INFECTIOUS ORGANISMS OF OPHTHALMIC IMPORTANCE

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OCULAR BACTERIOLOGY

Bacteria are prokaryotic organisms consisting of a cell membrane, cytoplasm, RNA, DNA, often a cell wall, and sometimes specialized surface structures such as capsules or pili. Bacteria lack a nuclear membrane and mitotic apparatus. The DNA of most bacteria is organized into a single circular chromosome. Additionally, the bacterial cytoplasm may contain smaller molecules of DNA– plasmids –that carry information for drug resistance or code for toxins that can affect host cellular functions.

Some physical characteristics of bacteria are variable. *Mycoplasma* lack a rigid cell wall, and some agents such as *Borrelia* and *Leptospira* have flexible, thin walls. Pili are short, hair-like extensions at the cell membrane of some bacteria that mediate adhesion to specific surfaces. While fimbriae or pili aid in initial colonization of the host, they may also increase susceptibility of bacteria to phagocytosis.

Bacteria reproduce by asexual binary fission. The bacterial growth cycle in a rate-limiting, closed environment or culture typically consists of four phases: lag phase, logarithmic growth phase, stationary growth phase, and decline phase. Iron is essential; its availability affects bacterial growth and can influence the nature of a bacterial infection. The fact that the eye is iron-deficient may aid in its resistance to bacteria.

Bacteria that are considered to be nonpathogenic or weakly pathogenic can cause infection in compromised hosts or present as co-infections. Some examples of opportunistic bacteria include *Staphylococcus epidermidis, Bacillus* spp., *Corynebacterium* spp., *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., and *Pseudomonas* spp. (other than *Pseudomonas aeruginosa,* depending on whose research you read).

The infectivity of pathogenic microorganisms is influenced by their ability to initiate the infectious process. Bacterial adhesion to epithelial cells is a critical initial step. Adhesins, which are protein determinates of adherence, have been identified for most bacterial pathogens. Some adhesins are expressed in bacterial pili. Additionally, production of virulent factors increases the ability of an organism to cause tissue inflammation and destruction. Virulent factors include proteases, elastases, hemolysins, and cytoxins.

Among the bacteria of the most interest in ocular bacteriology are *Pseudomonas aeruginosa* and *Moraxella bovis*.

While differences between species do exist, bacterial keratitis is the most common and dangerous ophthalmic manifestation of bacterial disease in most species. Typically, the epithelium provides a crucial barrier to infection, and its integrity requires efficient wound healing. Bacterial cells and secretomes from a subset of species of bacteria inhibited human and porcine corneal epithelial cell migration in vitro and ex vivo. Secretomes from 71% of *Pseudomonas aeruginosa* and 29% of *Staphylococcus aureus* strains, and other bacterial species inhibited epithelial cell migration. Transposon mutagenesis implicated lipopolysaccharide (LPS) core biosynthetic genes as being required to inhibit corneal epithelial cell migration. This demonstrated how bacteria impact wound healing and provided evidence that secreted LPS is a key factor in the inhibitory mechanism (Brothers 2015).

Primary bacterial conjunctivitis is more common in cats than other species, whereas in dogs, bacterial conjunctivitis is usually only secondary to keratoconjunctivitis sicca. Systemic diseases such as pyometra, prostatic abscesses, and periodontal disease may result in chorioretinitis, which often goes unnoticed. Other systemic diseases such as rickettsial diseases frequently cause ophthalmic signs in dogs.

Normal bacterial and fungal flora

Bacteria can be cultured from the conjunctival sac in 50–90% of normal dogs. Gram-positive aerobes are the most commonly cultured, with *Staphylococcus* spp., *Bacillus* spp., *Corynebacterium* spp., and *Streptococcus* spp. predominating. Gram-negative bacteria have been recovered from the conjunctival sac in 8% of normal dogs. Anaerobes are rarely isolated. The normal flora appears to vary with the season and the breed of dog. While fungi are rare, the most commonly cultured are *Alternaria*, *Cladosporium oxysporum*, *Curvularia lunata, Penicillium*, and *Aspergillus*. In one study, 11/50 dogs (22%) had a positive mycological culture from the conjunctiva from at least one eye. There was no significant effect of sex or age on frequency of fungal isolation. The presence of conjunctival fungal organisms was correlated to the presence of fungi on the skin (Verneuil 2014). We must keep in mind that we can find only what we look for. For example, anaerobes will be isolated only under the correct conditions. Also, organisms isolated by culture may represent transient environmental contaminants rather than true resident flora.

Conjunctival flora is altered in dogs with ulcerative keratitis. Therefore, bacteria are more likely to be isolated from the conjunctiva of dogs with ulcerative keratitis than dogs with healthy eyes. In one study, *Malassezia pachydermatis*, a lipophilic yeast that is most commonly associated with otitis and dermatitis, was found in 23% of eyes with corneal ulceration and only 3% of healthy eyes. The increased number of bacteria and yeast could be due to decreased ocular defenses, increased phospholipids secondary to inflammation, or due to decreased concentration of tear lysozyme from increased tear production secondary to ulceration (Prado 2004).

The equine conjunctiva, like other species, has multiple types of bacteria residing as normal flora. *Corynebacterium* spp., beta-hemolytic *Streptococcus*, *Staphylococcus* spp., *Klebsiella* spp., *Bacillus cereus,* and *Moraxella* spp. have all been recovered from normal eyes of healthy horses. Unlike other species, fungi are also a common component of the normal flora of horses. Unidentifiable molds, dematiaceous molds, *Chrysosporium* spp., *Cladosporium* spp., *Aspergillus* spp. and *Penicillium* spp. are the most commonly reported isolates from normal horses; however, there may be variability depending on season, housing, and geographic location. A study from the United Kingdom showed slightly different results from those from the United States. The most frequently isolated bacterial species in the UK was *Acinetobacter* sp. Three genera of fungi (*Mucor, Absidia* and *Aspergillus* spp.) were isolated from 13% of horses. There was no significant effect of geographic location, sex, age, or housing on frequency of microbial isolation. Horses from which Gram-negative bacteria were isolated were significantly older than horses from which Gram-positive bacteria were isolated (Johns 2011). The flora of donkeys is similar to that of horses.

The fungal flora of cattle has also been studied. *Cladosporium* spp. and *Penicillium* spp. were found in the conjunctival fornix of the great majority of cattle cultured. There was no seasonal or housing difference. This might represent transient seeding from the environment, including the hay, as suspected in other species (Sgorbini 2010).

Bacterial microbiota of the ocular surface of bats was described, and *Staphylococcus* spp. were the most frequently isolated type of microorganism from healthy bat eyes (Leigue Dos Santos 2013).

Bacteria were recovered from 96.7% of 46 alpaca eyes. A total of 190 bacterial isolates were cultured with a mean of 2 bacterial isolates per eye. Seventy percent of isolates were Gram-positive. *Staphylococcus xylosus* predominated, followed by viridans streptococci and *Pantoea agglomerans*. Other isolated bacteria included *Rothia mucilaginosa*, *Staphylococcus equorum*, Bacillus species, *Moraxella ovis*, and *Moraxella catarrhalis*. Statistical analysis showed that alpacas harboring viridans streptococci and Moraxella species were significantly younger. Gender did not significantly affect type of bacterial isolation. There appeared to be no significant effect of age or gender on number of bacteria isolated (Storms 2016).

One study investigated the bacterial and fungal flora present in the eyes of healthy and pathological chelonians and compared findings in captive bred turtles with those in tortoises. In captive breed turtles and tortoises conjunctival disease is common. Bacteria were recovered from 100% of 34 healthy and diseased chelonian eyes. Thirteen animals harbored a single bacterial species as sole isolate and 21 animals harbored more than one species. Detection of multiple bacterial infection was clearly greater in tortoises compared to turtles. Most frequently isolated bacterial species were *Bacillus* spp., *Staphylococcus xylosus, Sphingomonas paucimobilis, Staphylococcus sciuri* and *Aeromonas hydrophila/caviae, Ochrobactrum anthropi, Citrobacter freundii, Enterobacter cloacae* and *Pseudomonas luteola*. Only one isolate of each of 13 other species was cultured. The presence in 8 animals of Mycoplasma spp. and in 1 animal with severe conjunctivitis of Chlamydia spp. was detected by PCR. Candida spp. was also isolated from two healthy animals. A clear predominance of Gram positive isolates in tortoises and Gram negative isolates in turtles was found. However, the observed difference could not be associated with the diversity of animal species, as other factors, including especially different characteristics of the living environments, may play a role. Almost all bacterial species are considered to be opportunistic pathogens. Salmonella spp. was isolated in the eye of only one of the animals (Di Ianni 2015).

Aerobic Pathogens (Including Facultative)
Gram-positive bacteria
Staphylococcus spp.

Staphylococci are ubiquitous and are part of the microflora of the skin and mucous membranes. Staphylococci are Gram-positive organisms that appear cytologically as individuals, pairs, small groups, or grape-like clusters. They are facultative anaerobes and fermentative.

Staphylococcal isolates commonly recovered from ocular sources are coagulase-positive species. *Staphylococcus aureus* is isolated from about 5% of horses with infectious keratitis, and *Staphylococcus intermedius* has been isolated from 2% of horses and 29% of dogs with infectious keratitis. *Staphylococcus epidermidis,* a coagulase-negative species, has been isolated from 6% of affected horses (Sauer 2003, Keller 2005).

Isolates of *S. intermedius* from dogs are sensitive to cefazolin, ciprofloxacin, and chloramphenicol. Equine isolates are sensitive to bacitracin, chloramphenicol, neomycin, and enrofloxacin (Tolar 2006).

Streptococcus spp. and related cocci

Streptococci are ubiquitous, suppurative Gram-positive cocci that may be found among normal mucosal flora. Enterococci are opportunists and are found in the intestinal tract of most mammals. Streptococcal keratitis is relatively common in domestic animals, especially the horse, in which streptococcal endophthalmitis may also occur. Most pathogenic streptococci produce hemolysins; the type of hemolysis produced varies with the species. Generally, β-hemolytic *S. equi* subspecies *zooepidemicus* in horses or β-hemolytic *Streptococcus* sp. in dogs are the most pathogenic streptococci. β-hemolytic *Streptococcus* sp. was isolated from 17% of dogs with bacterial keratitis (Tolar 2006). *S. equi* subspecies zooepidemicus accounted for 12% and 22% of the bacterial isolates from horses in two studies (Sauer 2003, Keller 2005). These streptococci can digest through a conjunctival graft; therefore, antimicrobial treatment prior to surgery should be attempted.

All the β-hemolytic *Streptococcal* isolates from dogs and horses in University of Tennessee studies are susceptible to ciprofloxacin, cefazolin, or chloramphenicol. However, there is resistance to neomycin, bacitracin, polymyxin B, and the aminoglycosides. There is increasing resistance of *S. equi* subsp. *zooepidemicus* to gentamicin at the University of Florida (Sauer 2003, Tolar 2006, Keller 2005). A more recent study from Australia showed that β-Hemolytic *Streptococcus* spp. >80% were resistant to ciprofloxacin but remained susceptible to chloramphenicol and cephalexin (Hindley 2015). This may be an indication of increased resistance or varied environments.

