identified viral DNA in trigeminal ganglia, autonomic ganglia, optic nerves, olfactory bulbs, vestibular ganglia, conjunctiva, and corneas of latently infected cats. Although this virus may have been latent, gene expression, antigen immunohistochemistry, and histologic responses were not always assessed. In one study, FHV-1 DNA was detected in almost 50% of corneas of cats without clinical evidence of ocular disease; however none contained detectable LATs suggesting that a state other than latency may explain viral presence in the cornea of these cats. The subsequent finding of virulent and viable virus in the corneas of normal cats (albeit within a shelter) reopens this issue and has important clinical implications for diagnostic testing and corneal transplantation in cats. Figure 2 illustrates and summarizes the viral states associated with latency, and primary, recrudescent, and persistent infections.

![FHV-1 Pathogenesis](image)

**Incubation and shedding**
The incubation period for FHV-1 after initial exposure is 2-10 days but experimentally it has been shown that this and the severity of the disease induced are both dose-dependent. Viral shedding has been observed in the ocular, oropharyngeal and nasal secretions as early as 24 hours after inoculation and can persist continuously for 1-3 weeks. Subsequent, intermittent, shedding is characteristic of the lifelong carrier state.

**Immune Responses to FHV-1**
Serum neutralizing antibody responses occur to several major viral glycoproteins (60, 68, and 105 KD molecular weight). Neutralizing titers are usually of low magnitude (< 1:64) after primary infection and may rise with re-exposure or recrudescent disease. However there is a questionable relationship between titer magnitude and disease status, due perhaps in part to the inability of serological methods to discriminate between exposure to vaccine and wild-type virus. Similarly, correlation between circulating antibody titer and protection from disease is not clear. This may reflect the less major role that systemic humoral immunity is presumed to play relative to that of surface (mucosal) and cell-mediated arms of the immune response. Humoral immunity to FHV-1 has been shown to diminish the severity of infections, but appears incapable of preventing infection or the establishment of latency. Following vaccination, detectable titers appear to persist for at least 4 years and to be associated with a 52% reduction in clinical signs upon re-exposure. Seroconversion may occur faster in FHV-1-naïve cats receiving a subcutaneously-administered attenuated vaccine than in those receiving a modified-live FVRCP vaccine. Vaccines and vaccination are discussed more fully in the section on immunotherapy below.

**COMPARATIVE SIGNIFICANCE**
Infection of human beings with herpes simplex virus type 1 (HSV-1) is almost identical in most respects to FHV-1 infection of cats:

- Primary infection manifests by 5 years of age, most commonly producing self-limiting upper respiratory signs.
- Ninety percent of people are seropositive by age 15, and circulating antibodies are found in 95% of adults.
- Recovery from primary infection is almost always spontaneous.
- In the USA, 30 million people are affected, and 100 million episodes of labial, genital, and ocular infections occur annually. There are five hundred thousand cases of recurrent HSV keratitis annually, and HSV-1 is the number one infectious cause of corneal blindness in developed countries. (*Chlamydia trachomatis* is the leading infectious cause of corneal blindness in developing nations).

These similarities mean that much information can be gleaned from one virus and applied to the other. A recent review By Dr. Maes provides a valuable summary. In the case of therapies, the vast majority of measures used for FHV-1 have been “borrowed” from HSV-1. In some situations their efficacy has actually been tested in FHV-1 and sometimes found to be as expected from data generated with HSV-1. In other cases, these investigations have highlighted the toxicity or poor efficacy (or both) of certain compounds. This reminds us that knowledge gained
from the study and treatment of HSV-1 can improve our approach to cats with FHV-1 only if that information is carefully investigated, thoroughly tested, and judiciously applied.

Whenever we take a drug developed for the treatment of HSV-1 in humans and use it for treating a cat infected with FHV-1 we are making 2 giant leaps of faith unless placebo-controlled, masked, prospective trials of its safety (for cats) and efficacy (against FHV-1) have been performed.

PATHOGENIC MECHANISMS

Primary Infection
Virus replicates in epithelium of nasal mucosa, conjunctiva, tonsil, and turbinates. Tissue damage is due to viral cytolysis. The ability of FHV-1 to induce rapid and severe lysis of susceptible cells presumably is responsible for symblepharon formation in young cats. FHV-1 replicates to a limited extent in corneal epithelium where it can produce dendritic lesions. The cause of the branching pattern is unknown, but is considered to be pathognomonic for herpetic infections of all species. Dendritic corneal lesions occur in a biphasic pattern on days 3 and 12 of primary infection, the latter peak likely reflecting virus released from replication within and rupture of conjunctival epithelium. Little, if any, viral replication occurs within the corneal stroma.

Recrudescent disease
Recrudescent disease occurs following periods of viral reactivation in only some latently infected animals. The severity of disease and the tissues involved in these recrudescent episodes range widely among individuals and even among disease episodes. Conjunctivitis is usually milder and less ulcerative than seen in the acute infection. However, substantial conjunctival thickening and hyperemia can occur secondary to inflammatory cell infiltration. Corneal infections may again involve epithelial tissues, in which case dendritic and later geographic corneal ulceration may be seen, as in primary infections. Stromal involvement is also reasonably common. Data produced for FHV-1 (like HSV-1) suggest that stromal damage is immunopathological (i.e., immune-mediated but not necessarily autoimmune) in origin. Histologic observations from cats with chronic, naturally-occurring stromal keratitis reveal fibrosis, collagen degeneration, and numerous lymphocytes, plasma cells, and macrophages. Experimentally, stromal keratitis does not occur during primary infection unless the normal immune response is suppressed by corticosteroids. In this scenario, the development of stromal keratitis is preceded by chronic epithelial ulceration and delayed viral clearance (with prolonged viral shedding), and acquisition of viral antigen by the corneal stroma. The subsequent stromal accumulation of polymorphonuclear cells, and T- and B-lymphocytes, correlates temporally with the return of normal immune function, eventually leading to stromal inflammation and fibrosis. The events surrounding experimental FHV-1 stromal keratitis are most compatible with a delayed type hypersensitivity response (Th1 cell-mediated, macrophage effector cells). Despite the intensity of study of HSK and the consensus regarding its immunological mechanism, there is still a degree of controversy regarding the antigen responsible for initiating and maintaining the immunological response. The difficulty isolating virus from the cornea, the clinical response of patients to topical corticosteroid application and their lack of response to antiviral therapy, and the so-called immunologically privileged features unique to the cornea have caused some authors to suggest that auto-immunity is responsible. The suggested mechanisms are autoantigens (host antigens altered due to viral infection) or molecular mimicry (i.e., viral sensitization of the immune system to extremely similar but unaltered host antigens). By contrast, the detection of persistent virus in these tissues, and the demonstration of similar mechanisms of inflammation in non-corneal tissues such as eyelid skin and conjunctiva have provided evidence that undetectable viral antigens may be responsible.

FHV-1-Associated Disease Syndromes

Keratoconjunctivitis Sicca (KCS). In one study, experimental FHV-1 infection was associated with decreased STT results in cats. Although the mechanism was not determined, 2 mechanisms were proposed: (i) secretory ductule destruction or obstruction as a result of conjunctivitis, or (ii) destruction of lacrimal acini as a result of viral lacrimal adenitis. More recently, there has been contradictory evidence that FHV-1 infection increases STT values
in association with reduced tear film quality; specifically, reduced tear film break-up time (TFBUT), mucin content of the tear film, and conjunctival goblet cell density (GCD). Following primary experimental infection in cats, TFBUT and GCD remained abnormal for at least 1 month following infection, despite apparent normalization of clinical and histological examination findings suggesting that FHV-1 infection induces persistent qualitative tear film abnormalities that are not detected without measurement of TFBUT or GCD. This suggests that mucinomimetic therapy should continue after apparent clinical recovery from FHV-1 infection and until TFBUT has returned to normal. Despite the remarkable antiviral efficacy of famciclovir, it does not reduce this marked goblet cell depletion suggesting that mucinomimetic therapy should be used in addition to an antiviral drug.

**Corneal Sequestration.** Experimentally, chronic FHV-1 infection can result in corneal sequestration. However, the prevalence of detectable FHV-1 in samples collected from cats with sequestra has varied widely in the clinical setting and the link between FHV-1 and sequestra is not proven to be causative. In the author’s opinion, sequestration is a non-specific response to stromal exposure or damage and FHV-1 is just one possible cause of this. This is borne out by identification of FHV-1 DNA less frequently in sequestra from Persian and Himalayan cats than those from domestic shorthaired cats that had better lid anatomy and function. This suggests that other non-viral causes of sequestration are more likely or prevalent in brachycephalic breeds.

**Eosinophilic Keratitis.** Clinical studies have suggested a link between FHV-1 infection and eosinophilic keratitis, and PCR testing of ocular surface scrapings from cats with cytology-confirmed eosinophilic keratitis has revealed FHV-1 DNA in 55-76% of cases. By contrast, PCR performed on tears collected using a STT strip failed to detect FHV-1 DNA in 10 cats with cytologically-proven eosinophilic keratitis. In one study, FHV-1 DNA was more likely to be detected if corneal ulceration preceded the diagnosis of the eosinophilic keratitis. As with corneal sequestra, the role of the virus in the initiation of this disease has not been determined.

**Symblepharon.** There is little question that symblepharon can be a sequela to severe primary FHV-1 infection. It is commonly seen in young animals, and presumably occurs as a result of widespread epitherial cytolysis with exposure of the conjunctival substantia propria and sometimes also the corneal stroma. It is the author’s belief that FHV-1 is the predominant cause of symblepharon formation in cats and that other infectious agents are unlikely to cause symblepharon formation.

**Uveitis.** HSV-1 is a well-documented cause of uveitis in humans. Given the shared biological behavior of these 2 alphaherpesviruses, we examined the role of FHV-1 in feline idiopathic uveitis. The PCR assay used demonstrated FHV-1 DNA in the aqueous humor of 12/86 cats, all but one of which had uveitis. The same study also used ELISA to examine FHV-1-specific antibody concentrations in aqueous humor and serum. While seropositivity did not vary among cats, intraocular antibody production, as determined by a Goldman-Witmer coefficient (C-value) > 1, was detected only in cats with uveitis. Additionally, a C-value > 8, which is frequently quoted as a more clinically useful indicator of intraocular antibody production, was found only in cats with idiopathic uveitis. A subsequent investigation also demonstrated FHV-1 DNA could be detected in the aqueous humor of cats and more often in the blood of cats with uveitis than those without uveitis. And an experimental inoculation model revealed that viral DNA could be found within, and live virus isolated from, the uveal tract (and retina) of all 4 cats at 6 and 10 but not 30 days after inoculation. Taken together, these data suggest that intraocular FHV-1 infection occurs and that, at least in some cats, stimulates a specific local intraocular antibody response. Because the trigeminal nerve supplies the uveal tract, it is possible that virus may reactivate spontaneously or via induction and arrive in the uvea (and aqueous humor) via the “round trip theory”, as for surface ocular disease. Viral pathogenic mechanisms similar to those reported in surface disease are therefore plausible explanations for the uveal pathology seen. That is, virally mediated cytolysis and immunopathological responses directed at auto or viral antigens are possible. However, proving a casual association remains difficult.

**Dermatitis.** Periodically, FHV-1 has been identified as a cause of dermatological lesions, particularly those involving facial skin of domestic and wild felidae. This is not surprising when one considers the marked epithelial tropism of this virus and the reliability with which HSV-1 causes dermal lesions. Unlike for ocular disease where a high rate of viral shedding in normal cats dramatically reduces the diagnostic utility of PCR, FHV-1 PCR appears to be extremely useful for herpetic dermatitis. In one study, FHV-1 DNA was detected in all 9 biopsy specimens from 5 cats with herpetic dermatitis but in only 1 of 17 biopsy specimens from the 14 cats with nonherpetic dermatitis, and in none of 21 biopsy specimens from 8 cats without dermatitis. When results of histologic examination were used as the gold standard in this study, sensitivity and specificity of the PCR assay were...
100% and 95%, respectively. We concluded that FHV-1 DNA can be detected in the skin of cats with herpetic dermatitis, that the virus may play a causative role in the disease, and that this PCR assay may be useful in confirming a diagnosis of herpetic dermatitis. An Italian group using a different PCR assay to assess biopsies from cats with a variety of eosinophilic dermatopathies found FHV-1 DNA in 12/64 biopsies.

Interactions with Other Agents

There appears to be a real but relatively minor relationship between chronic FHV-1 diseases and co-infection with FeLV and FIV. Cats with chronic FHV-1 infection are more likely to be infected with FeLV or FIV than normal cats. Experimentally, FIV-infected cats exposed to FHV-1 develop more severe disease, however, the length of the illness or level of FHV-1 shedding is not different. In vitro, FHV-1 has been shown to enhance activation of gene sequences of FIV. Co-infection rates of FHV-1 with other agents of upper respiratory disease such as FCV, Chlamydia felis, Bordetella bronchiseptica, and Mycoplasma spp. range widely, and the likely clinical significance of coinfections is not known. No potential interactions between FHV-1, Bartonella henselae, and Toxoplasma gondii in the induction of uveitis in cats have been detected.

DIAGNOSIS OF FHV-1 OCULAR DISEASE

A major paradox exists with respect to the diagnosis of FHV-1. Cats experiencing primary FHV-1 infection shed virus in sufficient quantities that viral detection is relatively easy. However, clinical signs during this phase of infection tend to be characteristic and self-limiting, making definitive diagnosis less necessary. By contrast, during the more chronic FHV-1-associated syndromes, the diversity and ambiguity of clinical signs make viral identification more desirable, especially if specific antiviral therapy is being considered. However, the elusive nature of the virus in these chronic syndromes makes this difficult. Indeed, the diagnosis of FHV-1 in individual cats represents one of the greatest challenges in the management of chronic FHV-1-related diseases.