Strangles

Strangles is caused by *Streptococcus equi* subsp. *equi*. Infection occurs via direct contact and fomites. Typically this organism colonizes the pharyngeal and nasal mucosa and leads to lymphadenopathy with the clinical signs of pyrexia, malaise, purulent discharge, pharyngitis, and abscessed lymph nodes. Bastard strangles refers to cases in which the infection involves any area other than the pharyngeal area. Ocular abnormalities associated with bastard strangles may include serous then mucopurulent ocular discharge, panophthalmitis, and chorioretinitis. Central blindness may develop secondary to brain abscesses. *S. equi* subsp. *equi* can be diagnosed via culture or real-time PCR. Real-time PCR has a diagnostic sensitivity of 95% and specificity of 86%, has no cross-reactivity with any of the bacterial species tested (including *S. equi zooepidemicus),* and detects as few as three gene copies. This may aid in the rapid detection of subclinical shedders of *S. equi* subsp. *equi*, enabling more rapid treatment and helping to limit the spread of strangles in equine populations (North 2014).

Corynebacterium spp.

Corynebacterium may be found as flora of normal skin, mucous membranes, and the intestine and may propagate in soil following fecal dissemination. They are Gram-positive rods that often appear singly or in pairs +/- clubbed ends. Corynebacteria have been recovered from corneal ulcers in domestic animals, usually as part of a mixed infection. In those cases, the specific etiologic role of corynebacterial organisms is not clear. Following periocular trauma, *Corynebacterium* may cause blepharitis, orbital cellulitis, or abscesses, especially in large animal species.

Bacillus spp.

Bacillus spp. are Gram-positive rods found singly, in pairs, or chains. They may have a single endospore. They are commonly isolated from corneal ulcers, but they are not believed to play a major pathogenic role because other, more pathogenic organisms usually present as co-infections. This is the most common organism isolated from endophthalmitis in humans.

Listeriosis

Listeriosis is caused by a small rod-shaped, Gram-positive bacterium. The most common organism causing disease in domestic animals is *Listeria monocytogenes*. Spoiled or incompletely fermented corn or hay silage is the main source of infection in outbreaks. Listeriosis of the central nervous system is most likely to be associated with ocular signs in food animal species. Clinical signs include vestibular ataxia, cranial nerve deficits, brain stem involvement with facial nerve paralysis, and keratoconjunctivitis sicca. While keratitis is the main ocular lesion, anterior uveitis with hypopyon or purulent endophthalmitis may also be seen (silage eye) (Alexander 2010). Conjunctivitis, neurologic signs, and pancytopenia have occurred in a dog with generalized *Listeria monocytogenes* infection.

In sheep and goats, scleral hyperemia, unilateral keratitis with or without ulceration, bilateral mydriasis, vertical or horizontal nystagmus, ventrolateral or ventromedial strabismus and absent menace responses, lack of palpebral reflexes, and diminished or absent pupillary light responses may also occur. Swine listeriosis is rapidly fatal, causing septicemia in newborn piglets and encephalitis in older pigs. The diagnosis of listeriosis is made on clinical signs, PCR, culture, and identification of the organism from body fluids. Histologically, meningoencephalitis with mononuclear perivascular cuffing, neutrophilic and macrophagic microabscesses, and neuroparenchymal necrosis is seen (Headley 2104).

Gram-negative bacteria
Pseudomonas spp.

Pseudomonas spp. are Gram-negative rods widely distributed in nature, including soil and water, as saprophytic bacteria. They are also found on the skin and mucous membranes. On microscopic examination of smears collected from corneal lesions, *Pseudomonas* is morphologically indistinguishable from other Gram-negative bacilli. Antibiotic susceptibility testing is especially important with *Pseudomonas* isolates because multiple drug resistance associated with plasmids is common.

P. aeruginosa is isolated from about 15% of horses with bacterial keratitis and from 21% of dogs with bacterial keratitis.

Pathogenic mechanisms of *Pseudomonas aeruginosa*

P. aeruginosa is recognized as the most virulent corneal pathogen even though it is considered opportunistic. The normal cornea is exquisitely resistant to microbial attack; the inoculation of extremely large inocula (a thick bacterial suspension) of either invasive or cytotoxic *P. aeruginosa* onto intact mouse or rat corneas in vivo results in rapid bacterial clearance from the ocular surface without pathology (Evans 2013).

It is well recognized that tear fluid and blinking can physically cleanse the ocular surface and wash away potential pathogens, and that tear fluid also contains molecules with direct antimicrobial activity against many microbes, for example, lysozyme and lactoferrin. Although many *P. aeruginosa* strains grow readily in undiluted human tear fluid, tear fluid can still protect corneal epithelial cells against them. In this case, it may be that tear fluid acts directly upon corneal epithelial cells to make them more resistant to *P. aeruginosa* virulence strategies.

Corneal epithelial-associated barriers to *P. aeruginosa* consist of defenses against adhesion and against microbial penetration (traversal). The players involved likely include junctional complexes, secreted and internal antimicrobial peptides, mucins, and the basal lamina foundation that provides a physical barrier while also supporting epithelial homeostasis. During and after *P. aeruginosa* challenge, corneal epithelial defenses are enhanced and regulated by epithelial-derived cytokines and chemokines that can facilitate communication with cells of the immune system to boost corneal defenses.

The pathogenic factors associated with *P. aeruginosa* contribute to invasiveness and tissue destruction and include proteases, exotoxins, and hemolysins. Pseudomonas also have pili that can bind to corneal epithelial glycosylated proteins that provide receptor sites. Additionally, they excrete a biofilm, which anchors the cell to a substrate and confers resistance to physical disruption and antimicrobial treatment.

Adherence to the epithelial surface is the first step of pathogenesis, and *P. aeruginosa* adheres to corneal epithelial cells more rapidly than any other bacterial species. The organisms adhere poorly to intact epithelium or bare stroma, but they adhere readily to injured epithelium at the edge of an epithelial defect. Therefore, trauma is a necessary prerequisite for bacterial adherence and subsequent corneal infection. After only 30 minutes, organisms are engulfed by epithelial cells and reach the corneal stroma through transcellular migration.

Adherent bacteria secrete proteases to create openings in cells, thus exposing intracellular proteins that enhance pathogenesis by furthering adherence and colonization of the host tissue. *P. aeruginosa* produces at least two major matrix metalloproteinases: elastase and alkaline protease. These are shown to attack the helical structure of native types I, III, and IV collagen, generating specific fragments. Additionally, these proteinases interfere with the host defense systems by degrading complement components, immunoglobulins, interferon, IL 1 and 2, and tumor necrosis factor (Twining 2001). It has also been shown that the pseudomonal elastase strongly activates proMMPs. During active ulceration due to infection, concentrations of latent and active forms of MMP-2 and MMP-9 are higher than in contralateral unaffected eyes and control dogs (Wang 2008). Another protease, MucD, suppresses IL-1b, KC, and MIP2 during early stages of infection and inhibits neutrophil recruitment in the cornea (Mochizuki 2014).

Additionally, there are 2 strains of *P. aeruginosa:* cytotoxic and invasive (Lee 2003). During corneal infection, cytotoxic *P. aeruginosa* strains remain mostly extracellular, while invasive strains can enter corneal cells and replicate within them. A study was done evaluating the hypothesis that ofloxacin, which easily penetrates host cell membranes, would be more effective than the less cell-permeable antibiotic tobramycin for treatment of corneal infection by an invasive *P. aeruginosa* strain. Results showed that tobramycin was less effective at eradicating viable bacteria from corneas infected with the invasive strain. However, despite rapid sterilization of corneas in the ofloxacin group, disease progression occurred during the 12-hour treatment period. Both antibiotics hastened disease resolution over the next 7 days for infections caused by either strain. Corticosteroid use during the 12-hour treatment period was of little benefit and decreased eradication of bacteria. The results suggest that management might be improved by addressing factors contributing to disease progression during sterilization of the cornea by antibiotics, but steroids are not the answer.

Factors influencing barrier function of the corneal epithelium to an invasive strain of *P. aeruginosa* in vivo and ex vivo were investigated by introducing subtle forms of injury/compromise and studying their impact. Bacterial traversal and pathology occurred only in older mice that had corneas blotted, were treated with ethylene glycol tetraacetic acid, and were also SP-D deficient. These results highlight that defenses against infection in the cornea are extremely robust and multifactorial and that there is significant redundancy built into the system (Alarcon 2011).

Pseudomonas aeruginosa and antibiotics

Almost all *P. aeruginosa* isolates from dogs and horses are sensitive to gentamicin, tobramycin, and ciprofloxacin (Keller 2005, Tolar 2006, Sauer 2003).

Susceptibility of isolates from dogs to seven fluoroquinolones from 2nd, 3rd and 4th generations was evaluated (Ledbetter 2007). In vitro, bacterial resistance to the tested fluoroquinolones was infrequently identified (24/27 isolates were susceptible to all fluoroquinolones evaluated); susceptibility percentages ranged from 88.9–100% for individual antimicrobials. There were no significant differences among isolate susceptibilities to the individual antimicrobials or among generations of fluoroquinolones.

Multi-drug resistant, extensively drug resistant, and pan-drug resistant strains of *Pseudomonas aeruginosa* are associated with risk factors such as bandage contact lens, topical steroids, previous therapeutic graft, preservative-free lubricant ointment and ocular surface disorders. Of 15 isolates, six were sensitive only to imipenem, three to colistin, two to neomycin, one each to imipenem and colistin, imipenem and ceftazidime, and azithromycin, respectively. One isolate was resistant to all antibiotics. Success with medical therapy alone was not common. These cases are more likely to require the use of tissue adhesives and keratoplasty and are likely to have treatment failure (Fernandes 2016, Vazirani 2015). Another study compared the efficacy of topical 1.5% and 0.5% levofloxacin for the treatment of multidrug-resistant Pseudomonas aeruginosa (MDRP) keratitis in rabbits and showed based on viable bacterial counts topical 0.5% levofloxacin is not adequately effective, while 1.5% levofloxacin is efficacious in controlling MDRP keratitis (Tajima 2015).

Bacteriophages (or phages), the viruses of prokaryotes, are attractive therapeutic alternatives to conventional drugs in the control of bacterial pathogens that are resistant to clinically approved antimicrobials.
Isolation and characterization of two bacteriophages showed that one phage, P5U5, was active against all 26 *P. aeruginosa* isolates from dogs with ocular infections, whereas P2S2 formed lytic plaques on plates of 21 (80.8%) isolates. These may play a part in future treatment regimens (Santos 2011).

Predatory prokaryotes might also be used as live antibiotics to control infections. One study evaluated *Pseudomonas aeruginosa* ocular isolates exposed to the predatory bacteria *Micavibrio aeruginosavorus* and *Bdellovibrio bacteriovorus*. Seven of the 10 *P. aeruginosa* isolates were susceptible to predation by *B. bacteriovorus* 109J, with 80% being attacked by *M. aeruginosavorus*. To further investigate the effect of the predators on eukaryotic cells, human corneal-limbal epithelial (HCLE) cells were exposed to high concentrations of the predators. Cytotoxicity assays demonstrated that predatory bacteria do not damage ocular surface cells *in vitro*, whereas the *P. aeruginosa* used as a positive control was highly toxic. Additionally, levels of pro-inflammatory cytokines IL-8 and TNF-alpha in HCLE cells were measured after exposure to the predators, and there was no increase. These results suggest that predatory bacteria could be considered in the future as a safe topical bio-control agent to treat ocular infections (Shanks 2013).

Moraxella spp.
Moraxella bovis, a large, plump, Gram-negative coccobacillus, is the primary cause of infectious bovine keratoconjunctivitis (IBK), and is of great economic importance. IBK or "pinkeye" is a significant and highly contagious ocular infection of cattle. Though IBK is rarely fatal, it causes considerable losses to the cattle and dairy industries because of decreased weight gain, decreased milk production, devaluation because of eye disfigurement, and because of the high cost of treatment. Losses total around $150 million per year. Weaning weight losses of IBK-affected calves can range between 20–40 lb., and up to 80% of the herd can have disease during an epizootic outbreak. Approximately 2% of calves are blinded.

Transmission of *M. bovis*
M .bovis is an opportunistic pathogen whose virulence is influenced by both host and environmental factors. Environmental factors include ultraviolet light exposure, face fly populations, climate, and pasture conditions. Host factors include genetics, breed, age, nutrition, immune status, and current infections. Non-piliated, nonpathogenic forms can exist in a carrier state in the host. Carrier animals are asymptomatic but shed the organism. *M. bovis* may be harbored in the nasal, ocular, and vaginal secretions, and it may be transmitted by direct contact, aerosol, or fomites. Cattle are the primary natural reservoir for *M. bovis,* and there is a high nasal carrier rate.

The face fly, *Musca autumnalis*, is a primary mechanical vector for IBK and also serves as an irritant. These organisms can live on the fly's legs for up to 3 days. Additionally, certain stimuli such as ultraviolet light can cause direct conversion of nonhemolytic, non-piliated organisms to pathogenic forms in carrier animals. A rapid logarithmic growth phase of the organism accompanies this transformation.

Some breeds of cattle are more susceptible to disease. *Bos taurus* is more susceptible to IBK than is *Bos indicus* (such as Zebu and Brahman), and calves are more prone to disease than are adults. IBK is more common in summer and fall months; this is attributed to high-level exposure to ultraviolet light, which predisposes the eye for infection and alters the bacteria.

Pathogenic Mechanisms of *M bovis*

M. bovis has several pathogenic mechanisms; however, only two, pili and the secretion of a β-hemolytic cytotoxin, have been determined to cause clinical disease. The pili allow the bacteria to attach to the dark cells of the corneal epithelium. These cells, as seen on electronic microscopy, have few microplicae and may represent cells in their final stages. Q-pili are specific for colonization of the bovine corneal epithelium, whereas I-pili enable maintenance of an established infection. The pili demonstrate interstrain and intrastrain antigenic heterogeneity, and a single strain can produce both pili types. The orientation of an invertible DNA determines which pili gene will be transcribed. The presence of pili alone is insufficient to cause disease following infection with *M. bovis* (Rogers 1987).

Where bacteria are seen to be attached to the epithelial cells, a small depression is present. This pit may be associated with the β-hemolysin of *M. bovis* that is also required for disease. The hemolysin is a pore-forming toxin that lyses corneal epithelial cells leading to ulceration and causes lysis of bovine leukocytes. With the lysis of leukocytes, enzymes are released that cause fragmentation and aggregation of collagen fibrils, which delay healing. Hemolysin, which belongs to the RTX family of toxins, causes clinical signs both directly, as a result of damaging ocular cells, or indirectly through lysis of the leukocytes attracted to the affected site. This latter effect results in delayed corneal healing and may contribute to corneal damage due to the release of endogenous enzymes (Postma 2008). A recent study showed that type IV pili not only allow attachment of *M. bovis* to the epithelium but also are necessary for biofilm production. This biofilm is quite resistant to antibiotics. $MgCl_2$ not only removed the pili, but it also disrupted the biofilm (Prieto 2013).

Clinical Disease of *M. bovis*

Pinkeye most commonly occurs in summer and fall. Younger cattle are more susceptible to disease because older animals have usually developed acquired surface immunity as a result of previous exposure. The incubation period is usually 2 to 3 days.

Affected cattle have a decreased appetite and moderate pyrexia. Conjunctival edema and hyperemia, epiphora, and blepharospasm are initial clinical signs. A small opaque area appears in the center of the cornea in about 2 days, and by day 6 the entire cornea can be gray-white to yellow in color with axial corneal ulceration. Corneal vascularization may occur. Severe ulceration and corneal rupture with iris prolapse, conical bulging of the cornea, and blindness are infrequent complications of pinkeye. More often, complete recovery occurs in 3 to 5 weeks, with only a few affected eyes having a persistent white corneal scar.

Treatment of *M. bovis*

Elimination of *M. bovis* in calves with IBK has been demonstrated following parenteral treatment with oxytetracycline or florfenicol. The most common treatments are intramuscular oxytetracycline (LA200 2 injections, 20 mg/kg at 72-hour intervals) or Tetradur (300 mg/mL, 1–2 mL IM, lasts 7–10 days.) Oxytetracycline is an amphoteric molecule that should theoretically diffuse into tears; however, parenteral administration of long-acting oxytetracycline leads to a tear concentration less than 1 g/mL although conjunctival levels are > 2 g/mL for 72 hours. Substantial conjunctival concentrations of oxytetracycline are present for as long as 20 hours after a single 20 mg/kg IM injection. The drug localizes within the lacrimal gland, conjunctiva, and cornea but not within tear film or aqueous humor. The efficacy of parenterally-administered oxytetracycline is likely linked to these higher tissue levels. Treatment with two doses of long-acting oxytetracycline has been shown to cause a reduction in *M. bovis* organisms and decrease in corneal ulcer healing time and recurrence. Some antimicrobial resistance to tetracycline has been reported in *M. bovis* isolates in the United States.

Florfenicol is given at 2 IM dosages of 20 mg/kg 48 hours apart or as a single 40 mg/kg SC dose. Florfenicol provides a treatment option in *Anaplasma*-endemic regions where oxytetracycline use is restricted. Simultaneous administration of these drugs to all animals reduces the incidence of IBK in the herd (McConnel 2007).

Subconjunctival treatment is also effective. Subconjunctival procaine penicillin (1–2 mL) +/- subconjunctival dexamethasone (1–2 mL) or 100 mg/mL subconjunctival oxytetracycline (1–2 mL) can be given. In calves given a bulbar subconjunctival injection of a conventional (100 mg/mL) oxytetracycline formulation, the antibiotic concentration in tears is above the minimum inhibitory concentration (MIC) for 24 hours. Although a single subconjunctival dose of a long-acting

oxytetracycline formulation achieved tear concentrations above MIC for longer than 72 hours, severe tissue necrosis at the injection site precludes such therapy.

Ceftiofur crystalline-free acid (CCFA) is administered in the posterior aspect of the ear to reduce the risk for tissue residue and lower the potential for injection-site trimming at slaughter. Concentrations of ceftiofur metabolites in plasma remain above published *M. bovis* modal MIC values for more than 7 days when administered via this particular route. A single dose of CCFA (6.6 mg ceftiofur equivalents/kg, SC) is effective in beef calves, decreasing clinical signs and leading to a 50% increase in the percentage of healed calves in 14 days. Tulathromycin at 1 dose SC and tilmicosin administered SC (5 or 10 mg/kg) are also both effective (McConnel 2007).

Nictitating membrane flaps or a temporary tarsorrhaphy for deep ulcers or perforation can be done. Also, keeping down the fly population and minimizing exposure to UV radiation are beneficial.

Autogenous vaccines have been tried over the years. One study evaluated a farm-of-origin *M. bovis* autogenous vaccine that was also shown to be ineffective for preventing IBK and associated weight loss (O'Connor, 2011). Some work is being done to try to preserve the cellular piliation level of *M. bovis* during the growth of bacteria in stirred bioreactors (Prieto 2009).

Another study evaluated two cytokines in combination with inactivated bacteria (bacterin) and their effect on the conjunctival immune response to *M. bovis*. Treatments using the bacterin vaccine combined with interleukin-2 and interferon-α as adjuvants, the bacterin vaccine only, and controls without treatment were applied by ocular spraying to evaluate cattle experimentally infected with *M. bovis*. Fewer of the animals in the group exposed to the bacterin with the cytokines were affected, and those that were affected had less severe clinical signs than animals in the other groups. This suggests that the addition of cytokines to treatments can reduce not only eye injuries caused by IBK, but also the number of diseased animals (di Girolamo 2011).

Recently, an intranasal vaccine was tested in healthy beef cattle using the carboxy terminus of recombinant *M. bovis* cytotoxin adjuvanted with the mucoadhesive polymer polyacrylic acid. It was found that the vaccine stimulated changes in antigen-specific ocular mucosal IgA; however, there was no statistical significant difference in the variables between the treatment groups in post-hoc testing (Angelos 2014).

The reason vaccines do not afford protection is not exactly known. There may be more than one organism responsible, the pili could be shearing from the bacteria used for the vaccine, or an ocular mucosal secretory response may not be forming because of systemically-administered vaccines. The latter was tested by evaluating IgA production against *M. bovis*-purified pili produced after intranasal inoculation of experimental vaccines. Significantly higher anti-pili IgA response was detected in calves vaccinated intranasally with two pili compared to control calves, although this specific immune response did not seem to be related to protection against infection or typical IBK lesion development (Zbrun 2011).

Moraxella bovoculi

M. bovoculi has been isolated from IBK cases in the United States and South America (Sosa 2012). Additionally, *M. bovoculi* was cultured from dairy cattle with ulcerative blepharitis and conjunctivitis. Examination revealed mild to severe ulceration of the lower and/or upper eyelids, mild to severe swelling surrounding affected eyes, and profuse lacrimation. Lesions typically affected one eye and involved the eyelid skin and conjunctiva. Oxytetracycline treatment topically once daily for 3 days led to resolution in 2 weeks (Klibs 2011). Genetic sequencing or PCR is necessary to differentiate *M. bovis* from *M. bovoculi* (Shen 2011). The low MIC (90) of *M. bovoculi* isolates suggests that antibiotics commonly used for treatment of IBK associated with *M. bovis* should also be effective against *M. bovoculi* (Angelos 2011).

The association of *M. bovoculi* and IBK was evaluated using a corneal scarification model in calves. A study was designed with calves receiving corneal scarification only, corneal scarification and inoculation with *M. bovoculi,* and corneal scarification and inoculation with *M. bovis*. Of the enrolled calves, 90% of *M. bovis* calves, none of the *M. bovoculi* calves, and 9% of control calves developed corneal ulcerations consistent with IBK in the scarified eyes. The absence of corneal ulcerations in *M. bovoculi*-inoculated calves suggests it is not a causal organism for IBK in this model (Gould 2013).