Although the extreme sensitivity (and specificity) of PCR has improved detection of virus, it has also confirmed that virus can be demonstrated in up to 49% of apparently normal cats. This must be considered when interpreting results of diagnostic assays in individual cats with disease. Additionally, we know that HSV-1 (and therefore possibly FHV-1) can be stimulated to reactivate by irritation of the peripheral sensory neurons. Therefore, it is possible that virus detected at a peripheral site in a diseased animal may be there as a result rather than a cause of the disease being investigated. Finally, PCR assay in common usage can differentiate vaccine from wild-type virus. Therefore, whenever virus is detected in a cat with disease, there are at least 4 possible explanations:

1. Its presence is coincidental (i.e., unrelated to the primary disease process)
2. Its presence is a consequence of the primary disease process
3. It is the cause of the primary disease process
4. It is vaccine virus

Whether virus is found or not (i.e. irrespective of the PCR test result), the clinician must still decide whether specific antiviral treatment is warranted. Currently, other than clinical acumen, there is no “test” that will answer that question!

Perhaps one of the best ways to diagnose FHV-1 is to maintain a strong clinical suspicion of its involvement in any cat with ocular surface disease and to be well aware of its classical clinical features, but to be questioning of its role in that disease process whenever it is detected using any currently available diagnostic assays.

The following discussion highlights suggestive and pathognomonic clinical signs and the role of laboratory assays for FHV-1 diagnosis. Currently-available tests rely on demonstration of an immunological response (usually in serum) to the organism, or detection of whole, cultivable virus by virus isolation (VI), its antigens by immunofluorescent antibody test (IFA), or its DNA by PCR.
Clinical Signs.
Primary ocular FHV-1 infection of the FHV-naïve host is characterized by conjunctival hyperemia (sometimes with conjunctival ulceration), serous ocular discharge that becomes purulent by day 5-7 of infection, mild chemosis, and moderate to marked blepharospasm. Primary infection is always associated with typical signs of upper respiratory infection. The uncomplicated clinical course usually is only 10-14 days; however tear film changes may persist longer than 1 month. The only pathognomonic clinical sign during primary infection is the presence of dendritic lesions, which are inconsistently observed, especially without rose bengal/lissamine green stain. Dendritic corneal ulcers, erosions, or scars are also considered a pathognomonic clinical feature in recrudescent FHV-1 infections.

The major causes of feline keratoconjunctivitis are infectious in nature and the major differential diagnoses are FHV-1 and *Chlamydia felis* (previously *Chlamyphila felis* and, before that, *Chlamydia psittaci*). Based upon Koch’s postulates and epidemiological studies in naturally infected cats, feline calicivirus (FCV) has traditionally been considered an unlikely and minor primary conjunctival pathogen and is not a recognized corneal pathogen. However, cats with marked upper respiratory tract disease due to FCV will sometimes have conjunctivitis as part of that syndrome, especially when they reside in shelters. As a result of the difficulty interpreting diagnostic tests for FHV-1, one of the most important steps in the diagnostic process and instead of requesting laboratory confirmation is “old fashioned” clinical suspicion. Data presented in Table 2 are intended to assist with the distinction of primary infection with *C. felis* or FHV-1. Differentiation of chronic or recurrent syndromes utilizes the same criteria but is more difficult.

Table 2. Clinical signs that may assist with differential diagnosis of the cause of acute keratoconjunctivitis in cats.

<table>
<thead>
<tr>
<th>Clinical Signs</th>
<th>FHV-1</th>
<th>C. felis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conjunctival hyperaemia</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Chemosis</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Conjunctival ulceration</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>Keratitis</td>
<td>+/-</td>
<td>-</td>
</tr>
<tr>
<td>Dendrites</td>
<td>Pathognomonic</td>
<td>-</td>
</tr>
<tr>
<td>Respiratory signs/malaise</td>
<td>++ (primary)</td>
<td>+/- (reactivation)</td>
</tr>
</tbody>
</table>

Cytology.
In humans, the presence of multinucleate epithelial cells is considered compatible with HSV-1 infection. In cats this appears not to be the case. Although intranuclear inclusions are very common during primary FHV-1 infection, they are seen infrequently in clinical cases. The inflammatory cell infiltrate seen in acute FHV-1 infections consists predominantly of neutrophils and is of little diagnostic significance. Intranuclear inclusions are extremely helpful in histologic diagnosis of herpetic dermatitis.

Immunofluorescence Antibody Testing.
Immunofluorescent antibody (IFA or FA) testing was one of the original methods used to detect FHV-1 in cats. Samples for IFA testing can be gathered by conjunctival or corneal scraping or from biopsy sections. Smears should be acetone-fixed to preserve reactive epitopes. The IFA test utilizes an anti-FHV-1 antibody which is directly or indirectly labeled with a fluorescent marker and observed with a fluorescent microscope. Therefore, it is essential to collect samples for IFA testing before the instillation of fluorescein dye, as this will interfere with interpretation of the assay. The principal reported limitations of the IFA test for FHV-1 diagnosis include lack of sensitivity and, like all other viral detection methods, the number of normal cats which are positive by this assay. In one study, the IFA test was positive in 50% of cats in which acute FHV-1 infection was suspected and 36% in which chronic FHV-1 infection was suspected; however 28% of clinically normal cats were also IFA positive. In addition, interpretation of IFA test results requires subjective judgment by the technician and discerning true versus non-specific fluorescence can affect test accuracy. Additionally, because a positive IFA result is dependent upon the identification of a fluorescing cell, sensitivity is greatly diminished unless an adequate number of cells is examined. Finally, a positive IFA test is also dependent upon there being recognizable viral epitopes. In chronic, naturally occurring infections, it is possible that viral epitopes are bound by secretory antibody, and unavailable for binding with the diagnostic antibody. Due to these limitations and the availability of alternate diagnostic methods, IFA
testing appears to be of limited clinical application in ophthalmic practice.

Virus isolation.

Virus isolation (VI) has long been the gold standard for alphaherpesvirus diagnosis. Because the virus replicates so rapidly and produces characteristic cytopathic effects in cell culture, VI is relatively rapid and simple to perform. Although many cell lines may be used, the most common is Crandell-Rees feline kidney (CRFK) cells. Samples should be collected with Dacron swabs with plastic handles since cotton and alginate swabs, as well as wooden swab handles, can be inhibitory to some herpesviruses. Samples must also be collected before application of fluorescein or rose bengal (and likely lissamine green) stains as vital stains can inhibit viral replication. Ideally, samples should be adsorbed onto cells as soon as possible after collection to avoid viral death. If samples cannot be processed immediately, they should be maintained at refrigerator temperature (4°C) pending adsorption. The best approach is to moisten the swab with viral transport media, swab the conjunctival sac aggressively, and break the swab off into a tube containing 1-2 ml of the viral transport media. After vortexing, aliquots of the sample are allowed to adsorb onto confluent layers of CRFK cells, after which the media is replenished, the flask incubated, and observed daily for cytopathic effect. Freezing and re-thawing samples can result in loss of viral titer sufficient to preclude a positive test result. Despite its sensitivity, VI is not routinely advocated for clinical specimens due to the logistical difficulty of transporting and processing samples in a timely fashion. Additionally (and as for other viral detection tests), FHV-1 can be detected in approximately 11%–24% of normal cats and only 0%–18% of cats showing clinical evidence of FHV-1-related disease. Due to the number of normal cats that shed FHV-1, VI is not useful in the diagnosis of individual cats but remains a useful research and epidemiological tool.

Serology.

Unfortunately, serologic titers have not proven useful in the diagnosis of FHV-1 infection. Although there are several reasons for this, 2 major reasons are the inability to separate vaccinal titers from response to wild-type virus and the fact that FHV-1 titers tend to be of low magnitude (especially when measured by serum neutralization), even after primary infection. Interest in vaccinology has stimulated new investigations of the value of titers for predicting resistance to infection.

Serum Neutralization

Depending upon which assay methods are used, serum neutralizing (SN) titers reach only 1:16 - 1:64, 30-60 days after exposure. Although SN titers predictably rise after experimental reactivation of latent virus, titers in cats with recrudescent infections rarely achieve great magnitude. Likewise, no correlation was demonstrated between FHV-1 (SN) seropositivity or titer magnitude and the likelihood of detecting virus or the presence of clinical signs. In a prior study, serum titer was found to be inversely proportional to the likelihood of getting a positive IFA or VI result perhaps confirming that serum antibodies can make virus difficult to detect using VI or IFA.

Immunonassays (ELISA’s)

Immunonassays have been used for detecting HSV-1 antigen or antibody. A commercial ELISA for HSV-1 (Herpchek®) utilizes a polyclonal capture antibody and a biotinylated monoclonal anti-HSV detector antibody and has a sensitivity of 90%, and a specificity of 99% when compared with tissue culture methods. To date, a FHV-1 antigen-capture ELISA has not been developed. However, ELISA’s for the detection of antibodies to viral nucleocapsid and nuclear antigens have been described. These tests are more sensitive than SN and at least one was used to successfully detect FHV-1-specific antibody in aqueous humor and CSF, as well as serum. Seroprevalence, as assessed by ELISA in one study, was high (97%) and did not vary significantly between normal cats and cats suspected to be undergoing acute or chronic FHV-1 infections; nor did it correlate with the likelihood of detecting virus using IFA or VI. In that study, mean ELISA titer was significantly greater in clinically normal cats, while cats with chronic disease had the lowest mean titers, suggesting that IgG titers may be in some way protective. Importantly however, vaccination status was not considered in that study and serologic methods are unable to differentiate between titers arising from natural or vaccination exposure.

Polymerase Chain Reaction (PCR)

The advent of PCR technology has significantly improved detection of ocular pathogens in veterinary and human medicine. The principle of the PCR assay is that small amounts of DNA (conceivably one molecule) can be exponentially amplified under appropriate conditions. The specificity of PCR can be increased through a process termed “nesting”, in which the product generated from the first series of cycles is cycled again in the presence of a second set of primers that identify a shorter, internal sequence of template DNA. Nesting also dramatically
increases PCR sensitivity, often to an extent where the potential for contamination (and consequently the false positive rate) is unacceptably high. This introduces the concept that assay sensitivity and diagnostic sensitivity may be inverse at higher levels of detection of FHV-1 because of the potential for contamination and because of the number of normal animals that shed virus in very low quantities. Based upon in vitro assessment, it appears that (unlike VI or IFA) prior application of a topical anesthetic agent or fluorescein stain does not inhibit PCR results for FHV-1; however these results need to be verified in vivo.128

A large number and variety of PCR assays for the detection of FHV-1 have been described and these have produced a very wide range of data regarding FHV-1 detection in normal and abnormal eyes (Table 3).

### Table 3. Reported PCR detection rates for various tissue types, pathology, and sample types. (Results are reported on a per eye or per sample basis; not on a per cat basis)

<table>
<thead>
<tr>
<th>Tissue and Pathology</th>
<th>Swab/brush</th>
<th>Scraping</th>
<th>Biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal cornea</td>
<td>6%90, 32%31, 46%59, 49%37</td>
<td>3%102, 8%53, 20%100, 31%55</td>
<td>12%59</td>
</tr>
<tr>
<td>Sequestrum</td>
<td>27%85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Feline eosinophil keratitis/conjunctivitis</td>
<td>55%, 87%76%, 60%</td>
<td>18%, 59%, 44%, 51%, 55%60</td>
<td></td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>10%105, 14%55, 18%50, 33%100</td>
<td></td>
<td>54%59</td>
</tr>
<tr>
<td>Diseased but not specified</td>
<td>18%125, 25%65, 33%120</td>
<td>47%108, 58%106, 89%129</td>
<td>91%131</td>
</tr>
</tbody>
</table>

Although these studies were conducted on different populations with different clinical syndromes in different geographic areas, it is likely that some variation is also due to sampling and testing methodologies that might be controlled for by the clinician collecting and shipping the sample and the laboratory processing and reading out the sample. For example, variability among labs has been shown, whereas variation among sampling sites seems less important.106  Minimization of such variation will enable the clinician to better interpret the test result for their individual patient. Not unexpectedly, a major component of variability in PCR test result appears to be attributable to the PCR assay used, with calculated detection rates for the same samples tested with 6 different assays varying from 29-86%, and sample results being concordant in only 9 of 15 conjunctival biopsies.133  The type of sample collected, as well as the way in which the DNA is extracted and the PCR assay is performed also appears to be important.134,135  Although PCR may be used to look for FHV-1 DNA in any biological sample, the test is expected to be more likely to detect virus as more host cells are tested because FHV-1 is an obligate intracellular virus. Therefore, it is expected that biopsy samples are more likely to be positive than samples gathered by surface scraping, which in turn are more likely to be positive than swab samples; however this has not been tested yet. We did however compare cytobrushes and swabs and concluded that a swab was preferable to a cytobrush and that it should be submitted in a dry tube. Once it arrives at the lab, a defined volume (rather than a defined mass) of sample extract should be submitted to PCR.134  By contrast, sample handling and shipping protocols appear to exert less influence on FHV-1 PCR assay results and shipping at ambient temperature (not on ice) seemed appropriate.135

The main “take-home” message is that with shedding of FHV-1 at the peripheral surfaces of up to 50% of normal cats, individual testing seems of little benefit.37  Partly for this reason, quantitative PCR (qPCR) methods for FHV-1 detection have been assessed in experimentally infected animals.136  The authors’ hypothesis was that there is a critical concentration of viral DNA within host tissues that, below which, is likely to represent coincident or consequential virus, and above which is more likely to represent causative virus. The authors demonstrated good correlation between results of conventional methods of quantifying virus (viral titration) and the qPCR. Subsequently, SPF cats experimentally infected for the first time showed gradually reducing viral loads, as has been previously demonstrated using less sensitive techniques, such as VI. Relatively low-cellularity (STT and cytobrush) ocular samples from 20 cats which were demonstrating upper respiratory or ocular signs (keratitis and conjunctivitis) and in which natural FHV-1 infection was suspected then were assessed using the same technology. Reasonable correlation was again demonstrated between viral titer and DNA concentrations and, based on these
data, the authors characterized cats into three “stages” of disease. However, another group has since reported the clinical utility of a qPCR method in cats without history of conjunctivitis, cats with presumed herpetic conjunctivitis that had resolved at least 3 weeks prior to testing and those with active presumed herpetic conjunctivitis. They found no difference among the 3 groups in either the number of cats that tested positive or the amount of FHV-1 DNA detected. Most recently severity of histologically graded rhinitis was found to be significantly correlated with amount of FHV-1 DNA detected using qPCR, however whether these results are repeatable or applicable for ocular signs is not clear.