Another study found genetic diversity of *M. bovis* and *M. bovoculi* associated with IBK cases even within an outbreak, and antibiotic resistance patterns showed differences between *M. bovis* and *M. bovoculi*. This variation within isolates could explain the partial protection induced by commercial vaccines (Sosa 2013).

Other diseases caused by Moraxella

Moraxella spp. have also been reported to cause other ocular infections of small ruminants and horses. *Moraxella (Branhamella) ovis* is a Gram-negative diplococcus similar to *M. bovis*. This agent has been cultured from normal small

ruminants and from eyes of sheep and goats with keratoconjunctivitis. *M. ovis* may occur as a co-infection with chlamydial or mycoplasmal conjunctivitis, and it may complicate other ocular diseases.

Pasteurellosis

Pasteurella multocida is a very small, non-motile, Gram-negative ovoid, coccoid, or short rod that shows bipolar staining. It is aerobic and facultatively anaerobic. Disease occurs when predisposing factors give the bacteria the opportunity to multiply and overwhelm the physiological and immunologic defenses of the respiratory tract. During these episodes, clones of virulent bacteria increase and are transmitted to neighboring animals. *P. multocida* produces an endotoxin that varies with serotype. It also produces adhesins that allow the bacteria to adhere to epithelial tissues. Filamentous appendages elaborated by the bacteria may help *P. multocida* colonize mucous membranes.

Clinical Signs of Pasteurellosis

Clinical signs associated with pasteurellosis in rabbits include rhinitis (or snuffles), pneumonia, genital infections, wound infections and abscesses, and otitis media. Ocular signs include dacryocystitis and conjunctivitis. Dacryocystitis is characterized by mucopurulent discharge, which can be profuse and malodorous and is most marked at the medial canthus of the eye. Pressure on the skin beneath the medial canthus expresses a string of purulent material through the lacrimal puncta into the conjunctival sac. In some cases, the lacrimal sac is visually and palpably distended. Secondary conjunctivitis may occur, and keratitis may be found in association with the mucopurulent discharge. While *Pasteurella* is often incriminated in dacryocystitis, one publication of 28 cases of dacryocystitis in rabbits did not grow *Pasteurella* on any of the 6/8 positive cultures (Florin 2009).

Brucellosis

Brucella canis is a zoonotic aerobic Gram-negative coccobacillus that can survive in mononuclear cells. *B. canis* naturally infects dogs by penetrating mucous membranes. Ingestion and venereal transmission are common, but *Brucella* spp. can be transmitted via fomites, such as cages or equipment. Ocular signs may occur in ~14% of dogs with brucellosis. Some of the more common ocular signs include endophthalmitis, chronic uveitis, hyphema, and chorioretinitis. Other signs may include diskospondylitis, glomerulopathy, and meningoencephalitis. Abortion and infertility are common clinical signs that occur in breeding dogs; however, dogs that are not used for breeding may have undetected disease for long periods of time due to the prolonged bacteremia and secondary localization.

Diagnosis is difficult, but due to the zoonotic potential, it is especially important. The only definitive diagnosis is isolation and identification of the organism by blood culture, centesis of ocular fluids, etc. Serologic screening usually involves the rapid slide agglutination test with and without 2-mercaptoethanol. Tube agglutination, ELISA, or IFA tests that employ multiple dilutions or the cytoplasmic protein agar gel immunodiffusion test have greater specificity and are used as confirmatory serologic tests. Because of the zoonotic potential of brucellosis and the difficulty in eradicating the organism, euthanasia may be elected.

One report seems to debunk some previous thoughts of *Brucella*. Three neutered dogs presented with chronic or recurrent uveitis in the absence of overt systemic disease. All dogs had unilateral mild-to-moderate anterior uveitis, iris hyperpigmentation, marked vitreal infiltrates, and multifocal chorioretinitis. Dogs were diagnosed with canine brucellosis serologically and by blood culture or PCR on aqueous humor and blood. Dogs were treated symptomatically with topical ophthalmic anti-inflammatories and a novel antimicrobial protocol that included doxycycline, enrofloxacin, rifampin, and streptomycin. Ocular inflammation resolved in all dogs during treatment, with preservation of vision in two dogs. Following treatment, *B. canis* could not be cultured from blood, and serological values declined, with seronegativity achieved in all dogs after a median of 96 weeks of therapy (Ledbetter 2009).

Haemophilus spp.

Haemophilus spp. require factors from blood for growth. *Haemophilus* or *Haemophilus*-like organisms can be normal flora of the oral cavity and nasopharynx but have also been a primary etiologic agent for acute mucopurulent conjunctivitis in humans. The organisms have little affinity for the avascular cornea, and corneal involvement is a rare complication of conjunctivitis. However, ocular lesions of thromboembolic meningoencephalitis (TEME), which is caused by *Haemophilus somnus* (or *Histophilus somni*), are typically found in the fundus. Acute septic TEME most commonly occurs in feedlot cattle and may cause thromboembolic chorioretinitis, resulting in funduscopically visible exudates and hemorrhages. Retinal detachments may result from retinal edema, and sub-retinal exudate and blindness may occur either from the ocular lesions or septic thrombosis of brain tissues. When infected animals survive the acute septicemia, the foci of chorioretinal exudate become areas of necrosis and chorioretinal scarring.

Mycobacterium bovis

M. bovis, an acid-fast bacterium, is the causative organism of bovine tuberculosis. Ocular tuberculosis is rare in cattle and swine and causes sub-retinal exudation, retinal hemorrhages, and anterior uveitis. Histologically, endophthalmitis with a

granulomatous response is seen. Generally, affected small animals are in farm settings and are drinking unpasteurized milk. This is a zoonotic disease.

Mycoplasma

Members of the class Mollicutes (often referred to as "mycoplasmas") are the smallest prokaryotic cells capable of self-replication. They are ubiquitous free-living saprophytes (e.g., members of the genus *Acholeplasma*) and animal pathogens (members of the family Mycoplasmatacae, which includes the genera *Mycoplasma* and *Ureaplasma*). The mycoplasmas lack a true cell wall but have a plasma membrane. This plasma membrane accounts for their plasticity and pleomorphism, including cocci, spiral filament, and ring-like structures. They stain poorly with Gram stain. Giemsa and other Romanowsky stains produce better results. The fragility, pleomorphism and weak staining characteristics make direct examination of stained smears of limited value in making a diagnosis. *Mycoplasma* spp. adhere to host mucous membranes where they remain extracellular, producing hemolysins, proteases, nucleases, and other toxic factors. Latency can occur, and stresses can predispose animals to low-grade, chronic mycoplasma infections. Most of these organisms are host specific. Eye infections are characterized by serous discharge and conjunctival hyperemia.

Numerous species, including *M. felis, M. gateae, M. arginini,* and *Acholeplasma laidlawii,* have been recovered from the eyes of cats with ocular disease. Numerous studies confirm that these organisms can also be recovered from the normal conjunctiva, and Koch's postulates have not been fulfilled with these organisms, with the exception of *M, felis*. Inoculating kittens with *M. felis* causes clinical signs, and it is more commonly cultured from ill cats versus normal cats. Clinical signs associated with *M. felis* are conjunctivitis, lymphoid follicle formation, and development of pseudomembranes.

Mycoplasma conjunctivae and *Mycoplasma agalactiae*

Mycoplasma conjunctivae is one etiology of infectious keratoconjunctivitis (IKC), commonly known as pink eye) in domestic sheep, goats and other wild animals in many parts of the world. In sheep, *M. conjunctivae* and *M. agalactiae* are important causes of keratoconjunctivitis (with corneal vascularization but not corneal ulcers). In goats (and maybe sheep) *M. agalactiae* causes systemic disease including arthritis, mastitis, or abortion. Also in goats, *M. mycoides* subsp. *mycoides* (large-colony type) causes keratoconjunctivitis, perilimbal corneal opacities, septicemia, mastitis, and arthritis. Eye lesions may occur in goats without systemic signs. This has also been cultured from an ibex in Italy (Giangaspero 2010). In general, ophthalmic signs of mycoplasma include keratoconjunctivitis, anterior uveitis, chorioiditis, and hyalitis in sheep and goats. *M. conjunctivae* can be diagnosed via culture and PCR. Topical application of 0.5% sterile solution of gentamycin (100 mg/mL) proved suitable for the treatment in Lohi lambs; all clinical signs of disappeared after 5 days of treatment (Shahzad 2013).

Additionally, *M. bovoculi, Ureaplasma* spp., *M. laidlawii,* and *M. bovirhinis* have been associated with bovine conjunctivitis. The association with *Moraxella bovis* has not been confirmed. Typically, bovine mycoplasma conjunctivitis tends to occur in the summer and is usually mild and self-limiting. *Mycoplasma bovis* has been reported to cause pneumonia, arthritis, mastitis, meningitis, infertility, and subcutaneous abscesses in cattle. It may also cause keratoconjunctivitis.

Chlamydophila sp. (Chlamydiae)

Chlamydophila are obligate intracellular organisms that possess cell walls similar to those of other Gram-negative bacteria. They lack the machinery that allows autonomous survival and replication. Their cell replication cycle involves extracellular (elementary body) and intracellular (reticulate or initial body) forms. The elementary bodies are 0.2–0.6 µm in size with rigid cell walls and are resistant, metabolically inactive, and infectious. They undergo extracellular migration and transform within vacuolar inclusions into larger vegetative replicating forms. These reticulate bodies (0.5–1.5 µm) lack cell walls and are noninfectious. They proliferate by budding and fission within the cytoplasmic vesicle of the host cell. After rapid division, the initial bodies transform again and become a large membrane-bound population of elementary bodies. After this 2-day cycle, the elementary bodies are released from the cell after lysis and infect new host cells. Previously, all members of the family Chlamydiaceae were known as one species: *Chlamydia psittaci*. Currently there are two genera, *Chlamydia* and *Chlamydophila*, with multiple species in each.

Disease in Cats

Chlamydophila felis is a highly contagious disease of cats and spreads rapidly by direct contact or aerosol. The organism survives a few days in the environment. Cellular and hormonal mechanisms play a role in immunity. Cats under 8 weeks and older than 5 years are unlikely to become infected with *C. felis*. In general, chlamydiae are considered to have a restricted host range; however, there are several documented cases involving transmission of *C. felis* from cats to humans.

The primary ocular manifestation is conjunctivitis. Apparently, a low dose of bacteria incites unilateral disease and a high dose incites bilateral disease. After infection, the organisms spread internally to colonize many tissues including the tonsil, lung, liver, spleen, and kidney. Organisms are shed in the tears and nasal secretions and may persist in the ocular tissues for

several months following remission of ocular signs. Initial chemosis followed by suppurative conjunctivitis is typical, and petechial hemorrhages may occur along with conjunctival lymphoid follicles. Only occasionally are respiratory signs seen.

Experimental infection is characterized by unilateral conjunctivitis within 5–10 days of exposure. Serous ocular discharge becomes mucoid or mucopurulent within 3–5 days, while cats that become bilaterally affected have clinical signs persist for 22–25 days. Histologically, a neutrophilic infiltrate is seen, with macrophages, lymphocytes, and plasma cells becoming more numerous between days 10 and 21. Recovery from *Chlamydophila* disease can result in persistent infection. Experimental ocular exposure has resulted in conjunctival shedding for up to 8 months. Chronic shedding of organisms from the urogenital and gastrointestinal tract has also been documented.

C. felis in cats, in contrast to chlamidial infections in other species, is not associated with keratitis. Isolation using conjunctival cotton swabs (without wooden sticks) is best. The swabbing must be vigorous to acquire enough cells. ELISA, PCR, and FA are also used for diagnosis. Cytology can reveal the intracytoplasmic elementary bodies, but they are low in number.

One study evaluated conjunctival smears from 88 cats with conjunctivitis and 10 healthy control cats. The smears were stained with a Romanowsky stain, and cytologic results were compared with PCR analysis for FHV-1, *C. felis*, and *M. felis*. Inclusions interpreted as chlamydial inclusions were found in all eight cytologic smears from cats positive for *C. felis* by PCR analysis and in three PCR-negative cats. Inclusions interpreted as *Mycoplasma* organisms were found in 3/6 cats that were PCR-positive for *M. felis* and in one PCR-negative cat. FHV-1 inclusion bodies were not detected in the nine cats that were positive for herpes (Hillstrom 2012).

The most common treatment for *Chlamydophila* in cats is topical oxytetracycline QID for 2 weeks past resolution of clinical signs; however, erythromycin and chloramphenicol are also effective. The efficacy of systemic pradofloxacin against *C. felis* and *Mycoplasma* spp. in comparison to doxycycline has been studied. Complete elimination of *Mycoplasma* spp. was achieved in both groups. All cats that received doxycycline eliminated *C. felis*, but some cats treated with pradofloxacin remained PCR-positive (Hartmann 2008).

Disease in Farm Animals
Chlamydiosis in food animals is most commonly caused by *C pecorum*, *C abortus*, and *Chlamydia suis*. *C pecorum* may cause encephalomyelitis, enteritis, polyarthritis, metritis, pneumonia, and conjunctivitis in cattle, sheep, and swine. Typically, young animals are affected in sporadic outbreaks.

In sheep, *C. abortus* has been associated with conjunctivitis, keratitis, polyarthritis, pneumonia, orchitis, epididymitis, and abortion. Chlamydophilosis among lambs and kids may produce both ocular signs and polyarthritis. Ocular signs are bilateral in 80% of the cases. Conjunctival lesions include conjunctivitis, petechial hemorrhage, epiphora, purulent exudation and lymphoid follicle proliferation, which may become confluent, producing folds. Peripheral edema and neovascularization may develop in the cornea, especially dorsally. Corneal ulceration rarely develops. Topical oxytetracycline is used to treat lambs and kids, but LA200 is an effective option. Usually, the condition is self limiting with resolution in 2–3 weeks. Conjunctival cytology, culture, ELISA, and PCR can all be used for diagnosis. Persistent apparent infection with intermittent shedding is common among ovine chlamydial diseases. The elementary bodies are relatively resistant and may remain viable for several days.

In swine, *C. suis* has been isolated from the conjunctiva and intestinal and respiratory tracts in association with conjunctivitis, keratoconjunctivitis, enteritis, and pneumonia. Lymphofollicular hyperplasia of the palpebral conjunctiva is common. *C. suis* has, however, also been isolated from the conjunctiva of healthy pigs. *C. suis* was found via histopathologic and microbiologic analysis of a wild boar piglet with severe bilateral keratoconjunctivitis. A mixed inflammatory infiltrate was seen in the cornea, choroid, and optic nerve, and *C. suis* was detected in the eyes bilaterally (Risco 2013).

Disease in Birds
Avian chlamydiosis, caused by *Chlamydophila psittaci*, can be an unapparent subclinical infection or acute, subacute, or chronic disease of wild and domestic birds; the disease is characterized by respiratory, digestive, or systemic infection. Infections occur worldwide and have been identified in at least 150 avian species, particularly colonial nesting birds (e.g., egrets, herons), ratites, caged birds (primarily psittacines), raptors, and poultry (except chickens). Long-term unapparent infections lasting for months to years are common and considered the normal chlamydia-host relationship; 10–30% of surveyed avian populations are positive. The same strain may cause mild disease or asymptomatic infection in one species, but severe or fatal disease in another species.

Avian chlamydiosis is a zoonotic disease and can infect other mammals. Human disease most often results from exposure to psittacines or pigeons and is usually respiratory and characterized by abrupt onset of flu-like symptoms. Pneumonia, organ failure, and death can result if the disease is severe or left untreated. Also, apparent transmission of chlamydia from a macaw to a cat has been reported (Lipman 1994).

All chlamydiae share an identical genus-specific antigen in their lipopolysaccharide but often differ in the composition of other cell-wall antigens, providing a basis for serotypic identification. Currently, eight serotypes are recognized; six (A–F) infect avian species and are distinct from mammalian chlamydia serotypes. Each avian serotype tends to be associated with certain types of birds.

Airborne particles from respiratory secretions and dust spread the elementary bodies, which are resistant to drying. Additionally, disease is spread by fecal-oral transmission. After inhalation or ingestion, elementary bodies attach to microvilli on mucosal epithelial cells and are internalized by endocytosis. Stress can initiate shedding in latently-infected birds and may cause recurrence of clinical disease. Carriers can shed the organism for extended periods. The incubation period is typically 3–10 days. Nasal and ocular discharge, conjunctivitis, sinusitis, green to yellow-green droppings, fever, inactivity, ruffled feathers, weakness, inappetence, and weight loss can be seen in clinically-affected birds. Asymptomatic infections are common.

Clinical findings, hematology, clinical chemistries, and radiology are often adequate for a tentative diagnosis in ill birds. Additionally, the organism can often be identified in impression smears of affected tissues stained by Giemsa, Gimenez, or Macchiavello's method. Immunohistochemistry, ELISA, and PCR are also useful. Confirmation requires isolation and identification of *C. psittaci*.

Severity of clinical signs and lesions depends on the virulence of the organism and susceptibility of the bird. Necropsy findings in acute infections include serofibrinous polyserositis (airsacculitis, pericarditis, perihepatitis, peritonitis), pneumonia, hepatomegaly, and splenomegaly. Multiple pale foci and/or petechial hemorrhages can be seen in the liver and spleen. Similar lesions are seen in other systemic bacterial infections and are not specific for avian chlamydiosis. Necrosis results from direct cell lysis or vascular damage. The latter is also the source of the generalized serofibrinous exudation. Chronic infections show enlargement and discoloration of the spleen or liver with a mononuclear cell response (not inclusions). Lesions are usually absent in latently-infected birds, even though .*C psittaci* is often being shed.

Disease in Koalas
C. pecorum causes kerato-conjunctivitis, urinary tract disease, reproductive tract disease, and a rhinitis/pneumonia complex in koalas (Blanshard 2008). Infection of the mucosal surfaces of the eye results in inflammation, characterized in the early stages by serous discharge, blepharospasm, and hyperemia of the conjunctiva and sclera, progressing to purulent discharge, conjunctival hyperplasia, and fibrosis. One or both eyes may be affected. In some severe and chronic cases, the cornea is affected by opacity caused by edema and vascularization with or without pigmentation, and severe end-stage cases may have corneal rupture. Clinical presentations are highly variable in severity and chronicity between koalas, and lesions may be active, with copious exudate, or inactive, with no exudate and mature scarring (Wan 2011).

Mild and acute cases respond well to treatment, but response to therapy in more chronic cases depends on the degree of scarring. Extensive and advanced fibrosis of the conjunctiva leads to reduction in the size of the palpebral fissure and sometimes entropion. Systemic antimicrobial drugs used for chlamydial infections at koala treatment facilities include enrofloxacin and chloramphenicol (Blanshard 2008). Tetracycline and macrolide antibiotics that are used in humans and other species can be potentially fatal because of the effect on the microflora of the koala gastrointestinal tract (Polkinghorne 2013). A vaccine to control chlamydial infections in the koala is currently under development and showing promise (Carey 2010, Kollipara 2012).

Bartonella
Bartonella is a Gram-negative, facultative intracellular rod or coccobacillus belonging to the Bartonellaceae family. There are approximately 20 members of this genus.

Bartonella spp. are transmitted by arthropods, and the primary vector for *Bartonella henselae* in cats is the cat flea. In dogs, tick transmission via the brown dog tick, *Rhipicephalus sanguineus*, is thought to be the mode of infection. Although *Bartonella* spp. are intracellular bacteria and the infection is suspected to be primarily in red blood cells, an infection of vascular endothelium also occurs. Relapsing bacteremia occurs, but where the bacteria are harbored in the body has not been elucidated (Guptill 2010).

Disease in Cats

Existing data indicate that few cats naturally infected with *Bartonella* have notable clinical signs. Some small epidemiologic studies have evaluated the association between exposure and clinical conditions, such as neurologic conditions, stomatitis, uveitis, and fever, but a statistically significant association of documented active infection with those conditions in naturally-infected cats has not been found (Guptill 2010). Naturally-occurring infection is generally thought to be mild and transient, and clinical findings may include pyrexia, lymphadenopathy, lethargy, anorexia, CNS disorders, urologic diseases, and endocarditis. Ocular diseases in cats may include, uveitis, keratitis, and chorioretinitis. *Bartonella henselae* is the species most frequently reported to infect cats.

Disease in Dogs

The species most commonly associated with canine disease are *B. henselae, B. vinsonii, B. clarridgeiae*, and *B. elizabethae*. Canine *Bartonella* spp. have been associated with anterior uveitis, hyphema, chorioretinitis, and retinal detachment due to systemic hypertension. Additionally, lethargy, weight loss, muscle and joint pain, hind-limb paresis, fever, endocarditis, cutaneous vasculitis, granulomatous lymphadenitis, and myocarditis can be seen. Various other neurologic and dermatologic signs may also be observed. In one case, identifying the bacterium from aqueous humor using PCR was unsuccessful, but the dog did respond to therapy with azithromycin. In dogs, diagnosis is based on serologic testing. Endocarditis appears to be the clinical condition having the strongest association with *Bartonella* infection in dogs, and it appears as if many infected dogs show no signs. Whether bartonellae are primary pathogens of dogs, or opportunistic pathogens, is not completely clear.(Guptill 2010)

Diagnosis

Diagnostic tests include serology, blood culture, and PCR. Epidemiologic studies show that there are high numbers of cats that are seropositive for *Bartonella* spp. in many countries. In the United States, 12–67 % of cats are seropositive for *Bartonella*. A higher prevalence of affected cats occurs in warm, humid areas that have more fleas. Because of the high percentage of chronically bacteremic yet healthy cats, drawing conclusions between presence of the bacteria and disease states is problematic (Lappin 2000).

B. henselae may be a cause of uveitis in some cats. Specific, intraocular IgG antibody production was documented in 8/50 cats with naturally occurring uveitis, in 4/9 experimentally-infected cats, and in 0/49 healthy shelter cats. The C value considered significant in these papers, however, was >1. Typically, C values >8 are considered more likely to be associated with local antibody production. *B. henselae* DNA was identified by PCR from the aqueous humor of 3/24 cats with uveitis, 1 healthy shelter control cat, and 4/9 experimentally-infected cats. Although these data are interesting, it must be kept in mind that an organism that naturally infects red blood cells may easily be included in an aqueocentesis sample, confounding PCR results (Lappin 1999).