TREATMENT OF FHV-1 OCULAR DISEASE

Targets for Antiviral Pharmacology.
Conceivably any step in the virus replicative process could be a target for antiviral intervention. These include adsorption, penetration, uncoating, early transcription, early translation, replication, late transcription, late translation, virus assembly, and release from the host cell. To date however, most of the effective antiviral therapies that have been developed target viral enzymes and proteins responsible for DNA synthesis. For a review of many available agents and their proposed mechanisms of action see this article by De Clercq, and for their specific applications in feline medicine, see the review by Thomasy & Maggs.

Specific Antiviral Agents and FHV-1.
FHV-1 is susceptible to inhibition by all commercially available antiviral ophthalmic medications studied thus far, however their safety in cats is not readily predicted from their behavior in other host species (principally humans), and their efficacy against FHV-1 is not predicted from their efficacy against other viruses (principally HSV-1 and -2). In addition, there are no antiherpetic drugs currently approved in the USA for use against FHV-1 in cats. All those in common use are “borrowed” from the human pharmacy.

Whenever we take a drug developed for the treatment of HSV-1 in humans and use it to treat a cat infected with FHV-1 we are making 2 giant leaps of faith unless placebo-controlled, masked, prospective trials of its safety (for cats) and efficacy (against FHV-1) have been performed. For these reasons, careful in vitro investigation of efficacy against FHV-1, followed by safety and pharmacokinetic trials, subsequent placebo-controlled efficacy studies in experimental animals, and finally judicious clinical trials in client-owned animals should always precede widespread clinical use and anecdotal reporting.

Although opinions vary as to when and why antiviral therapy should be instituted in cats believed to be experiencing herpetic diseases, it appears reasonable that antiviral agents should be considered when signs are severe, persistent, or recurrent, particularly when there is corneal involvement, and especially ulceration. Because epithelial replication, latency and reactivation, and persistence are such interdependent and sequential phases of herpetic disease, interruption of any one of them is expected to limit the virus’ abilities to cause subsequent disease. Therefore, aggressive treatment of FHV-1-associated disease may limit disease progression and minimize frequency and severity of recurrences. This seems to be borne out by data that shows a significant direct (positive) correlation between clinical score and viral DNA copy number 30 days after experimental infection, which suggests that the quantity of viral DNA that becomes latently established in the ganglia is related to the severity of the acute clinical infection.

Some important general concepts about antiviral agents assist with prescribing and expectations of this class of drugs:
- Because viruses reside intracellularly and utilize host cellular machinery, antiviral agents tend to exhibit greater host toxicity than do antibacterial drugs. This sometimes limits topical application of these drugs but especially limits their systemic use.
- All antiviral agents to date are virostatic; therefore they require replicating virus and must be dosed (orally or topically) relatively frequently.
- No antiviral drug has proven antibacterial activity.
- Antiviral drugs safe in humans are not necessarily safe in cats.
Antiviral drugs effective against human herpesviruses are not necessarily effective against FHV-1.

Antiviral prodrugs metabolized to their active form by humans are not predictably metabolized by cats.

The effect of some antiviral drugs on FHV-1 replication in vitro has been studied and their potency relative to each other and relative to HSV-1 reported (Table 4). Antiviral efficacy is expressed as the IC_{50} (or the concentration at which viral replication is inhibited by 50%). Note, therefore, that a lower IC_{50} equates to a more efficacious antiviral drug.

### Table 4. Efficacy of various antiviral drugs against FHV-1 and HSV-1. Efficacy is reported as median (range) concentration (µM) at which in vitro viral replication is inhibited by 50% (IC_{50}), therefore a lower IC_{50} equates to greater efficacy. Drugs are ranked (left to right) in order of decreasing efficacy against FHV-1. Note the different ranking for HSV-1.

<table>
<thead>
<tr>
<th>IC_{50} (µM)</th>
<th>HPMPA</th>
<th>IDU</th>
<th>GCV</th>
<th>PCV</th>
<th>PMEDAP</th>
<th>BDVU</th>
<th>TFU</th>
<th>CDV</th>
<th>VDB</th>
<th>ADV</th>
<th>ACV</th>
<th>PFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHV-1</td>
<td>0.23</td>
<td>5.6 (4.3-6.8)</td>
<td>8.9 (5.2-13)</td>
<td>14 (1.2-130)</td>
<td>14</td>
<td>18 (5.1-30.1)</td>
<td>19 (0.67-1350)</td>
<td>19 (7.9-168)</td>
<td>21</td>
<td>73</td>
<td>150 (16-11100)</td>
<td>187 (140-230)</td>
</tr>
<tr>
<td>HSV-1</td>
<td>22</td>
<td>2.1</td>
<td>0.39</td>
<td>1.5</td>
<td>6.9</td>
<td>0.3</td>
<td>0.5</td>
<td>19</td>
<td>30</td>
<td>21</td>
<td>0.8</td>
<td>68</td>
</tr>
<tr>
<td>Ref</td>
<td>139,140</td>
<td>70,72,141</td>
<td>72,142,145</td>
<td>72,139,144,147</td>
<td>142,148</td>
<td>70,139,149</td>
<td>70,139,150</td>
<td>15</td>
<td>42,142,154</td>
<td>72,139,142,144,147,154</td>
<td>49,150,155</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** ACV: Acyclovir; ADV: Adefovir; BDVU: Bromovinyldeoxyuridine; CDV: Cidofovir; GCV: Ganciclovir; HPMPA: (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl) adenine; IDU: Idoxuridine; PCV: Penciclovir; PFA: Foscarnet; PMEDAP: 9-(2-phosphonylmethoxyethyl)-2, 6-diaminopurine; TFU: Trifluridine; VDB: Vidarabine.

The following antiviral agents have been studied to varying degrees for their efficacy against FHV-1, their pharmacokinetics in cats, and/or their safety and efficacy in treating cats infected with FHV-1.

**Idoxuridine** (5-iodo-2'-deoxyuridine; IDU) is a thymidine analogue originally developed for treatment of humans infected with herpes simplex virus (HSV) type 1. It differs from thymidine by having a single iodide substitution at the 5 position of the pyrimidine ring. Following intracellular phosphorylation, it competes with thymidine for incorporation into viral DNA rendering the resultant virus incapable of replication. IDU is a nonspecific inhibitor of DNA synthesis, affecting any process requiring thymidine. Host cells, therefore, are similarly affected, systemic therapy is not possible, and corneal toxicity can occur. It has been used as a topical (ophthalmic) 0.1% solution or 0.5% ointment. This drug is reasonably well tolerated by most cats and seems efficacious in many. It is no longer commercially available in the USA but can be obtained from a compounding pharmacist. No veterinary data regarding dose are available but, based on pharmacologic principles and data from humans, it likely should be applied to the affected eye at least 5-6 times daily. In a retrospective case series of cats with ocular disease attributed to FHV-1, 0.1% idoxuridine solution was used every 4-6 hours with improvement or resolution of clinical signs in 3 cats and no improvement or worsening in 4 cats.156

**Vidarabine** (Adenine arabinoside; 9-β-D-arabinofuranosyladenine; Ara-A®; Vira-A®) Vidarabine is an adenosine analogue that, following triphosphorylation, appears to affect viral DNA synthesis by interfering with DNA polymerase. However, like idoxuridine, vidarabine is non-selective in its effect and so is associated with notable host toxicity – especially if administered systemically. Because it affects a viral replication step different from that targeted by idoxuridine, vidarabine may be effective in patients whose disease seems resistant to idoxuridine. As a 3% ophthalmic ointment, vidarabine often appears to be better tolerated than many of the antiviral solutions. Where it is not available commercially, it can be obtained from a compounding pharmacist. Like idoxuridine, no veterinary data are available regarding dose but it should probably be applied to the affected eye at least 5-6 times daily. In a
retrospective case series of cats with ocular disease attributed to FHV-1, 3% vidarabine ointment was used every 4 to 6 hours with improvement noted in 1 cat and no improvement or worsening noted in 2 cats.

Trifluridine (trifluorothymidine; 5-trifluoromethyl-2′-deoxyuridine; Viroptic®, generic) is a fluorinated nucleoside analogue of thymidine. Its specific mechanism of action against HSV-1 (for which it was developed) is not completely understood and has not been studied in FHV-1. However, following intracellular phosphorylation it reduces DNA synthesis via inhibition of thymidylate synthetase. It is too toxic to be administered systemically but topically-administered trifluridine is considered one of the most effective drugs for treating HSV-1 keratitis. This is in part due to its superior corneal epithelial penetration. In vitro, it is also one of the more potent antiviral drugs for FHV-1. It is commercially available in the USA as a 1% ophthalmic solution that based on human recommendations should probably be applied to the affected eye at least 5-6 times daily. Unfortunately, it is expensive, and often causes marked ocular irritation. In a retrospective case series of cats with ocular disease attributed to FHV-1, 1% trifluridine solution was used every 4-8 hours with improvement in 1 cat and no improvement or worsening in 2 cats.

Acyclovir (9-(2-hydroxyethoxymethyl)guanine; ACV; Zovirax®) is the prototype of a group of antiviral drugs known as acyclic nucleoside analogues. Members of this group of antiviral agents all require 3 phosphorylation steps for activation. The first of these steps must be catalyzed by a viral enzyme, thymidine kinase. This fact increases their safety and permits them to be systemically administered to humans. Unfortunately, the activity of the FHV-1 thymidine kinase enzyme on acyclovir is much less than that of the HSV-1-encoded enzyme, which likely explains the relative lack of efficacy of ACV against FHV-1. The second and third phosphorylation steps must be performed by host enzymes. To my knowledge the affinity of these enzymes for the acyclic nucleoside analogues has not been studied in cats. In addition to relatively low antiviral potency against FHV-1, acyclovir has poor bioavailability and is potentially toxic when systemically administered to cats. Oral administration of 50 mg/kg acyclovir to cats was associated with peak plasma levels of only 33 μM (approximately one third the IC₅₀ for this virus). Common signs of toxicity are referable to bone marrow suppression. With the advent of the apparently safer and more effective famciclovir, administration of acyclovir seems more difficult to justify. Acyclovir is also available as an ophthalmic ointment in some countries which, while it may overcome systemic toxicity concerns, need not necessarily increase antiviral efficacy against FHV-1. Regardless, a 0.5% ointment used 5 times daily in naturally infected cats was associated with a median time to resolution of clinical signs of 10 days. Cats treated only 3 times daily took approximately twice as long to resolve and did so only once therapy was increased to 5 times daily. Taken together, these data suggest that frequent (at least 5 times daily) topical application of acyclovir may produce concentrations at the corneal surface that exceed the IC₅₀ for this virus but are not associated with clinically appreciable toxicity. There are also in vitro data suggesting that interferon exerts a synergistic effect with acyclovir that could permit an approximately 8-fold reduction in acyclovir dose. In vivo investigation and validation of these data are needed.

Valacyclovir (L-valine, 2-[(2-amino-1,6-dihydro-6-oxo-9H-purin-9-yl)methoxy]ethyl ester, monochloride; Valtrex®) is another acyclic nucleoside analogue. As a prodrug of acyclovir, it is - in humans and cats - more efficiently absorbed from the gastrointestinal tract than acyclovir is and, following absorption, is converted to acyclovir by a hepatic hydrolase. Plasma concentrations of acyclovir that surpass the IC₅₀ for this virus can be achieved after oral administration of this drug. However, in cats experimentally infected with FHV-1, valacyclovir induced fatal hepatic and renal necrosis, along with bone marrow suppression, and did not reduce viral shedding or clinical disease severity. Therefore, despite its superior pharmacokinetics, valacyclovir should never be administered to cats. This also reminds us how toxic acyclovir is in cats once a sufficiently high plasma concentration is achieved.

Ganciclovir (Cytovene®, Zirgan®) is another acyclic nucleoside analogue that, like acyclovir, requires triphosphorylation to achieve its active form with the first phosphorylation step mediated by viral thymidine kinase. It appears to be approximately 10-fold more effective against FHV-1 than is acyclovir. It is available for oral or intravenous administration in humans, where it is associated with more severe neurologic toxicity, neutropenia, and bacterial infections than is acyclovir. Like acyclovir, a prodrug of ganciclovir (valganciclovir) is available. To my knowledge, the safety and pharmacokinetics of ganciclovir or valganciclovir have not yet been studied in cats. An ophthalmic gel preparation (Zirgan®; 0.15%) is available for human use but is currently very expensive in the USA. However there are anecdotal reports of good efficacy and tolerability in cats with FHV-1 in Europe (where it is cheaper). Given the in vitro efficacy of ganciclovir, this product definitely warrants efficacy and safety trials in
cats. However, to the author’s knowledge, neither the safety nor pharmacokinetics of valganciclovir or ganciclovir in any form has been reported in cats.

**Penciclovir and Famciclovir**

Penciclovir (9-(4-hydroxy-3-hydroxymethylbut-1-yl)guanine; BRL39123; Denavir®, Vectavir®) is a nucleoside deoxyguanosine analogue with a similar mechanism of action to acyclovir and with potent antiviral activity against a number of human herpesviruses. Like acyclovir, it requires viral and cellular phosphorylation but is highly effective against FHV-1 in vitro and in vivo. In a rabbit model of human HSV-1 keratitis, a 3% penciclovir ointment administered once, twice or four times daily decreased epithelial keratitis severity. Thus, a topical ophthalmic penciclovir ointment may be effective in cats with FHV-1 keratitis and/or conjunctivitis, but, to the authors’ knowledge, there are no commercial or compounded preparations available for ophthalmic use. Penciclovir is available as a 1% dermatologic cream for humans, but that should not be applied to the eye.

Famciclovir (2-(2-(2-amino-9H-Purin-9-yl)ethyl)-1,3-propanediol diacetate; Famvir®) is a highly bioavailable prodrug of penciclovir, which – once absorbed – is metabolized to penciclovir. In humans this metabolism is complex; requiring di-deacetylation to BRL42359, in the blood, liver, or small intestine, with subsequent oxidation to penciclovir by aldehyde oxidase in the liver. Neither famciclovir nor BRL42359 has any in vitro antiviral activity against FHV-1, therefore complete metabolism to penciclovir is required. However, hepatic aldehyde oxidase activity in cats is about 2% of that seen in humans and lower than in any other species reported to date. Famciclovir pharmacokinetics in the cat are extremely complex and nonlinear (i.e., doubling of famciclovir dose does not lead to doubling of plasma penciclovir concentration) presumably due to saturation of the hepatic oxidase. As a result very high plasma concentrations of BRL42359 accumulate in the cat. Fortunately, this compound demonstrates very little cytotoxicity in vitro. Table 5 summarizes the pharmacokinetic data available to date for penciclovir in tears and plasma of cats receiving one of numerous famciclovir dose regimens. Tissue concentration data are not yet available.

**Table 5.** Maximum (C max) and minimum plasma and tear penciclovir concentrations and time to plasma and tear C max in cats administered a variety of famciclovir doses at various dose frequencies.

<table>
<thead>
<tr>
<th>FCV dose</th>
<th>Dose frequency</th>
<th>Plasma [PCV] (ng/mL)</th>
<th>Plasma PCV C max</th>
<th>C ss(min)</th>
<th>Tmax (h)</th>
<th>Tear [PCV] (ng/mL)</th>
<th>Tear PCV C max</th>
<th>C ss(min)</th>
<th>T max (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9-18 mg/kg</td>
<td>BID</td>
<td>330</td>
<td>64</td>
<td>5.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>TID</td>
<td>680</td>
<td>180</td>
<td>3.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>BID</td>
<td>2010</td>
<td>345</td>
<td>1.3</td>
<td>305</td>
<td>65</td>
<td>4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TID</td>
<td>1755</td>
<td>570</td>
<td>1.7</td>
<td>395</td>
<td>160</td>
<td>2.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 mg/kg</td>
<td>BID</td>
<td>1945</td>
<td>445</td>
<td>2.3</td>
<td>375</td>
<td>55</td>
<td>3.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TID</td>
<td>2210</td>
<td>790</td>
<td>2.5</td>
<td>750</td>
<td>150</td>
<td>2.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 mg/kg</td>
<td>BID</td>
<td>2720</td>
<td>630</td>
<td>2.7</td>
<td>680</td>
<td>200</td>
<td>2.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TID</td>
<td>2015</td>
<td>905</td>
<td>2.7</td>
<td>555</td>
<td>275</td>
<td>3.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: BID: twice daily; C max: maximum observed drug concentration; C ss(min): minimum observed drug concentration during the dosing interval at steady state; FCV: famciclovir; ND: not done; PCV: penciclovir; TID: thrice daily; Tmax: time to C max.

In addition to these pharmacokinetic data, recommendation of an appropriate famciclovir dose requires:

- Knowledge of whether penciclovir concentrations in plasma, tears, or the infected tissues themselves are most relevant
- Selection of an appropriate target penciclovir concentration based on in vitro IC_{50} (which reportedly range from 304 to 3500 ng/mL.)
- Knowledge of whether the targeted IC_{50} should be exceeded by the trough or the peak penciclovir concentrations, and for how long.

Together, these uncertainties have led to much controversy about the optimum famciclovir dose in cats, with reported doses ranging from 8 mg/kg once daily to 140 mg/kg thrice daily.
The following data are provided to inform dose selection.

In the only masked, placebo-controlled efficacy trial to date, cats known to be infected with FHV-1 and given approximately 90 mg famciclovir/kg thrice daily per os achieved an approximate peak plasma penciclovir concentration of 2100 ng/mL. Relative to control cats, treated cats had significantly reduced clinical signs, decreased serum globulin concentrations, reduced histologic evidence of conjunctivitis, decreased viral shedding, and reduced serum FHV-1 titers, as well as increased goblet cell density. A subsequent study showed that administration of a single dose of 40 mg/kg to uninfected healthy cats achieved nearly identical plasma penciclovir concentrations to those achieved with a single dose of 90 mg/kg. A third study revealed that cats receiving 40 mg/kg thrice daily had tear penciclovir concentrations likely to be effective against FHV-1 (using a target IC50 of 304 ng/mL) for at least 3 hours after each dose (i.e., for ≥ 9 hours/day). In the most comprehensive pharmacokinetic study to date, healthy cats were administered famciclovir at 30, 40 or 90 mg/kg twice or thrice daily, and plasma and tear famciclovir, BRL42359, and penciclovir concentrations were measured. This resulted in the recommendation that cats should receive 90 mg famciclovir/kg twice daily because this regimen achieved comparable plasma and tear penciclovir concentrations to those achieved with 90 mg/kg thrice daily, whereas the lower doses tested did not result in adequate tear penciclovir concentrations, even when administered thrice daily.

Perhaps most revealing so far, are data from a retrospective study comparing outcomes when famciclovir was administered thrice daily to cats with presumed herpetic disease at approximately 40 (n = 33 cats) or 90 mg/kg (n = 26 cats). Median duration of therapy required for clinical improvement was significantly longer in cats administered 40 versus 90 mg/kg. Furthermore, cats in the 90 mg/kg group showed significantly greater and faster improvement than did cats in the 40 mg/kg group. The reduction in treatment duration with the higher famciclovir dose was estimated to decrease overall client costs due to a reduction in total famciclovir administered (and potentially the number of recheck examinations required). These data, suggest that 90 mg/kg TID is clinically and cost effective. Meanwhile, pharmacokinetic data suggest that tear and plasma penciclovir concentrations are similar whether cats receive 90 mg famciclovir/kg 2 or 3 times daily. Taken together, data from these 2 studies suggest that 90 mg famciclovir/kg twice daily is likely to be effective in treating cats with herpetic disease. These data, combined with those from pharmacokinetic studies suggest that 90 mg/kg twice daily is likely to be effective in treating cats with FHV-1. Adverse events (most commonly gastrointestinal) potentially attributable to famciclovir were reported in 17% of cats receiving 40 or 90 mg famciclovir/kg thrice daily, but the prevalence was not different between the 2 dose groups. Assessing all in vivo tolerance data for famciclovir, this drug appears to be markedly safer than acyclovir and valacyclovir - the only other systemic antiviral drugs to be orally administered to cats. However, patients administered famciclovir should be closely monitored, and assessment of a complete blood count, serum biochemistry panel and urinalysis should be considered in cats with known intercurrent concurrent disease or cats expected to receive famciclovir for long periods. As in humans, reduction in dose frequency should be considered in cats with renal insufficiency.

Cidofovir is a cytosine analogue that requires 2 host-mediated phosphorylation steps but does not require virally-mediated phosphorylation. Its safety arises from its relatively high affinity for viral DNA polymerase compared with human DNA polymerase. It is available in injectable form in the United States and is administered intravenously or intravitreally to humans infected with herpesviruses; principally cytomegalovirus. This drug has also been topically applied as a 0.5% or 1.0% solution in experimental animal models of human herpetic keratoconjunctivitis and found to be equally effective when administered only twice daily as trifluridine was when administered 4-9 times daily. This is believed to be due to the long tissue half-lives of the metabolites of this drug. The efficacy of a 0.5% solution compounded in methylcellulose artificial tears and applied topically twice daily to cats experimentally infected with FHV-1 was associated with reduced viral shedding and clinical disease in a prospective, masked placebo-controlled study. There are occasional reports of its experimental topical use in humans being associated with stenosis of the nasolacrimal drainage system components, and it is not commercially available as an ophthalmic agent in humans. Therefore, although the in vitro and short-term in vivo efficacy of cidofovir against FHV-1 are proven, cats should be monitored for nasolacrimal cicatrization. Unlike most compounded agents, there are also some excellent data on the efficacy over time and using various storage conditions for cidofovir. Cidofovir 0.5% compounded in normal saline (not methylcellulose as was studied in vivo) retained efficacy under all combinations of storage conditions tested: in plastic or glass; refrigerated (4C), or frozen at -20C or -80C; and for 1, 2, 4, or 6 months. Importantly, safety was not studied.

**Lambda-carrageenan** is a red seaweed extract containing sulfated polysaccharides with demonstrated antiviral
activity against numerous enveloped viruses including HSV-1. Activity of λ-carrageenan against FHV-1 replication in vitro and in vaccinated cats exposed for the first time to wild type FHV-1 has been examined in a placebo-controlled masked study.\textsuperscript{183} λ-carrageenan demonstrated some antiviral activity in vitro when used prior to but not following viral adsorption. Safety/tolerance trials revealed no adverse effects in uninfected cats when 1 drop of a 250 µg/mL (or less concentrated) solution was applied topically 4 times per day. Efficacy was tested using 1 drop of a 250 µg/mL λ-carrageenan solution applied before and after infection (n = 6 cats) or after infection only (n = 6 cats). The drug did not reduce clinical signs, regardless of whether it was applied prior to and after infection or only after infection and a significant reduction in virus isolation was noted only on day 21 following inoculation. Other plant extracts with antiviral activity have also been studied in vitro.\textsuperscript{184}

**Leflunomide** is an immunosuppressive agent that appears to have some antiviral effects against human herpesviruses including HSV-1.\textsuperscript{185} Its effects against FHV-1 in vitro have been reported.\textsuperscript{186} Simultaneous inoculation of CRFK cells with virus and treatment with leflunomide was associated with a significant and dose-dependent reduction in plaque number and - at higher concentrations - viral load. However (curiously) viral load was not reduced in a dose dependent manner at very low concentrations of this drug. At higher concentrations, some cytotoxicity was observed. Electron microscopy revealed an apparent failure in viral assembly, especially of the tegument and external membrane, which may indicate the mode of action of this drug.\textsuperscript{186}

**Lactoferrin** is a mammalian iron-binding glycoprotein that has antibacterial, antifungal, antiprotazoal, and antiviral properties. It is produced by mucosal epithelial cells of many mammalian species and is present in secretions such as tears, saliva, seminal and vaginal fluids, and in low concentrations in plasma. Breast milk, especially colostrum, is a major source of lactoferrin. Lactoferrin has been demonstrated to exert a very potent antiviral effect against FHV-1 replication in vitro.\textsuperscript{2} Further analysis of the data in this study reveals that lactoferrin appears to inhibit FHV-1 adsorption to the cell surface and/or penetration of the virus into the cell. At a molecular level, this may occur due to interaction of lactoferrin with receptors on the cell surface or by direct neutralization or inhibition of the viral particle.

The interferons (IFNs) are a group of cytokines that have diverse immunological and antiviral functions. The IFNs are divided into 4 groups; α, β, γ, and ω interferons, and numerous subtypes. Viral infection stimulates cells to secrete IFN into the extracellular space where it binds to specific receptors on neighboring cells and, through mechanisms not fully understood, prevents or limits the spread of infection (i.e., it is not virucidal). Knowledge and consideration of this mode of action allows the clinician to set and maintain reasonable expectations of how effectively the IFNs may work when used therapeutically, and in which patients with which stage of disease they might be expected to be most effective.

Although IFNs may play important physiological roles in the control of viral infections, in vitro and clinical trials investigating potential therapeutic applications have produced conflicting and not solidly supportive results. In in vitro studies, $1 \times 10^5 - 5 \times 10^5$ IU/mL of recombinant human IFNα or recombinant feline IFNω significantly reduced FHV-1 titer and/or cytopathic effect while not producing any detectable cytotoxic changes in the feline corneal cell line\textsuperscript{187} or CRFK cells\textsuperscript{188} on which the virus was grown. At higher concentrations, the effect of the recombinant feline IFNω was greater than that of IFNα.\textsuperscript{188} In a separate in vitro study, notable synergistic activity against FHV-1 was demonstrated when 10 - 62.5 µg/mL of acyclovir was combined with 10 or 100 IU/mL of human recombinant IFNα. The combined use of the 2 compounds did not cause increased cytotoxicity but permitted a nearly eightfold reduction in the dose of acyclovir required to achieve maximal inhibition of FHV-1. Significant synergistic interactions resulted when the IFNα was given before or after infection at the lower doses of acyclovir; however pretreatment was more effective.\textsuperscript{159}

To my knowledge, there are relatively few peer-reviewed, placebo-controlled, prospective clinical trials of IFN administration to SPF cats experimentally infected with FHV-1 (the gold standard for the other antiviral products described here). One study utilized 10,000 IU of recombinant feline IFNω administered topically (OU) q 12 hours and 2,000 IU administered PO q 24 hours.\textsuperscript{189} Based on data generated in studies utilizing other viruses and knowledge of the mode of action of IFNs, IFN administration in this feline study\textsuperscript{189} was initiated 2 days prior to viral inoculation but was not continued after inoculation. No beneficial effects were shown. The effects of very high-dose systemic administration of IFNα prior to experimental FHV-1 infection have also been studied; $10^9$ IU/kg were administered subcutaneously BID on two consecutive days prior to inoculation.\textsuperscript{190} Although disease was not prevented, cumulative clinical scores were lower for cats treated with IFNα. An abstract has also been presented.
was effective. However, intracellular delivery of these agents is essential but proving complex. While delivery effect and warranted further study. A subsequent study of siRNAs that targeted the glycoprotein and DNA polymerase genes of FHV-1 showed that the siRNAs directed against the FHV-1 glycoprotein D (gD) gene had some antiviral effect and warranted further study. A subsequent in vivo study assessed efficacy of some additional siRNAs alone or in combination and revealed that combined use of siRNAs targeting the FHV-1 DNA polymerase and gD genes was effective.