Another study also looked at the relationship between bartonellosis and feline anterior uveitis. The hypothesis was that *Bartonella* spp. can cause uveitis in cats. The retrospective study, using a database, identified 116 cats with uveitis and 156 controls. The state of origin of the cats was determined and categorized as either a high or low flea risk state. Those cats (with and without uveitis) in which ELISA-IgG antibodies to *Bartonella* in serum had been examined were identified. A significant negative association between a positive antibody titer to *Bartonella* and clinical uveitis was found. Cats with uveitis were less likely to have *Bartonella* titers ≥1:64. Because of the limitations of this study, it does not support or deny the premise that cats affected with *Bartonella* are likely to have uveitis (Fontenelle 2008). In another study, PCR was done on aqueous humor and serum from cats with uveitis, and several more cats were positive on PCR than on serology (Powell 2010). For a review of *Bartonella* and feline ocular disease, see Stiles 2011.

Neonatal Septicemia/Bacteremia
Disease in Foals

Septicemia and bacteremia are typically seen in neonatal foals. The predominant bacteria involved in neonatal foal septicemia are the Gram-negative organisms *Escherichia coli, Klebsiella* spp*., Enterobacter* spp*., Actinobacillus* spp*.,* and *Pseudomonas* spp*.* In a recent study, only Gram-negative bacteria were detected in the uveitic bacteremic foals. Ocular disease could be associated with direct bacterial spreading to the uveal tissue or with local vascular alterations induced by secondary endotoxemia, causing inflammation and exudation of proteins and cells into the aqueous humor (Leiva 2009). Another study had four foals with culture-positive aqueous humor indicating *Rhodococcus equi;* most of those foals were bilaterally affected (Labelle 2011).

In foals, the typical routes of entry for bacteria include the placenta, umbilicus, lungs, and gastrointestinal tract. The major risk factor for septicemia in foals is failure to receive an adequate quality and quantity of colostral antibodies. Other factors that influence disease incidence include unsanitary environmental conditions, gestational age of the foal (prematurity), health and condition of the dam, difficulty of parturition, and the presence of new pathogens in the environment against which the mare has no antibodies.

Frequently affected organ systems include the umbilical remnants, central nervous system, respiratory, cardiovascular, musculoskeletal, renal, ophthalmic, hepatobiliary, and gastrointestinal organs. Foals in the early stages of sepsis are depressed and lethargic and do not nurse with normal frequency. Fibrin in the anterior chamber or hypopyon, severe miosis, and entropion (if the foal is dehydrated) are commonly seen.

Anterior uveitis is seen in a significantly higher number of septic/bacteremic foals than in septic/ nonbacteremic foals, nonseptic foals, and control foals. Anterior chamber fibrin was observed in only 4/14 (29%) septic/bacteremic foals with anterior uveitis. Anterior uveitis was also associated with posterior uveitis in 6/35 (19%) septic/bacteremic foals. Uveitis was related to nonsurvival (Leiva 2009, Labelle 2011).

Disease in farm animals

Calves, piglets, kids, and lambs may develop septicemia from umbilical infections or ingesting bacteria. Commonly isolated bacteria are *Streptococcus* species, *Escherichia coli*, *Arcanobacterium pyogenes*, *Salmonella* spp., *Actinobacillus* spp., *Klebsiella* spp., and *Pasteurella* spp. Systemic clinical signs include polyarthritis, meningitis, and/or diarrhea. Ocular lesions typically include episcleral or conjunctival injection, fibrin clots in the anterior chamber, hypopyon or hyphema, miosis, and chorioretinal embolic lesions of multifocal hemorrhages, exudate, and focal retinal detachment.

ANAEROBIC PATHOGENS

Anaerobic bacteria possess complex, species-dependent virulent mechanisms. They may induce corneal damage directly by the elaboration of toxins, metabolites, enzymes, and degradation products, and indirectly by the stimulation of corneal immune responses. Obligate anaerobic bacteria have been isolated from dogs, cats, horses, and alpacas with ulcerative keratitis. In fact, they were isolated from 13% of corneal samples from these species. The most commonly cultured bacteria were from the genera *Clostridium, Peptostreptococcus, Actinomyces, Fusobacterium,* and *Bacteroides*. A positive correlation was found between the isolation of aerobic bacteria and ocular trauma, preexisting corneal disease, and chronic dermatologic disease (Ledbetter 2008).

Concurrent or prior facultative aerobic bacterial multiplication in the cornea produces a habitat more supportive of anaerobic bacteria invasion and growth through bacterial oxygen scavenging and lowering of oxidation reduction potentials within host tissue. Additionally, aerobic bacteria may enhance anaerobic bacterial growth by the production of essential nutrients, growth factors, energy substrates, and protective enzymes. Mixed infections provide mutual protection from phagocytosis and intracellular killing.

In many cases, prescribed antimicrobials had limited to no microbial action against anaerobic pathogens, including aminoglycosides and fluoroquinolones, and may have eliminated the aerobic bacterial flora of the corneal ulcers, while sparing the anaerobes. With the exception of *Bacteroides* species, antimicrobial susceptibility patterns are relatively predictable for obligate anaerobic bacteria. There is a high frequency of susceptibility to chloramphenicol, metronidazole, amoxicillin, clavulanic acid, and clindamycin. The reason that many cases have a favorable clinical outcome in the absence of direct antimicrobial action against the anaerobes may include elimination of synergistic aerobic bacteria and disruption of low oxygen corneal microenvironment.

A case of stromal abscession associated with *Actinomyces bowdenii* was described in a diabetic mixed-breed dog that had undergone phacoemulsification and was being treated with topical cyclosporine. This was controlled but not cured with topical fluoroquinolone therapy. Eventually a superficial keratectomy with conjunctival graft and treatment with triple antibiotic was employed (Sherman 2013).

Non-spore-forming obligate anaerobes are also implicated in necrotic and suppurative deep tissue infections, often as mixed infections with facultatively anaerobic bacteria. In veterinary ophthalmology, *Peptostreptococcus, Bacteroides,* and *Fusobacterium* have been isolated from cases of orbital cellulitis or orbital abscesses.

OTHER BACTERIAL INFECTIONS
Rickettsial species

Rickettsial species consist of minute, obligate intracellular bacteria that are transmitted by ticks. They are rod-shaped or coccobacilli, and they vary from 0.3 to 0.6 µm in length. Rickettsial organisms contain both RNA and DNA, and they replicate through binary fission primarily within the cytosol of target cells. They are non-motile and aerobic.

Rickettsia rickettsii
Transmission and Pathogenesis

Rickettsia rickettsii causes Rocky Mountain Spotted Fever (RMSF), an acute febrile illness in dogs and humans. The disease is seen primarily in the southeastern United States, but is found throughout the United States and in western Canada and Central and South America. The tick vectors are *Dermacentor variabilis* and *Dermacentor andersoni*. The ticks become

infected horizontally during feeding on infected mammals or following transtatal replication within the tick midgut and transovarial transmission between generations of ticks. Transmission to the host does not occur for 5–20 hours post attachment, so removal of ticks may prevent disease.

Vasculitis is the primary lesion caused initially by direct infection of the vascular endothelium and perithelial smooth muscle from spread through the circulatory system, and later by immunologic phenomena. The pathogenesis of ocular disease in RMSF relates directly to the vascular lesions, which initiate platelet activation and activation of the coagulation system.

Disease in Dogs

Common clinical signs include fever, neurologic dysfunction, polyarthritis, petechial and ecchymotic hemorrhages, thrombocytopenia, and nonregenerative anemia. Signs begin within 3 days of tick attachment. Hemorrhages most commonly occur on mucous membranes, but epistaxis, melena, and hematuria may be present in severely affected animals. The hemorrhage is due to the vasculitis and not the thrombocytopenia.

Altered vascular permeability in the conjunctiva, uvea, and retina result in ocular signs. Evidence of conjunctivitis usually begins with onset of fever and includes conjunctival hyperemia, chemosis, petechial hemorrhages, and a mucopurulent to purulent ocular discharge. Anterior segment findings include subconjunctival hemorrhage, iris stromal petechiations, anterior uveitis, and hyphema. Posterior segment findings include retinitis characterized by perivasculitis, focal areas of edema, and petechiation. Unilateral or bilateral optic neuritis can also occur. Because ocular disease can be confined to the retina, ophthalmoscopy should always be done in dogs with suspected RMSF. Generally the ophthalmic lesions are mild with RMSF.

In experimental infection, fluorescein angiography revealed increased permeability in retinal vessels beginning 6 days post infection and 2 days after onset of pyrexia. Venules were more permeable than arterioles (Davidson 1990).

The histologic appearance of RMSF is different from ehrlichiosis in that the most prominent lesion is one of necrotizing vasculitis with perivascular accumulations of polymorphonuclear and lymphoreticular cells. The progressive necrotizing vasculitis ensues, and organs with endarterial circulation such as skin, brain, heart, kidney and retina are often most affected.

Confirmation of RMSF involves the rising of serum titers on IFA or isolation of the organism.

Treatment with doxycycline, tetracycline, chloramphenicol, or enrofloxacin for 14–21 days is usually effective. Those with severe posterior segment lesions can be treated with anti-inflammatory doses of oral prednisone in conjunction with antibiotic therapy.

Ehrlichia canis

Ehrlichia canis causes canine monocytic ehrlichiosis that results in a spectrum of acute, subclinical, and chronic disease. They are Gram-negative bacteria, but they lack peptidoglycan and lipopolysaccharide components. *E canis* is an obligate intracellular parasite transmitted by the brown dog tick, *Rhipicephalus sanguineus*. The tick can still transmit the disease more than 5 months after detaching from the canine host.

The organism replicates in mononuclear inflammatory cells, which allows it to spread throughout the body. Circulating leukocytes are also targeted. As perivascular tissues become infected with the organisms, severe vasculitis may occur, resulting in bleeding and platelet consumption. The morulae, which are cytoplasmic clusters of dividing organisms, can be seen microscopically in monocytes.

Disease in Dogs

The acute phase begins 8–20 days post infection and lasts 2–4 weeks. Common clinical signs include fever and depression. Severe cases may show neurologic signs, petechial and ecchymotic hemorrhages, epistaxis, lymphadenopathy, limb edema, and vomiting. Ticks are found on 40% of dogs that present with signs. The subclinical phase lasts for weeks to months. Clinical signs may regress. The chronic phase may persist for years. Signs may include depression, weight loss, pale mucous membranes, abdominal tenderness, bleeding episodes, secondary infections, and limb edema.