Two additional areas of controversy exist regarding IFN administration. Firstly, the stability of INFα once thawed appears to be very limited and should be addressed with respect to dispensing practices for this drug. There has also been some concern raised regarding the efficacy of this drug when given orally. Since IFN is a simple peptide, it is presumably digested by gastric peptidases. However, the proposed route of absorption following oral administration is across the oropharyngeal mucosa, from where it is believed to be active in surrounding and distant lymphoid tissue. The dissemination of IFN activity is believed to occur via direct cell-to-cell transfer of the antiviral state. This provides a mechanism whereby low concentrations of IFN are able to produce potent antiviral activity even at sites distant from the route of entry, and are amplified via a cascade effect.

Small (or short) interfering RNAs (siRNAs) are short (about 20 nucleotide), double-stranded sections of RNA designed to transfect a cell and interfere with (“knockdown”) expression of specific gene(s). To overcome the short-lived effect of transfection of native siRNAs, they can be incorporated into plasmids and thus extend their longevity, especially within rapidly dividing cells. Three studies have now been published investigating the effects of these agents on FHV-1. An initial in vitro study of siRNAs that targeted the glycoprotein and DNA polymerase genes of FHV-1 showed that the siRNAs directed against the FHV-1 glycoprotein D (gD) gene had some antiviral effect and warranted further study. A subsequent in vitro study assessed efficacy of some additional siRNAs alone or in combination and revealed that combined use of siRNAs targeting the FHV-1 DNA polymerase and gD genes was effective.

Immunotherapy. A commercial hyperimmune serum containing antibodies against FCV, FHV-1, and feline panleukopenia virus is available in Europe for treatment of cats with upper respiratory disease and has been assessed in a randomized, placebo-controlled, double-masked clinical trial. Forty two cats received hyperimmune serum (n = 22) or saline (n = 20) for 3 consecutive days as well as symptomatic treatment. The serum was administered subcutaneously once daily and topically into eyes, nostrils, and mouth every 8 h. Clinical signs were recorded daily for 8 days and again on day 21. Clinical signs in both groups improved significantly over time (P < 0.001) with treated cats improving earlier (Day 3) than placebo-treated cats (Day 7). In a separate study, 2 days post inoculation with FHV-1, 9 cats received placebo (n=3), or 10 (n=3) or 30 (n=3) mg/kg of chimeric antibodies developed against FHV-1 in hybridoma cells. Treated cats at both doses showed remarkable reduction in clinical signs. Pre-treatment of cats 15 days before inoculation had similar results.

Probiotics. To date, I am aware of one prospective, placebo-controlled study of probiotic use for FHV-1 infection in cats. Cats were experimentally infected with FHV-1 for another study, and then administered as a dietary supplement an immune-enhancing probiotic (Enterococcus faecium strain SF68) or placebo. All cats in both groups showed such minimal evidence of disease that a statistically significant difference between groups could not be demonstrated.
**Anti-inflammatory therapy.** The use of anti-inflammatory drugs in the management of FHV-1 infections remains controversial. However, a return to basic virology and a review of the literature makes some comments possible. Recall that FHV-1 produces disease by (at least) two very different mechanisms that require markedly different (almost opposite) therapeutic approaches. Cytolytic infection represents active viral replication and is often ulcerative. Immunomodulation at this point is almost certainly contraindicated. By contrast, immunopathological (or immune-mediated) injury (such as feline eosinophilic keratitis) is mediated by host inflammatory responses “driven” by persistent viral and/or auto antigens.

The important therapeutic implication is that the immune-mediated disease may involve no or low-grade viral replication and so antiviral agents may not be effective as sole therapeutic agents. However, anti-inflammatory therapy may be indicated (presumably in addition to antiviral agents) for the treatment of immunopathological herpetic diseases.

**Corticosteroids**

Systemic administration of corticosteroids is a well-established and reliable means of inducing viral reactivation from latency. This must be considered whenever these drugs are considered in the clinical management of other disease in FHV-1-infected cats. The ability of locally-administered corticosteroids to exacerbate self-limiting primary conjunctival infection and to sometimes induce chronic herpetic keratitis has also been well established. Complications seen in corticosteroid treated eyes included deeper and more persistent corneal ulcers, corneal edema, corneal vascularization, sequestrum or band keratopathy formation, and protracted viral shedding. Topical corticosteroids are therefore contraindicated in primary ocular FHV-1 infection. The specific adverse effects of corticosteroids in ocular viral infection are diverse and include inhibition of RNA and DNA, suppression of protein synthesis, decreased antigen presentation by T cells, decreased production of IL2, and impairment of macrophage and cellular immune functions. Therefore, from a basic virological standpoint, corticosteroid administration is difficult to support if active viral replication is occurring. However, because corneal opacification in chronic stromal keratitis occurs secondary to an exuberant immune response, corticosteroids may be indicated in such cases. It would seem prudent, however, to always treat concurrently with a topical antiviral.

**NSAIDs and Cyclosporine**

The potential complications from using corticosteroids have prompted interest in the use of non-steroidal anti-inflammatory drugs (NSAIDs) for managing the inflammatory effects of ocular FHV-1 infection. Although flurbiprofen has been shown to exert some in vitro antiviral effect, HSV-1 clinical trials have demonstrated equivocal efficacy. In fact, in rabbits, flurbiprofen has been shown to exacerbate viral keratitis. Due to its relatively specific T-cell suppression, cyclosporine therapy has been investigated but has produced equivocal results. Although cyclosporine is capable of suppressing inflammatory events operative in viral stromal keratitis, this drug is also capable of impairing viral clearance from the eye, and beneficial immune responses. In vitro, cyclosporine exerts a dose dependent effect on HSV-1 replication. In some experimental model systems, however, cyclosporine therapy resulted in more severe and persistent keratitis. In clinical trials, cyclosporine and trifluridine were used in combination to treat HSK in humans with good results, and cyclosporine was used (often along with other therapies) for cats with eosinophilic keratitis, typically with good results. Other than that, use of NSAIDs and cyclosporine in chronic feline herpetic disease has been inadequately studied and I am unaware of any studies examining the effects of tacrolimus on ocular herpetic infections in any species. This suggests that use of these agents should, as a minimum, be restricted by the same principles that govern the use of corticosteroids in HSK.

**Lysine Therapy.**

Lysine is perhaps the best studied and yet maybe one of the more controversial of all of the other compounds with proven or putative antiviral efficacy against FHV-1 in cats. As with the antiviral drugs, initial interest arose from in vitro data and clinical trials in humans. Lysine’s antiviral effect is believed to arise because arginine is an essential amino acid for FHV-1 and HSV-1 replication, and assumes that lysine antagonizes arginine availability to or utilization by these viruses during protein synthesis. This was hypothesized to affect protein synthesis of the virus more than the host because viral proteins had a higher arginine-to-lysine content than did human (and feline) proteins. And recent analysis suggests that the difference in feline versus FHV-1 protein amino acid content is minimal. This may explain why in vitro replication of HSV-1 and FHV-1 is notably suppressed in the presence of markedly elevated lysine concentrations in combination with exceptionally low arginine concentrations, but that this is not borne out with more physiologic amino acid concentrations. Alternate mechanisms may
induce fatal encephalopathy. By adopting a common protocol of administering lysine to client-owned cats, it is should not be advised to restrict their cat’s arginine intake. Feeding a single arginine-deficient meal to a cat can demonstrate that oral lysine supplementation helps to prevent viral reactivation and/or shedding in latently infected cats. Despite significant elevations in plasma lysine concentration, no change in plasma arginine concentration or any ill effects attributable to lysine administration were observed in either study (other than perhaps some mild and reversible GI disturbance in Stiles’ study). I am aware of only one study in which bolus administration of lysine has been tested in naturally infected cats. This study examined the effects of lysine in 144 cats residing in a humane shelter. Cats received oral boluses of 250 mg (kittens) or 500 mg (adult cats) of lysine once daily for the duration of their stay at the shelter and outcomes were compared with those of an untreated control group. No significant treatment effect was detected on the incidence of infectious upper respiratory disease (IURD), the need for antimicrobial treatment for IURD, or the interval from admission to onset of IURD. However, it was not determined if and to what extent these cats were shedding or infected with FHV-1 (or other pathogens).

Bolus administration of lysine is sometimes impractical, for several reasons. Cats frequently require very protracted therapy (life-long in some cases), and often live in multiscat environments in which all cats should be treated. In some multicat situations, twice-daily oral administration techniques may stimulate further viral reactivation through stress or facilitate transfer of infectious organisms among cats by operators. Thus, we studied the safety and efficacy of incorporating lysine into cat food. Results of an initial safety trial were encouraging. Cats fed a diet supplemented with up to 8.6% (dry matter) lysine showed no signs of toxicity, had normal plasma arginine concentrations, and had normal food intake. Mean plasma lysine concentration of these cats was increased to levels similar to that achieved with bolus administration. In a subsequent study, 25 cats with enzootic upper respiratory tract disease were fed a diet supplemented to 5.1% lysine while 25 cats remained on a basal ration (~1% lysine). The study was conducted for 52 days at the beginning of which cats were subjected to rehousing stress (which is known to cause viral reactivation). Ironically, food (and therefore lysine) intake decreased coincident with peak disease and viral presence. As a result, cats did not receive lysine at the very time they needed it most. Perhaps partly because of this, disease in cats fed the supplemented ration was more severe than that in cats fed the basal diet. In addition, viral shedding was more frequent in cats receiving the supplemented diet. To further assess these unexpected outcomes, we performed a similarly designed experiment in a local human shelter with a more consistent “background” level of stress and with greater numbers enrolled compared to the initial rehousing study. We enrolled 261 cats; each for 4 weeks. Despite plasma lysine concentration in treated cats being greater than that in control cats, again more treated cats than control cats developed moderate to severe disease and shed FHV-1 DNA at certain points throughout the study.

In summary, there is considerable variability among these studies, especially with respect to methodology, study population, and dose and method of lysine administration. However, taken together, data from these studies suggest that lysine is safe when orally administered to cats and, provided that it is administered as a bolus, may reduce viral shedding in latently infected cats and clinical signs in cats undergoing primary exposure to the virus. However, the stress of bolus administration in shelter situations may well negate its effects and data do not support dietary supplementation. Unfortunately, no clinical trials have been conducted in the very group in which this drug is commonly used - client-owned cats with recurrent herpetic disease. Currently, I let clients help me decide whether they feel a trial of lysine in their cat was effective. I always administer L-lysine at 500 mg twice daily, and ensure that clients do not add it to food for animals to “graze” over an extended period. I also make owners aware that this is usually only an adjunctive or palliative therapy and that administration of antiviral drugs may also be necessary to gain better control of signs. Unlike the protocol for HSV-1-infected humans, owners of cats receiving lysine for FHV-1 should not be advised to restrict their cat’s arginine intake. Feeding a single arginine-deficient meal to a cat can induce fatal encephalopathy. By adopting a common protocol of administering lysine to client-owned cats, it is hoped that well designed, double-masked studies in clinically relevant populations will be forthcoming. Such data would be especially relevant if generated in populations with variable vaccination history, intercurrent disease, physiologic stresses, and high turnover of cats of diverse genetic composition and with varied exposure to infectious diseases.
Vaccination.
Vaccination plays a critical role in reduction of clinical signs attributable to FHV-1 in immunologically naïve animals. However, its role in treatment of FHV-1 infection remains controversial. A number of the basic virological issues already introduced, along with data from duration of immunity (DOI) studies bear particular relevance to discussions regarding the benefits and risks of FHV-1 vaccination. Because FHV-1 generally causes self-limiting disease with high morbidity but rare mortality, the benefits of vaccination must outweigh this relatively low risk.

The perfect vaccine for FHV-1 would prevent clinical evidence of disease and block establishment of latency in cats challenged with wild-type virus for a long period following vaccination. Additionally, it would do this without causing adverse side effects and without establishing latency and being shed itself. Currently, such a vaccine does not exist.

Vaccination Route, Immunology, and Safety
Typical vaccination strategies for FHV-1 have included use of modified-live, inactivated, or genetically modified virus administered by injection (subcutaneously or intramuscularly) or topically (conjunctivally and/or intranasally). (By the way, some topical vaccines were sometimes mislabeled as “intraocular” vaccines!) Topically administered vaccines appear to have certain merit in the prevention of FHV-1 because the virus gains entry, replicates, causes pathology, and recurs at mucosal sites and because mucosal associated lymphoid tissue (MALT) of the conjunctiva and respiratory tract fulfills distinct and frequently advantageous immunological functions (Table 6).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Vaccine Route</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mucosal</td>
</tr>
<tr>
<td>Onset of immunity</td>
<td>Rapid</td>
</tr>
<tr>
<td>Post-vaccinal sarcoma</td>
<td>Not recognized</td>
</tr>
<tr>
<td>Post vaccinal disease</td>
<td>2-4%</td>
</tr>
<tr>
<td>Potential for spread of vaccine virus</td>
<td>Possible</td>
</tr>
<tr>
<td>Generation of mucosal immunity</td>
<td>+</td>
</tr>
<tr>
<td>Generation of systemic immunity</td>
<td>+</td>
</tr>
<tr>
<td>Anamnestic response demonstrated</td>
<td>?</td>
</tr>
</tbody>
</table>

Mucosal vaccination induces systemic immunity and mucosal immunity both at the site of inoculation and at distant mucosal sites. The onset of mucosal immunity is relatively rapid, with development of partial immunity within 2 days and complete immunity in approximately 4 days. In addition, it is suggested that maternal immunity is less likely to interfere with mucosal vaccination than systemic vaccination allowing relatively early vaccination of kittens believed to be at risk. When compared with injectable vaccines, mucosal vaccination is believed to be associated with a higher but infrequent (1.6-4.3%) rate of usually mild side effects. Additionally, mucosal administration of vaccines would not be expected to induce postvaccinal sarcoma formation, whereas the development of postvaccinal sarcomas that are spatially and temporally related to trivalent (panleucopenia/ calicivirus/ herpesvirus) vaccine administration has been reported. There may also be some additive effect from simultaneous mucosal and subcutaneous vaccine administration relative to subcutaneous administration alone. Finally, there is some early evidence that cats vaccinated with a modified live intranasal FHV-1/feline calicivirus vaccine developed nonspecific protection against subsequent challenge with the completely unrelated pathogen—Bordetella bronchiseptica.

Vaccination and Latency
Although, some vaccines have been suggested to decrease viral load within the trigeminal ganglia, a vaccine that prevents disease or establishment of latency has not been developed. In fact, there are some disturbing data that suggest some vaccines may themselves become latent within and reactivate from the ganglia. Cats in this study were vaccinated topically with a commercially-available, temperature-sensitive mutant virus and monitored for FHV-1 shedding using VI and PCR for 13 weeks. During this period, half were challenged with a field strain of FHV-1 at 28 days post vaccination, and viral reactivation was induced in all cats at 56 days post vaccination. All cats shed the vaccine virus from oropharyngeal or conjunctival tissues at multiple times following vaccination; most
for the duration of the experiment (13 weeks). Vaccination did not significantly reduce the amount of virus shed following challenge with wild type virus, and vaccine virus comprised 32% of all virus shed. Vaccination also did not significantly reduce the amount of virus shed following reactivation. At necropsy, vaccine virus was identified in the same tissues and at the same frequency as wild type virus. Persistent or latent virus was detected in neuronal (trigeminal ganglia, optic nerve, olfactory bulb) and extra-neuronal (cornea, nasal turbinates, and tonsil) sites. Taken together, these data suggest that this intranasal vaccination did reduce clinical evidence of disease but did not limit establishment of latency, reactivation from latency, or viral shedding following challenge. The finding that vaccine virus itself establishes latency and is shed from conjunctival fornices and the oropharynx suggests that it could be a source of antigen and thereby cause or exacerbate chronic herpetic disease, infect in-contact cats, or be detected by diagnostic assays and alter their interpretation. Another pilot study assessed FHV-1 shedding 7 days prior to and 28 days following intranasal vaccination and found it to be infrequent during both time periods.

Vaccination and Disease Severity
Given, the questionable ability of vaccination to act as an epidemiological control tool via inhibition of latency, the major role for FHV-1 vaccination appears to be diminishing disease severity. Most vaccine studies have tended to emphasize the dramatic reduction in severity of clinical disease when animals are challenged at varying periods after vaccination. A study of duration of immunity for FHV-1 vaccination administered subcutaneously to young kittens provides further interesting information. While antibody titers appeared to wane rapidly between 6 and 7.5 years post vaccination, cats showed a rapid and exaggerated anamnestic humoral response upon challenge - even in the absence of a detectable pre-challenge titer. Even 7.5 years after vaccination, clinical evidence of disease following challenge was reduced by approximately half and fatalities were completely prevented. Importantly though from an epidemiological standpoint, vaccination did not reduce the amount or duration of virus shed following challenge. I am unaware of any reports demonstrating that vaccination is beneficial in cats that are already infected with FHV-1; however this is an area of growing interest, especially in light of early evidence that intranasal vaccines may infer resistance of a nonspecific nature.

FELINE INFECTIOUS PERITONITIS

Virus Features
FIP is taxonomically positioned in the family Coronaviridae. This is a large enveloped RNA virus, and the largest of all RNA genomes. For a review see the paired articles by Pedersen. No citation available yet. In that article, Pedersen succinctly summarizes the frustrations regarding this virus: “After a half century, FIP remains one of the last important infections of cats for which we have no single diagnostic test, no vaccine and no definitive explanations for how virus and host interact to cause disease. How can a ubiquitous and largely non-pathogenic enteric coronavirus transform into a highly lethal pathogen? What are the interactions between host and virus that determine both disease form (wet or dry) and outcome (death or resistance)? Why is it so difficult, and perhaps impossible, to develop a vaccine for FIP? What role do genetics play in disease susceptibility?” FIP has now also been diagnosed in ferrets.

The internal mutation theory, whereby a ubiquitous feline enteric coronavirus (FECV) mutates into a FIPV, is strongly supported by the literature, and at least three specific mutations have now been associated with the FECV-to-FIPV biotype conversion. The working hypothesis is that the FECV-to-FIPV transition involves positive selection for mutants that are increasingly fit for replication in macrophages and unfit for replication in enterocytes. The ultimate target cell is a distinct population of precursor monocytes/macrophages that have a specific affinity for the endothelium of venules in the serosa, omentum, pleura, meninges and uveal tract. Variability in genetic susceptibility has been identified in at least one breed of cats, but the genetics appear to be highly complex and cannot explain the entire disease incidence. Rather, it seems likely that FIP results from a confluence of numerous viral, host and environmental cofactors.

Pathogenic Mechanisms
Infection with the FECV results in mild, self-limiting, and presumably often unnoticed gastrointestinal signs. In a small percentage of cats mutation to the FIPV occurs. Although FIP can affect cats of any age, it is diagnosed most commonly in cats less than 3 years of age and especially from 4-16 months of age. Diseases is seen most commonly in cats living within multicat settings. Mortality is extremely high although some cats can live with the disease for weeks to months. In cats with relatively mild presenting signs of the dry form of FIP, the 1-year survival
rate was 5%. The basic pathology involves vasculitis and associated pyogranulomatous perivascular inflammation, both of which are thought to be due to an Arthus-type reaction (virus-antibody-complement). The immunopathogenesis of FIP is extremely complex and still not well understood. In his latest review, Pedersen provides an articulate summary: “It is widely assumed that immunity, when it occurs, is largely cell-mediated and that the production of antibodies is counterproductive. Antibodies enhance the uptake and replication of FIPVs in macrophages and also contribute to a type III hypersensitivity (antibody-mediated or Arthus-type) vasculitis. It is also assumed that much of the pathology occurring in FIP is associated with how macrophages respond to viral infection and how the immune system of the host responds to the infected cells. In this scenario, the effusive form of FIP results from a failure to mount T cell immunity in the face of a vigorous B cell response. At the opposite extreme, cats that resist disease presumably mount a vigorous cell-mediated immune response that is able to overcome any negative effects of antibodies. Cats with the dry form of FIP represent an intermediate state involving a cellular response that is partially effective in containing the virus to a relatively small number of macrophages in a few focal sites within specific target organs. The two forms of FIP are somewhat interchangeable; when it has been observed in experimental infection, the dry form always follows a brief bout of effusive disease. In the terminal stages of naturally occurring dry FIP, immunity can completely collapse and the disease reverts to a more effusive form. Although this scenario fits what is known about FIP, it must be emphasized that much of this scheme awaits confirmation and there are large gaps to be filled.” Ocular lesions occur more commonly with the dry form of FIP, result from the same mechanisms operative systemically, and are estimated to occur in 25% of infected cats. The principal target tissue is the uveal tract, particularly the choroid; the optic nerve may also be involved. Characteristic clinical signs are aqueous flare, keratic precipitates, iris swelling and color changes, hyphema, and retinal hemorrhages, perivascular exudates and detachment.

**Diagnosis of FIP**

Due to cross reactivity between the enteric coronavirus, serology is not useful as a sole test for diagnosing FIP infection. Rather, histopathological and immunohistochemical examination of affected tissues has long been considered the only reliable form of diagnosis. A large retrospective study appraised the commonly used diagnostic tests for FIP and calculated their positive (PPV) and negative (NPV) predictive values with respect to histopathologically confirmed cases. Individual serologic and hematological tests all had low diagnostic value. However, in cats with clinical signs suggestive of FIP, the presence of lymphopenia, hyperglobulinemia, and an antibody titer of 160 or greater had a PPV of 89%, whereas the absence of all 3 of these findings had a NPV of 99%. Nested RT-PCR directed toward a unique gene coding for peplomer protein E2 of the FIP virus produced sensitivity and specificity of 91.6% and 94% respectively. However, no diagnostic assay at this stage reliably differentiates FeCV from FIP virus. This, along with knowledge that FeCV (and FIP) can be detected in circulating blood cells, means that results of so-called “FIP” PCR conducted on aqueous humor samples collected from cats with uveitis must be interpreted with caution.

**FELINE IMMUNODEFICIENCY VIRUS**

**Virus Features**

The taxonomic classification of FIV is the family - Retroviridae, Subfamily - Lentivirinae, Group - Lentiviruses. FIV consists of a 100-nm diameter, enveloped virion. The genome consists of single-stranded RNA.

**Pathogenic Mechanisms**

*FIV Retinopathy.* FIV can cause geographic areas of thinning of the sensory retina, characterized ophthalmoscopically by hyper-reflectivity. Flame hemorrhages may also be present. Histologic findings are degeneration of all retinal layers with minimal inflammation. The lesion has been seen in naturally and experimentally infected cats, suggesting it is due to FIV and not opportunistic infections. Lesions occur during periods of higher viremia. These lesions have developed during the acute phase of infection (first 6 months) in experimentally infected cats, and during the AIDS-like stages in natural infection. The pathogenesis of this lesion is probably the same as the microvascular retinopathy that occurs in HIV infection, and the neurological degeneration that occurs in FIV infected cats and HIV infected humans. Two hypotheses have been proposed.

1. Neurotoxin; both FIV and HIV are capable of enhancing excitatory amino acid toxicity in cultured fetal neurons. This toxicity can be blocked using N-methyl-D-aspartate (NMDA) receptor antagonists. Glial cells must be present however for the toxicity to occur, suggesting the effect of the virus is indirect and mediated by
glial cells. These viruses do not appear to infect neurons, but can infect microglia, and possibly astrocytes.

2. The second hypothesis is that FIV/HIV results in increased vascular permeability and altered hemodynamics. The finding of cotton wool spots and retinal hemorrhages suggests vascular compromise does occur, but the pathogenesis of these lesions and their importance remains unclear.

**FIV Anterior Uveitis.** FIV is capable of causing anterior uveitis that can be transient or chronic and may persist despite anti-inflammatory therapy and lead to secondary glaucoma. Mononuclear inflammatory cell infiltrate adjacent to the base of the iris and anterior ciliary body are characteristic (but of course not pathognomonic) histologic features. The pathogenesis is unknown. Lappin et al suggests that opportunistic Toxoplasma infection plays a role.236 It is not known if this is a primary FIV-induced lesion or predominantly the result of opportunistic disease. FIV can be isolated from the aqueous humor of infected cats.

**FIV-Associated Anterior Hyalitis (vitritis).** An accumulation of WBC's in the anterior vitreous, especially around the lens zonules, may be seen in FIV infected cats, often producing a "snow bank" appearance.237 Although the lesion is not pathognomonic, most cats are FIV positive. The lesion is often an incidental finding and not associated with discomfort or other signs of anterior segment disease. Lens luxation and secondary glaucoma can result. Histologically these cells have been shown to be predominantly plasma cells. The pathogenesis of this lesion is not known but presumably arises from a pars planitis.

**FIV-Associated Conjunctivitis.** Mild, self-limiting conjunctivitis may be seen in acute FIV infection. FIV infection may also worsen the course of FHV-1 infection.97,238

**FIV-Associated Anisocoria.** Mild to moderate mydriasis may be seen in association with FIV infection, and both motor and sensory abnormalities have been described. The changes are usually permanent and slowly progressive. The pathogenesis is not known, but possibly related to CNS disease. Similar problems have been seen in HIV patients.

**FIV-Associated Neoplasia.** FIV-infected cats have a much higher rate of B-cell lymphoma than uninfected cats. These tumors tend to occur at atypical sites such as the eye or CNS, and can occur without lymph node or bone marrow involvement. Intestinal and renal lymphomas have developed in experimentally-infected SPF cats. The lymphomas examined to date using virus culture, PCR, and flow cytometry have been negative for virus, as is typical for HIV. The pathogenesis is unknown, but may be secondary to chronic type 1 immune activation occurring after HIV infection. A study that utilized nested PCR failed to demonstrate FIV DNA in any of 18 feline globes enucleated due to histologically confirmed uveal melanoma.239

**FELINE LEUKEMIA VIRUS**

**Virus Features**
FeLV is in the family Retroviridae, subfamily Oncovirinae, Group Mammalian C-Type. It has an enveloped RNA genome.

**Pathogenic Mechanisms**

**Systemic.** Viremia seeds virus to lymphoid and epithelial tissue, and bone marrow. Clinical signs are related to ability of the virus to induce hematological changes, immunosuppression, and tumor formation.

**Ocular.** The only ocular changes definitively linked to FeLV are retinal dysplasia, tumor formation, retinal hemorrhages, and bizarre pupillary changes.240-246

**Retinal dysplasia:** Exposure of fetal and neonatal kittens to FeLV can result in retinal dysplasia, which occurs secondary to diffuse retinal inflammation. Infection causes progressive disorganization and necrosis of retinal tissues, with subsequent reorganization into cell clumps and dysplastic rosettes. RPE proliferation and migration into the sensory retina were also described. The mature retina demonstrated full thickness folds and tubes associated with folding of the RPE. After experimental inoculation, retinal tumor formation is also possible.

**Anemic retinopathy:** Retinal hemorrhages have been seen in cats with FeLV and were originally attributed to
anemia. However a prospective study of the prevalence of retinal hemorrhage in dogs with anemia, thrombocytopenia, neither, or both reveals that anemia alone is not associated with retinal hemorrhage.\textsuperscript{247} Thus, further assessment of FeLV-infected cats with this finding is warranted.

\textbf{Lymphosarcoma:} Lymphosarcoma, especially of the anterior uvea, but also involving the conjunctival and retrobulbar tissues reflects the oncogenic effect of this virus. This is sometimes seen in the absence of identifiable tumor elsewhere in the body.

\textit{“Spastic Pupil” Syndrome:} Although the data supporting this are limited, in all probability this represents FeLV infection of the autonomic nerves (parasympathetic portion of CN III). A wide variety of spontaneously changing pupillary abnormalities is possible.