Ocular disease is associated with acute vasculitis, perivasculitis, and thrombocytopenia. Signs include conjunctival hyperemia, conjunctival hemorrhages, corneal edema, deep corneal vascularization, anterior uveitis, iridal congestion and petechiations, miosis, and fibrinous debris on the anterior lens capsule. Hyphema, chorioretinitis, vasculitis, vitreal and retinal hemorrhages and detachment, panuveitis, and optic neuritis have also been noted. The ocular hemorrhage associated with ehrlichiosis may be related to thrombocytopenia, platelet dysfunction, or hyperviscosity. As the disease becomes chronic,

retinal vascular engorgement and compromise of the blood/retinal barrier may result from hyperproteinemia and hyperviscosity syndrome.

Typical laboratory abnormalities include anemia, leukopenia, thrombocytopenia, monoclonal or polyclonal gammopathy, and bleeding tendencies. During the subclinical phase, thrombocytopenia persists and globulins rise. Morula may be seen on stained blood smears. Bone marrow cytology may show hypoplastic elements, plasmacytosis, and mastocytosis. Serology using IFA and PCR can be done. The most consistent ocular histopathologic finding is a predominantly monocytic or lymphocytic cellular infiltrate of the uveal tract and to a lesser extent, the retina and optic nerve.

Anaplasma platys
Anaplasma platys is a rickettsial parasite that causes infectious thrombocytopenia in dogs and has been reported to cause uveitis. *E chaffeensis, E ewingii*, and *E equi* (in dogs) may also cause anterior uveitis.

Borreliosis or Lyme's Disease
Lyme's disease is a tick-borne spirochetosis produced by *Borrelia burgdorferi*, which are small corkscrew-shaped, motile microaerophilic bacteria. These organisms do not survive free living in the environment. The highest incidence remains in the northeastern United States.

B. burgdorferi is transmitted primarily by ticks of the *Ixodes* sp. The primary reservoirs include small rodents and birds. Successful transmission of the agent relates directly to the contact time of the tick on the host, requiring 48 to 72 hours for a 38–92% transmission rate. Once inside the host, the organisms rarely spread hematogenously but rather use their specialized endoflagella to move through the connective tissues. Organisms can survive for years in skin, connective tissue, joints, and nervous system.

Disease in Dogs and Cats
Systemic signs include lameness, joint pain, pyrexia, and lymphadenopathy. Ocular lesions thought to be associated with borreliosis include conjunctivitis, corneal edema, anterior uveitis, retinal petechia, orbital myositis, chorioretinitis, and retinal detachment, although definitive proof of association is lacking (Raya 2010).

Disease in Other Species
Clinical signs of Lyme borreliosis are uncommon in horses; when present, they are often vague and nonspecific, but may include musculoskeletal, neurological, reproductive, and ocular disorders, including uveitis. Two horses had uveitis associated with *B. burgdorferi* based on the identification of spirochetes within ocular fluids and confirmed with PCR testing. Diagnosis can be challenging because the organism is rarely isolated, and serologic tests can be unreliable due to the possibility for false negative results and the failure of such tests to confirm active infection (Priest 2012). Organisms were found in the anterior chamber of one pony with bilateral panuveitis and lameness (Burgess 1986).

Humans have multiple ocular signs, including conjunctivitis, keratitis, panuveitis, chorioretinitis, retinal detachment, optic neuritis, and periorbital edema.

There is a high prevalence of serum-positive antibody titers (75%) with actual disease in approximately 5–10% of dogs. The diagnosis of Lyme's disease was previously problematic because vaccination resulted in positive results using ELISA and IFA tests. Current lab tests that employ the specific c6 lipoprotein antigen (EIA, ELISA) or Western blot procedure have overcome this problem. Antibodies may persist for months to years following vaccination or natural infection.

Leptospirosis
Members of the genus *Leptospira* are motile spirochetal bacteria. The predominant serovars responsible for causing clinical disease in dogs include *canicola, icterohemorrhagica, grippotyphosa, pomona,* and *bratislava. Leptospira* are the smallest spirochete bacteria, measuring < 0.3 µm in width and 6–30 µm in length. They have a tightly wound spiral shape. The organisms are motile and able to enter hosts by penetrating mucous membranes or abraded skin mechanically. They do not stain with conventional pathology stains and require a special silver impregnation stain for identification.

Leptospira are aquatic unicellular organisms found in river and lake waters and in sewage. Principle reservoirs are deer, cattle, swine, and rats. The organisms multiply in the kidneys of adapted hosts and are shed in the urine. Organisms have only a short life in soil; however, they can live in water for up to 6 weeks. Horses pastured next to unvaccinated cattle or pigs and horses that live on farms frequented by deer or infested with rats are at increased risk for exposure. Direct transmission can occur through contact with infected urine, bites, ingestion of affected material, and contact with contaminated water. Vasculitis and endotheliitis involving the kidneys, liver, spleen, muscles, central nervous system, and eyes occur.

Disease in Dogs

Renal failure is becoming the predominate clinical syndrome because of the bivalent vaccine decreasing the occurrence of *icterohemorrhagica* and *canicola,* which most commonly cause liver diseases. Even though acute leptospirosis causes vasculitis, ocular lesions are infrequently seen. Those reported, in conjunction with other systemic signs, are conjunctivitis with mucopurulent oculonasal discharge, scleritis, and anterior uveitis. Other signs include renal or hepatic failure or dysfunction.

Disease in Horses

Acute signs of leptospirosis in the horse include transient depression, fever, icterus, anemia, and anorexia. Serologic surveys of horses have shown that exposure to *Leptospira* is common, but variable, according to the geographic location or climate. Horses positive for leptospirosis are common in the Ohio, Delaware, Tennessee, and Mississippi river valleys.

L. interrogans most likely plays a role in many cases of equine recurrent uveitis (ERU). *L interrogans* is classified into more than 20 serovars. The serovar most commonly associated with ERU is *L. interrogans* serovar *pomona*. Leptospiral bacteria have been avidly sought in ocular tissues. Evidence of their presence has been intriguing, yet sparse. Usually, horses seem to develop ERU 18 to 24 months after an initial infection with leptospirosis.

Leptospira organisms are sequestered in various organs, particularly the kidneys, liver, spleen, central nervous system, and eyes. Diagnosis is dependent on clinical signs and detection of antibodies via the microscopic agglutination test (MAT), dark field, histology, or PCR; of these, only the MAT is serovar specific. *Leptospira* organisms may be cultured from the aqueous humor of some dogs. *Leptospira* can be shed in the urine for up to 3 months.

MYCOLOGY

Fungi are eukaryotic organisms that grow as either single-cell yeasts or multi-cellular filamentous molds. Yeasts are unicellular, elliptical to spherical and generally 3–5 µm in diameter. Yeasts reproduce by budding. Molds are characterized by branching tubular filaments or hyphae and are generally 2–10 µm in diameter. Fungi that can grow as either yeasts or molds are termed dimorphic. Some fungi can exhibit multiple forms simultaneously and these are considered polymorphic. Environmental temperature, nutrient factors, and kinetic factors determine the type of growth observed. The reproductive cycle of fungi may be sexual, asexual, or both. Fungal growth is by simple mitosis of somatic nuclei and budding or apical extension of the cell wall. Hyphae are often divided at regular intervals by cross partitions called septa, which have one or more small pores that allow for cytoplasmic communication. Fungal cell walls contain ergosterol, other lipids and glycoproteins and may serve as unique targets for antifungal drugs.

KERATOMYCOSIS

Fungi do not appear to be permanent floral residents of the ocular surface but only transient colonizers of the external eye. Filamentous fungi are the predominant fungal isolates reported from eyes of normal animals. In equine studies, the fungi most frequently isolated from normal eyes include *Aspergillus, Alternaria, Penicillium, Fusarium, Cladosporium,* and *Absidia*. Local defenses to fungi are generally quite effective because ocular infection is not common unless anatomic barriers are compromised. An intact corneal epithelium provides excellent resistance to fungal penetration and infection. Normal ocular surface flora, normal lacrimal flow, and mechanical movements of the eyelids and third eyelids create an environment unfavorable to the growth of many opportunistic fungi. In addition, because many fungi will not grow at elevated temperatures, normal body temperature is high enough to prevent some from becoming pathogenic. However, the lower temperature of the cornea may partially explain the predilection for keratomycosis.

Immunity to fungal infections is considered to be more cell mediated than antibody mediated. Local immunosuppression by corticosteroids may predispose an animal to a fungal infection by decreasing the cellular immune mechanisms. Additionally, systemic or topical antibacterial agents alter normal flora and can decrease natural microbial barriers as well as encourage colonization and growth of fungi. It appears that vascularization is required for the healing of many corneal fungal infections, and vascularization often appears to slow as it reaches the affected area. One study showed that certain fungal isolates produce metabolites that inhibit vascular tubule formation in vitro, thus altering the host vascular response to the fungi (Welch 2000). Keratomycosis is most commonly diagnosed in the horse but has been reported in the dog and cat as well. In dogs and cats, the infected cornea may have a brownish tinge.

Fungal infections of the equine cornea are usually attributed to environmentally common organisms such as *Aspergillus, Penicillium*, and *Fusarium*. Other organisms such as *Cylindrocarpon destructans* that have not been cultured from normal eyes occasionally cause disease. Proof of fungal disease may be difficult in some cases. Unfortunately, fungal hyphae tend to migrate into Descemet's membrane, making diagnosis more difficult.

Candida infections can occur on the conjunctiva and cornea. Candidiasis seems to be more of an opportunistic infection.

Diagnosis is routinely made from a combination of cytology, culture, or histopathology. Confocal microscopy has been shown to be a rapid non-invasive method to diagnose fungal keratitis, as well. Fungi were seen as linear, branching hyper-reflective structures. Main differentials are corneal nerves, but they are less haphazard, had less branching, and were less reflective. Fungi were harder to find in deep stromal abscesses (Ledbetter 2011).

Fungal sensitivities to natamycin, itraconazole, and natamycin vary by region. Voriconazole, a 1% solution administered topically, appears to have a broad spectrum of activity and can penetrate into the anterior chamber. Another inexpensive topical antifungal is silver sulfadiazine, which also has antibacterial properties. It can be applied every 4 to 5 hours. Fungi tend to be less sensitive to fluconazole than to other antifungal drugs (Brooks 1998). Treatment with antifungal agents must often continue for 6–8 weeks. Response to medical therapy can be very slow, and in cases that do not respond, surgery is indicated.

Dogs with fungal keratitis often have predisposing factors such as underlying pre-existing corneal disease such as KCS or vegetative foreign material, intraocular surgery, endocrinopathies, and/or prolonged use of either topical antibiotics or corticosteroids. Cytology in conjunction with culture offers the best likelihood of giving a definitive diagnosis. Many types of fungal organisms have been isolated, including *Cladosporium* spp., *Chrysosporium* spp., *Curvularia* spp., *Aspergillus* spp., *Penicillium* spp., *Scedosporium*, *Candida* and *Phialemonium* spp. (Scott 2014) (Nevile 2015).

Specific antifungal treatment can include 1% voriconazole solution, 1% itraconazole ointment or natamycin or others. Keratectomy and conjunctival grafting surgery may be necessary.