\textbf{Corneal Latency:} In a study of normal corneas, FeLV DNA was demonstrated (using PCR) in 65\% (11/17) FeLV-positive cats (as determined by p27 ELISA) and none of 17 FeLV-negative cats.\textsuperscript{248} Virus was localized to corneal epithelium using immunohistochemical staining techniques. This information must be considered when selecting suitable donor cats for corneal grafting procedures.

\section*{FELINE PANLEUKOPENIA}

\textbf{Virus Features}  
This virus is in the family Parvoviridae, Genus - Parvovirus. Characteristics: Non-enveloped virions, icosahedral symmetry, 18-26 nm in diameter, virions have 32 capsomeres. Replication and assembly take place in the nucleus. Virions are very stable.

\textbf{Pathogenic Mechanism.}  
Dysplastic ocular development has been attributed to intrauterine infection of kittens with the panleukopenia virus.\textsuperscript{249} In one case report of a 6-week-old kitten, lesions were characterized by retinal thinning, loss of the normal architecture, and rosette formation.\textsuperscript{250} The virus was recovered from the cerebrum. Cerebellar hypoplasia was also present. Hydrancephaly has also been reported in association with perinatal panleukopenia infection.\textsuperscript{251} The optic nerves were small, with increased perineural connective tissue.

\section*{FELINE SARCOMA VIRUS (FeSV)}

\textbf{Virus Features}  
Taxonomic classification: Family - Retroviridae, Subfamily - Oncovirinae, Group - Mammalian C-type. Horizontally transmitted oncogenic RNA virus. Replication-defective variant of FeLV.

\textbf{Pathogenic Mechanisms}  
\textit{Experimental Sarcoma Virus Uveitis.} In experimental studies, FeSV induced anterior uveitis 37 days after subcutaneous injection.\textsuperscript{252} Iritis, posterior synechia, and cataracts were seen. The cellular infiltrate was lymphocytic and plasmacytic. Detection of large quantities of virus in the aqueous humor suggests that intraocular viral replication occurs, but inflammatory changes have been attributed to virus-antibody interactions, or a cell-mediated immune response.

\textit{Experimental Sarcoma Virus-Induced Uveal Melanoma.} Intracameral injection of FeSV induces formation of uveal melanoma in cats.\textsuperscript{253,254} Tumors develop between 40 and 60 days after injection, and are composed of spindle and epithelioid cells. The tumors eventually enlarge to fill the anterior chamber. The posterior segment is involved only late in the course of the disease. More recently, nested PCR was used to examine globes enucleated due to feline uveal melanoma. DNA common to FeLV and FeSV was detected in only 3/36 globes.\textsuperscript{239}

\textit{FeSV-Associated Fibrosarcomas.} Fibrosarcomas of the eyelids in young cats have been associated with the oncogenic FeSV virus.\textsuperscript{243} However, in a study using PCR and immunohistochemistry, no association between FeLV or FeSV and ocular sarcoma could be demonstrated in biopsies from 6 cats.\textsuperscript{255}
**CANINE HERPESVIRUS**

**Virus Features**
Taxonomic classification: Family - Herpesviridae (DNA), subfamily Alphaherpesvirinae.
Host Range: Canine only. Viral replication is similar to FHV-1.

**Pathogenic Mechanism**
The virus shows marked host specificity for canidae. Traditionally, CHV-1 infection in dogs has been described as causing one of two relatively clearly defined syndromes believed to depend almost exclusively on the age of the affected dog – severe and often fatal systemic disease (sometimes with ocular signs) in young puppies, and mild conjunctivitis, vaginitis, or upper respiratory disease in adults.

Young, immunologically naïve puppies are believed to become infected in utero, or following perinatal contact with contaminated reproductive and respiratory secretions. Experimental infection of newborn puppies is associated with keratitis, uveitis, retinal necrosis and optic neuritis. The lesions are the result of virally-induced inflammation. Experimentally infected puppies develop panuveitis with the presence of intranuclear inclusion bodies within 4 days of infection. Necrosis, retardation of maturation, and reorganization into folds and tubes occur throughout the retina. Depigmentation and vacuolization of the RPE, with subsequent folding and hypertrophy are also seen. In some animals, ectopic retina was found within cystic spaces in the optic nerve. Death in puppies may occur from disseminated viral replication in parenchymal organs and the CNS. Like FHV-1 infection, lifelong neural latency is believed to occur in many affected animals with immunosuppression causing reactivation. By contrast, clinical signs in adults are typically very mild and include transient vaginitis, mild upper respiratory disease, or conjunctivitis.

However, reports have suggested that this clear age-related demarcation is not necessarily reliable with older dogs getting the fatal systemic forms of the disease and puppies and older getting mild ocular disease. For example, an adult dog receiving chemotherapy for lymphoma experienced the severe disseminated syndrome usually seen in puppies infected with CHV-1. Meanwhile, corneal disease has been described in 3 adult dogs with naturally-acquired CHV-1 infection. These dogs had classic dendritic ulcers, as well as nonspecific signs of ocular surface inflammation, which responded to cessation of topical immunomodulating drugs and initiation of topically administered antiviral agents. CHV-1 was isolated from ocular swabs. All dogs had altered local and/or systemic immunity (diabetes mellitus, KCS, topical administration of corticosteroids or cyclosporine, etc.). These cases suggest that these unusual presentations may, at least in part, be due to more regular local (ocular) and systemic immunosuppression of pet dogs as a result of or during treatment of other chronic diseases. However it is unlikely to be that simple. For example, the typical fatal form of hepatic necrosis described originally in only young puppies has been described in an apparently immunocompetent adult (9yo) dog. Likewise, a natural outbreak of CHV-1-induced ocular disease was described in a colony of 27 juvenile dogs but in the absence of the usual fatal systemic clinical syndromes and in which no source of immunosuppression was noted. Although, in affected dogs within the colony, marked bilateral conjunctivitis was a consistent sign (sometimes with petechiae), many dogs also developed ulcerative (26%) - often dendritic (19%) - or perilimbal stromal nonulcerative keratitis (19%). Antiviral treatment was not initiated. Another adult dog was presented with partial limbal stem cell deficiency and neurotrophic keratitis subsequent to a protracted episode of CHV-1 dendritic ulcerative keratitis (metaherpetic disease).

Despite these striking clinical reports and the susceptibility of the canine cornea to CHV-1 infection in vitro, canine herpetic disease and shedding at the ocular surface still appear to occur infrequently when compared with the prevalence of FHV-1 on the feline cornea or conjunctiva. For example, samples collected from 50 dogs without surface ocular disease and 50 dogs with non-dendritic corneal ulcers all were negative when tested for the presence of CHV-1. In a separate virologic survey assessing the prevalence of CHV-1 (and 6 other viruses) in conjunctival samples from dogs with or without conjunctivitis, no virus (of any type) was ever detected in dogs without conjunctivitis. In dogs with conjunctivitis, CHV-1 (5 dogs) or CAV-2 (2 dogs) was detected by VI and/or PCR occasionally but significantly more commonly than in normal dogs. Further evidence of the degree of natural resistance to corneal, systemic and severe recurrent ocular disease in most dogs is provided by a study in which adult SPF beagles were infected with CHV-1 with or without prior disturbance of the corneal epithelial surface and/or subconjunctival corticosteroid administration. Dogs developed mild to moderate bilateral conjunctivitis, shed virus, and seroconverted but corneal disease was not noted, epithelial disturbance or corticosteroid administration did not worsen signs, and all dogs spontaneously recovered without evidence of spontaneous
reactivation or recrudescence for 224 days post inoculation. 
Likewise, a single fraction 50 Gy radiation dose administered to the ocular surface by strontium-90 beta radiotherapy did not result in detectable recurrent ocular CHV-1 infection in mature dogs with experimentally induced latent infection.

By contrast, pharmacologically induced reactivation appears to be important in the pathogenesis of this virus, but highly dependent upon drug chosen and route administered. Dogs latently infected with CHV-1 via experimental inoculation 8 months previously and subsequently administered prednisolone systemically (3 mg prednisolone/kg/day x 7 days) experienced bilateral ocular disease (83%), viral shedding (50%), and 4-fold elevations in serum CHV-1 SN titers (100%). The same dogs where stimulated 12 months later (20 months after inoculation) by using topically applied 1% prednisolone acetate (1 drop OU QID x 28 days) and did not experience viral reactivation (detectable by quantitative PCR or SN) or recrudescent disease (detectable by slit lamp examination or ocular confocal microscopy).

When the potent immunosuppressive agent cyclophosphamide was administered at 200 mg/m² to mature dogs latently infected with CHV-1, evidence of myelosuppression was noted on Day 7, but no ocular signs suggestive of recurrent CHV-1 were noted clinically or with confocal microscopy, and ocular CHV-1 shedding was not detected by PCR. Finally, 8 mature Beagles with experimentally-induced latent CHV-1 infection proven to be reactivatable when prednisolone was systemically administered received 0.2% cyclosporine ointment in both eyes twice daily for 16 weeks. This failed to produce detectable virus at the ocular surface or signs of herpetic disease. (For a recent review of the ocular effects of this virus see the article by Ledbetter EC.)

To date, very little work has been done to define preferred antiviral drugs for dogs with herpetic disease. In his original case series, Ledbetter recounts cases improving while on topically applied idoxuridine or trifluridine, and a separate case report describes improvement of a dog treated with cidofovir. In a subsequent controlled study, the in vitro efficacy of cidofovir against CHV-1 was established, and a 0.5% solution was applied topically BID for 14 days in dogs with experimentally-induced recurrent ocular CHV-1 infection. Although cidofovir displayed similar in vitro antiviral activity against CHV-1 and HSV-1, and treated dogs had significantly reduced durations of ocular viral shedding compared to the vehicle group, ocular disease scores were significantly higher in dogs receiving cidofovir compared to those in the vehicle group. Marked conjunctival pigmentation and blepharitis were also detected in the cidofovir group, but not the vehicle group. Conjunctival and corneal leukocyte infiltration scores determined by in vivo confocal microscopy were significantly higher in the cidofovir group. By contrast, a placebo-controlled experimental infection model revealed that trifluridine applied 4-6 times daily reduced clinical signs and viral shedding in latently infected dogs undergoing pharmacologic reactivation.

CANINE DISTEMPER VIRUS (Carre's Disease, 1905)

Virus Features
Taxonomic classification: Family - Paramyxoviridae (RNA), Genus - Morbillivirus.
Structure: Negative strand RNA virus. Consists of 2 structural modules; an internal ribonucleoprotein core (nucleocapsid) containing single stranded viral RNA genome, and an outer spherical lipoprotein envelope.

Pathogenic Mechanism
General. Direct cytolysis. Target tissues are epithelial and neural.

Systemic. Route of infection is via the respiratory tract. Virus then spreads from oropharynx to regional lymph nodes and tonsil. Viremia disseminates virus to lymph tissues throughout the body. Virus persists in epithelial tissues of dogs unable to mount a protective immune response.

Ocular. Tissues affected are conjunctival epithelium, lacrimal gland, retina, optic nerve and optic tracts. In the earliest report, retinitis, optic neuritis, and destruction of the optic tracts were described. Acute retinitis is characterized by congestion, edema of the nerve fiber layer and inner plexiform layer, and perivascular cuffing. Intranuclear inclusions may be seen in retinal glia. Sequelae are optic nerve degeneration, and focal to diffuse areas of retinal degeneration characterized ophthalmoscopically in the tapetal fundus by hyperreflectivity, and by RPE pigment loss in the non-tapetal fundus. Distemper inclusions have also been found in the ciliary body of a dog. In a subsequent retrospective study, 41% (9/22) of dogs with distemper were noted to have retinochoroiditis, and 54% were positive by IFA testing of conjunctival smears.
CANINE ADENOVIRUS 1 (Infectious Hepatitis Virus, Rubarth's Disease, 1947)

**Virus Features**


**Pathogenic Mechanisms**

*Systemic.* Natural infection occurs via the oropharynx, with subsequent spread to regional lymph nodes. Principal tissues involved are the liver, kidney, lymph nodes, and vascular endothelium.

*Ocular.* Viral invasion of the eye occurs in acute infection. Mild anterior uveitis results from virus replication in vascular endothelium. Corneal lesions developed between 7 and 21 days after experimental subcutaneous inoculation.281 Moderate to severe iridocyclitis was the consistent finding in all eyes that developed edema. The presence of a loose fibrin clot in the anterior chamber was a consistent finding in opaque eyes. Using IFA, viral antigen was identified in the vascular endothelium of the iris, trabecular endothelial cells, and macrophages. Virus was also found in corneal endothelium. Uveal infiltrate includes lymphocytes and plasma cells. Relatively low levels of virus were recovered from the eyes compared to other organs.

Ocular damage has been suggested to be predominantly mediated by a Type III immune response (Arthus reaction).282,283 Twenty-four dogs were inoculated with ICH; 9 dogs received virulent virus subcutaneously, and 15 dogs received attenuated virus intravenously. After 4-8 days, aqueous humor was aspirated and the anterior chambers injected with 0.2 ml of anti-ICH serum. Fourteen of 24 dogs developed eye disease, and in 2, corneal edema was severe. Purified viral antigen and anti-ICH serum was then injected into the anterior chamber of 8 dogs; intense iritis with severe edema occurred. The reaction was found to be more severe if complement was added to the immune complexes. In a subsequent study, viral replication was found to occur consistently in the corneal endothelium.284 Virus infection was found to occur not by contagious spread, but as the result of endothelial adsorption of virus that was released into the aqueous humor. It was concluded that parenteral inoculation with ICH does not in itself cause severe or permanent corneal endothelial cell dysfunction.

*Prevalence.* Ocular disease is estimated to occur in 20% of naturally occurring cases, and in 0.4% of dogs given modified live vaccine. Afghan hounds have been suggested to be predisposed to more severe ocular lesions after inoculation with attenuated CAV-1 vaccine than are beagles.285

EQUINE HERPESVIRUS

**Virus Features**

Family: Herpesviridae.

To date, five genetically distinct equine herpesviruses (EHV) have been definitively identified: EHV-1 through EHV-5. As more accurate molecular techniques are used, it becomes obvious that previous reports often failed to differentiate between some of these viruses. Until relatively recently, EHV-1 and 4 were considered subtypes 1 and 2, respectively of EHV-1, while EHV-2 and 5 were not treated separately in the literature until around 1988, and some papers since then have continued to inadequately differentiate the two viruses.286,287 Interpretation of older data is therefore often difficult or impossible.

Although equine herpesviruses are frequently incriminated as ocular pathogens, little is known about their true role, and much evidence is based upon response to therapy. In fact, Koch’s postulates have rarely been demonstrated for the ocular signs currently blamed on the equine herpesviruses.288,289 A survey of ocular lesions and herpesvirus serology in a herd of 266 Lipizzaner horses in Austria sheds further light on the prevalence of these viruses but reinforces the difficulty of associating them with ocular syndromes.286 EHV-2 DNA was identified in 167 (62.8%) of the horses and EHV-5 in 136 (51.1%) horses; 105 (39.5%) were co-infected with EHV-2 and EHV-5. Horses carrying EHV-2 or EHV-5 DNA were significantly younger than the negative group. No significant association between any ocular disease pattern and presence of EHV-2 or EHV-5 was detected. A closely related study in the...
same population of horses revealed no association of ocular lesions and herpesviral infection status when horses were examined serially every 6 months for 18 months. A smaller study in Northern California assessed 12 horses suspected of having herpetic ocular surface disease and 6 normal horses. Prevalence of EHV-1, -2, -4, and -5 DNA as assessed by qPCR did not differ significantly between control horses and those with idiopathic keratoconjunctivitis. Neither EHV-1 nor EHV-4 DNA was detected in any normal or affected horse; EHV-2 DNA was detected in 2/12 affected horses but in no normal horses. EHV-5 DNA was commonly found in ophthalmically normal horses and horses with idiopathic keratoconjunctivitis. Taken together these data suggest that (just as in the cat) diagnostic testing in individual cats is of extremely limited value.

Antiviral Efficacy
Topically applied antiviral agents have been used to successfully treat herpetic keratitis in the horse, although no drugs are currently labeled for equine use. These drugs have undergone little testing with respect to their efficacy against the equine herpesviruses particularly EHV-2; however a recent review is comprehensive and very helpful for the alphaherpesviruses (EHV-1, -3, and -4) but has little information regarding EHV-2 and -5. When equine-specific data are not available, expected efficacy and application frequency are usually extrapolated from data generated in humans infected by HSV-1 and cats with FHV-1. Some general comments gained from those sources are likely to apply to horses and equine herpesviruses. All of the currently available antiviral agents are virostatic in action and therefore should be applied frequently. Although the treatment regimen recommended for humans (two-hourly application throughout the waking hours until the ulcer has healed) is usually not possible in horses, as frequent application as possible is recommended and therapy should be initiated early in the disease course. A minimum of four applications daily is probably indicated. The antiviral agents are variably toxic to corneal and conjunctival epithelium. In some cases this is noted as an apparent stinging reaction when the medications are applied. In more marked situations, delayed ulcer healing may occur.

There have been some studies assessing the in vitro antiviral efficacy against equine herpesviruses of some of the agents commonly used for human and feline herpesviruses. These are reviewed by Vissani. For EHV-1, these can be summarized (from most to least effective) as: ganciclovir ≅ cidovir > acyclovir ≅ penciclovir > adefovir > foscarnet > brivudin. The majority of clinical reports for ophthalmic disease describe topical application of either idoxuridine or trifluridine for the treatment of herpetic keratitis in horses. Some reports suggest antiviral drugs developed for HSV-1 and used in the treatment of FHV-1 are effective. Acyclovir given orally to horses is poorly bioavailable and reaches plasma concentrations that do not exceed the in vitro IC50 for EHV-1. However, oral administration of the acyclovir prodrug – valacyclovir – achieves concentrations within the sensitivity range of EHV-1. In spite of these promising results, studies in horses experimentally infected with EHV-1 have not yielded consistently positive data. Plasma penciclovir concentrations achieved after horses received a single oral dose of 20 mg famciclovir/kg were sufficient to exceed the IC50 for EHV-1. However, to my knowledge, none of these drugs have been tested in a prospective placebo-controlled therapeutic efficacy trial and tear pharmacokinetics have not been studied as they have in cats. The latter parameter seems particularly relevant in horses given that equine herpetic keratitis often lacks a robust vascular response. None of the antiviral agents has known antibacterial properties and therefore horses with ulcerative keratitis should probably be treated with a topical antibiotic agent in addition to any antiviral agent.

Clinical Syndromes
Ocular signs associated with equine herpesviruses may be divided into three broad categories:

- **Primary ocular disease in the absence of systemic signs** - for example, keratoconjunctivitis due to EHV-2 infection or chorioretinitis due to EHV-1.
- **Ocular signs secondary to neurological disease** - for example, keratitis secondary to facial nerve paralysis or blindness secondary to cerebral vasculitis seen with EHV-1.
- **Ocular signs as a minor and self-limiting component of a more serious upper respiratory syndrome** - such as mild and transient conjunctivitis seen in horses with severe rhinopneumonitis due to EHV-1 or -4 infection.

Keratoconjunctivitis
Herpetic conjunctivitis is seen in association with respiratory disease syndromes following infection with EHV-1 or -4, and alone or with keratitis with EHV-2. Clinical signs are non-specific and all may occur as outbreaks. In the case, of EHV-1 or -4, bilateral conjunctivitis is usually a relatively minor feature of a significant respiratory infection. Younger animals are more severely affected.
In horses with keratitis/conjunctivitis or both (in the absence of respiratory signs), some data seems supportive of a role for EHV-2. For example, the prevalence of EHV-2 in horses with keratoconjunctivitis (12 of 27) was significantly higher than in clinically healthy horses (2 of 12).\textsuperscript{303} Response to treatment with trifluridine in 9 of 16 cases further supported an etiological role for EHV-2. Similarly, herpetic keratitis due to corneal replication of virus has been suggested to occur with EHV-2 only.\textsuperscript{296,297,304} This syndrome seems to be diagnosed more commonly in the United Kingdom than in other parts of the world, although the reason for this is unclear. Herpetic keratitis, and often keratoconjunctivitis, may occur in individual horses or as small outbreaks, especially in young horses, and is often unilateral. Concurrent respiratory signs are usually absent or very mild. Recurrences are likely. Superficial ulcerative and non-ulcerative keratitis is described and may represent different stages of the same disease process. Corneal opacities range widely in appearance from ring-shaped lesions, a more generalized stippling or “orange-peel” appearance, or reticulated or lace-like (dendritic) networks. In all cases, their multifocal nature is highly suggestive. Variable corneal stromal inflammatory cell infiltration, edema, and neovascularization have been described. Uveitis, if present, appears to represent “reflex uveitis” mediated by the axonal reflex and is usually manifest as a miotic pupil without flare.

Corticosteroids are contraindicated in the treatment of ulcerative disease in the horse and active herpetic disease in all species studied so far. Some reports exist of topical or systemic corticosteroids causing exacerbation or recurrence of herpetic keratitis in horses. For these reasons, it seems wise to avoid the use of topical or systemic corticosteroids in horses with active herpetic keratitis. The use of topical non-steroidal anti-inflammatory agents is controversial but should also probably be best avoided in herpetic keratitis.

Anterior uveitis frequently occurs with herpetic keratitis and is a significant component of the ocular pain seen in this disease. This usually responds very well to one or two applications of topical applications of atropine ointment. Atropine should be given to effect (i.e., until wide mydriasis is achieved) and then given as often as necessary to maintain pupil dilation. Frequently, only once or twice weekly application is required.

**Ocular Signs Secondary to Neurological Disease**
Infection is generally sporadic but dramatic when it occurs. Ocular signs secondary to EHV-1 myeloencephalitis include nystagmus, blindness, strabismus, ptosis, exposure keratitis, optic neuritis, and retinal hemorrhage. These ocular signs represent manifestations of altered neurological function rather than virally induced ocular pathology. The ocular signs may result from CNS or peripheral cranial nerve injury, often secondary to vasculitis. The etiology of the exposure keratitis is presumably related to injury to the trigeminal (CN V) and/or facial (CN VII) nerve with resultant inability to sense corneal dryness, stimulate tear production by the lacrimal gland, and/or blink. Additionally, CN V is probably responsible for the supply and release of various trophic factors essential for normal corneal health. This means that the perceived need for, production of, and dispersion of tears and other corneal neurotrophic factors can all be affected leading to profound exposure keratitis.

**Uveitis**
Anterior uveitis or diffuse chorioretinitis has been described in horses in which EHV-1 infection has been diagnosed or experimentally induced. The exact causal association however remains undefined. In one case,\textsuperscript{305} a foal experimentally infected with EHV-1 developed severe visual impairment and bilateral, chronic diffuse chorioretinitis approximately one month after experimental inoculation with EHV-1. Anterior uveitis was not seen and a causal association with EHV-1 was not demonstrated. A separate report\textsuperscript{306} describes an otherwise typical outbreak of neurological disease and neonatal mortality attributed to EHV-1 in which a number of affected foals had evidence of anterior uveitis in addition to more classic pneumonic and/or gastrointestinal signs. Clinical signs sometimes included blindness and hypopyon, both of which resolved. In these animals, uveitis may be a manifestation of a more generalized vasculitis or may relate to super-infection with another pathogen secondary to EHV-1-mediated immunosuppression. An experimental study\textsuperscript{307} provides more definitive evidence that EHV-1 is a cause of multifocal chorioretinal lesions (typically classic “bullet hole” lesions in the peripapillary fundus) in 50-90% of experimentally infected horses. The lesions were evident between 3 weeks and 3 months after infection. The authors proposed that this was a vascular (ischemic) event. Chorioretinitis, optic neuritis, vitritis, retinal vasculitis, and encephalitis have also been described in camelids infected with EHV-1.\textsuperscript{308,309}
Equine viral arteritis - conjunctivitis, hypopyon, iridocyclitis, and keratitis.
African horse sickness - conjunctival and eyelid edema.
Equine adenovirus infection - conjunctivitis.
Borna Disease - retinal infection, optic neuritis.
Rabies - viral replication in retina and optic nerves.
Equine viral encephalitis - blindness due to encephalitis.
Equine infectious anemia - conjunctival hemorrhages.

BOVINE HERPESVIRUS I (Infectious Bovine Rhinotracheitis, IBR)

Virus Features
Taxonomic classification: Family - Herpesviridae, Subfamily - Alphaherpesvirinae. Structure, replication, and tissue damage is typical of that of other alphaherpesviruses.

Pathogenic Mechanisms
Systemic. Upper respiratory disease, tissue damage by direct cytolysis.

Ocular. Ocular disease may occur with or without respiratory signs. Conjunctivitis is the predominant feature, with formation of white conjunctival plaques composed of mononuclear cells (plasma cells and lymphocytes). Keratitis is also possible.

BOVINE VIRUS DIARRHEA VIRUS (BVD; Mucosal Disease)

Virus Features
Taxonomic classification: Family - Togaviridae, Genus - Pestivirus (mucosal disease viruses). Virus structure: Lipid containing envelope with surface projections surrounding a spherical nucleocapsid. Virions are 40-70 nm in diameter. Replication takes place in the cytoplasm, and assembly is assumed to occur during budding through the plasma membrane.

Pathogenic Mechanism
Ocular lesions occur as a result of exposure of the fetus to the virus between days 76 and 150 of gestation. Characteristic lesions are retinal dysplasia, cataracts, and microphthalmia. Reported histologic findings include swollen and multi-layered lens epithelium, microphthalmia, diffuse cataracts, retrolental fibrovascular membranes containing cartilage, and retinal dysplasia. Retinal dysplasia is described as severe, with absence of normal retinal architecture, diffusely fibroed nerve fiber layer, and gliotic optic nerves. Mononuclear cell infiltration may also be seen.

MALIGNANT CATARRHAL FEVER (MCF)

Virus Features
Taxonomic classification: Family - Herpesvirinae, Subfamily - Alcelaphinae.

Pathogenic Mechanisms

Ocular. Lymphocytic vasculitis of retinal, scleral, uveal vessels. Anterior uveitis is associated with corneal edema and keratitis. Predominantly a lymphocytic infiltrate, contrasting the disease from BVD, TEME, IBR, and IBK. Immunological mechanism suspected; Type IV (DTH) mechanism proposed.

MISCELLANEOUS CAUSES OF VIRAL INDUCED OCULAR DISEASE IN CATTLE:
Rabies - non-suppurative retinitis.

**BLUETONGUE**

**Virus Features**
Taxonomic classification: Family - Reoviridae, Genus - Orbivirus. Virus structure: Non-enveloped spherical virion, 70 nm in diameter, with 2 concentric capsids, the inner being icosahedral. RNA genome.

**Pathogenic Mechanism**
*Systemic.* Virus replicates in hematopoietic cells and vascular endothelium.

*Ocular.* Similar to BVD (both calves and lambs). Vaccination of ewes during the first trimester of gestation (attenuated live vaccine) produces necrotizing retinitis and CNS damage. Target tissues are brain, spinal cord, and retina. The severity of the disease depends on when in gestation the ewes are vaccinated. In lambs infected at 50-58 days, early lesions affect predominantly the retinal vasculature (endothelial nuclei rounded and vesicular).

**MAREK'S DISEASE**

**Virus Features**
Gamma herpesvirus of chickens.

**Viral Pathogenesis**
*Systemic.* Virus is slowly cytopathic but has ability to transform T lymphocytes to cause tumor formation. Systemic disease includes neurolymphomatosis (range paralysis), acute Marek's disease, and ocular lymphomatosis.

*Ocular.* Severe panuveitis to ocular tumor formation.
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