SYSTEMIC MYCOSES

The primary causes of ocular mycosis are the dimorphic fungi *Blastomyces, Coccidioides, Cryptococcus,* and *Histoplasma*. These may all cause uveitis, chorioretinitis, endophthalmitis, and optic neuritis. The host inflammatory response to ocular mycosis is generally suppurative in acute cases and pyogranulomatous in chronic cases. Host tissues can be damaged directly by inflammatory processes or by degradative enzymes produced by the fungal organisms.

Dimorphic fungi such as *Blastomyces dermatitidis, Coccidioides* sp (*C. immitis* and *C. posadasii*), and *Histoplasma capsulatum* have developed adaptations to circumvent the effectiveness of host defense responses. These are all primary fungal pathogens because they can infect humans and animals with normal or impaired immune defenses. Features that facilitate growth of dimorphic fungi in tissue include the production of small-sized conidia that can penetrate deep into the respiratory tree, ability to grow at 37°C (i.e., thermotolerance), conversion to yeast morphology, expression of yeast-phase–specific virulence factors, and evasion of host immune cells. The conversion between hyphal and yeast morphologies, known as the phase transition or dimorphic switching, is essential for the pathogenesis for all thermally dimorphic fungi.

A number of signalling pathways have been identified that induce the dimorphic switch. Since intracellular growth also necessitates survival through the macrophage phagolysosomal system, these signalling pathways also frequently co-regulate processes important for adaptation to this environment, such as adaptation to oxidative stress. Host or temperature-derived signals trigger dimorphic switching and are likely to be transmitted via signalling pathways, ultimately culminating in changes in gene expression. Phase (yeast or hyphal) specific genes have been identified, but little is known about how the expression of these genes is controlled. In the soil (22–25°C), *B. dermatitidis* grows as filamentous hyphae that produce conidia (asexualspores). Following soil disruption from human activities (e.g.,construction), conidia and hyphal fragments are aerosolized and when inhaled into the lungs of a human host (37°C) convert into budding yeast that cause pneumonia. The predominant stimulus that induces the morphologic switch between hyphal and yeast forms is temperature. Recent investigation has demonstrated that conversion to yeast is accelerated following phagocytosis of *B.dermatitidis* spores by alveolar macrophages. In addition to intracellular spore germination, yeasts are able to survive and replicate with in macrophages during the early stages of infection. Thus, *B. dermatitidis* exhibits an intracellular lifestyle, which is similar to other dimorphic pathogens including *H. capsulatum* and *Coccidioides* spp. (Smith 2015).

Residing within phagocytotic cells of the host shields organisms from the rest of the immune system. As protracted hyphal growth within phagocytes would lead to cell rupture, thus exposing the fungus to the host immune system, a number of fungi switch from the multicellular hyphal growth form found in the environment to a unicellular yeast growth form via dimorphic switching. Other dimorphic fungi use the yeast cell form to avoid phagocytosis and the cytotoxic environment of the phagolysosomal system; instead, they are adapted to tolerating the adaptive immune responses. Thus, dimorphic switching allows for the colonization of unique environmental niches within the host and the failure to switch almost always attenuates pathogenicity in these fungi. In other dimorphic fungi that exist predominately as a yeast vegetative growth form outside the host, such as *Candida albicans*, the dimorphic switch from a yeast to a filamentous growth form can facilitate tissue penetration during infection.

Surviving within the macrophage phagolysosome poses many metabolic challenges for intracellular dimorphic pathogens, including a lack of essential trace elements such as iron and zinc. Host cells restrict available iron through sequestration by high-affinity iron-binding proteins such as transferrin and ferritin to prevent intracellular microbial proliferation. Host T-cells produce the cytokine interferon gamma (IFNγ) which downregulates surface transferrin receptors and NO production by activated macrophages, which also restricts iron availability. Under iron-limiting conditions, fungi utilize high-affinity iron uptake systems such as reductive iron assimilation and non-reductive, siderophore-based iron assimilation. It has been known for some time that calcium plays an important role in *H. capsulatum* infection. Large amounts of calcium are essential for *H. capsulatum* hyphal but not yeast growth suggesting that yeast cells are adapted to surviving in low calcium environments such as the macrophage phagolysosome (Batanghari 1997). Bad1 is a yeast-specific calcium-binding protein which is essential for pathogenicity in *B. dermatiditis* (Brandhorst et al. 1999). Bad1 contains 41 copies of a tandem repeat each with a calcium-binding EF-hand motif, facilitating calcium binding and growth in a poor calcium environment (Boyce 2015).

Another common survival strategy used by dimorphic fungi is to rapidly remodel the cell wall during infection in order to prevent recognition by phagocytic cell PRRs. For example, *B. dermatitidis* decreases the ß(1,3)-glucan content and increases the α(1,3)-glucan content of the cell wall during infection and the hyphal-to-yeast dimorphic switch. This may prevent recognition of ß(1,3)-glucan, which is hidden by layer of α(1,3)-glucan, by the dectin-1 PPR. Additionally, proteins required for oxidative stress resistance were recently found to be components of the extracellular proteome of *H. capsulatum* yeast cells and heat shock proteins have developed to allow for a response to changes in environmental temperature (Caruso et al. 1987).

BAD-1 protein also mediates virulence in part by binding host lung tissue, the extracellular matrix, and cellular receptors via glycosaminoglycans, such as heparan sulfate. Pathogenic fungi and other microbes must adhere to host tissue to initiate infection. Surface adhesins promote this event and may be required for disease pathogenesis (Beaussart 2015).

Blastomycosis

Blastomycosis, a disease caused by the dimorphic fungus *Blastomyces dermatitidis*, occurs primarily in dogs and humans. Blastomycosis is primarily found in North America, and occurs most frequently in the Mississippi, Missouri, and Ohio River valleys, the mid-Atlantic states, and the Canadian provinces of Quebec, Manitoba, and Ontario. It also occurs in India and Africa. While the precise microecology is unknown, it has been found in harsh or rapidly changing environmental conditions and associated with changing water levels, sand soils, relatively low elevation waterways, relative drought, with ground-dwelling mammals that use burrows containing latrine chambers, and in other avian and mammalian waste products (Baumgardner 2009).

Growth and sporulation of strains have been observed at low glucose concentration and calculated ammonia concentrations of 4.2 mmol/l when plates were inoculated with either mold or yeast forms, and 4/5 strains tolerated ammonia concentrations of 42-62 mmol/l. Growth of virtually all soil fungi from the northern USA and Canada was inhibited at ammonia concentrations of 2.1-4.2 mmol/l. The ability to survive and grow in organic carbon-poor, high ammonia microenvironments may be important to the competitive success of this fungus (Baumgardner 2009).

One study that only looked at cases from Knox County, TN found that important risk factors associated with blastomycosis were sex, breed, age, and proximity to water. Soil type, pH, and organic matter content had no significant associations in this study area. The discrepancy with other studies may be due to little variation in pH and organic matter content of soils in this study area. It is also possible that these factors are not predictors of risk of blastomycosis in that study area thus strengthening the hypothesis that different variables may be more important predictors of the disease in certain geographical locales and not others. One other possibility is that exposure was not soil related. It has been noted that many isolates are collected above ground in manure or decomposed plant debris (Chen 2008).

Another study evaluated trends in prevalence and seasonal distribution of blastomycosis cases submitted to a veterinary diagnostic laboratory in Saskatchewan. Signalment was similar to that reported previously. All cases originated in southern Saskatchewan and Manitoba. Case numbers showed a significant increase in the period 2001 to 2010 compared to 1990 to 2000. Seasonally, there was an increasing trend in the number of diagnoses from February to November. There was no correlation between average seasonal temperature or average seasonal total precipitation and the number of cases of blastomycosis (Davies 2013).

One human study found six clusters of counties in 13 endemic states in which there was an elevated incidence of blastomycosis hospitalizations. The odds of a county being part of a high-risk cluster was associated with increasing percentage of population over age 65, decreasing maximum temperature, increasing mercury, and decreasing copper soil content (Seitz 2015).

The most significant route of transmission for *B. dermatitidis* is inhalation of airborne conidia that occurs from disruption of wet soil or organic matter containing micro-foci of mycelia. The mycelia release infectious conidia, which are subsequently inhaled by a susceptible host. Shared environmental exposures explain the occurrence of disease in humans and their canine companions (Saccente 2010). In one study, no cultures from the nares of 110 dogs in an area of Wisconsin that is highly endemic for blastomycosis grew *B. dermatitidis,* suggesting that carriers are not common (Varani 2009).

Initially *B. dermatitidis* establishes infection in the lungs, from which the organisms spread hematogenously or via the lymphatics to the skin, eyes, bones, lymph nodes, brain, and testes. The yeasts are 5 to 20 μm in diameter, have a thick, refractile, double-contoured cell wall, and are often seen in a characteristic broad-based budding form.

The characteristic host response to infection with *B. dermatitidis* is polymorphonuclear leukocytes and granulomas, with epithelioid histiocytes and giant cells. Early in the infection, polymorphonuclear leukocytes predominate, and organisms are generally found easily (Saccente 2010).

B. dermatitidis is widely considered an extracellular pathogen, with little evidence for a facultative intracellular lifestyle. However, one study infected mice with spores via the typical pulmonary route and studied intracellular residence, transition to pathogenic yeast, and replication inside lung cells. Nearly 80% of spores were inside cells at 24 h postinfection. Most spores were located inside of alveolar macrophages, with smaller numbers in neutrophils and dendritic cells. Real-time imaging showed rapid uptake of spores into alveolar macrophages, conversion to yeast, and intracellular multiplication during in vitro coculture. The finding of multiple yeast in a macrophage was chiefly due to intracellular replication rather than multiple phagocytic events or fusion of macrophages. Depletion of alveolar macrophages actually curtailed infection. And

dimorphic switching of the spores to yeast was delayed in these depleted. Thus, although advanced *B. dermatitidis* infection may exhibit extracellular residence in tissue, early lung infection with infectious spores reveals its unappreciated facultative intracellular lifestyle (Sterkel 2015).

Disease in Dogs

Young, large-breed dogs are more commonly infected. Infection typically involves the lungs, skin, bones, lymph nodes, brain, prostate, mammary glands, and testes, in addition to the eyes. Ocular disease occurs in 30% to 43% of dogs with systemic blastomycosis. Ocular infection causes anterior uveitis, panophthalmitis, chorioretinitis, retinal detachment, secondary glaucoma, and optic neuritis. Infection can also cause nodule formation in the inferior conjunctiva and orbital cellulitis. Orbital lesions can masquerade as neoplasia (Baron 2011). Neurologic signs can be caused by axial lesions, spread through the cribriform plate from the nose or spread from orbital disease (Hecht 2011).

Disease in Cats

Blastomycosis is much less common in cats than dogs. The most common clinical signs are associated with the respiratory, central nervous, lymph nodes, skin, eye, gastrointestinal tract and urinary systems. Ocular signs are similar to dogs and include anterior uveitis, rubeosis iridis, keratic precipitates, retinal detachment, and chorioretinitis with granulomas.

Disease in Other Species

Blastomycosis has also rarely been diagnosed in a dolphin, bats, horses, a polar bear, a black bear, a rat, and nondomestic felids. Ocular lesions have not been reported. Zoonotic transmission is rare but has been reported in association with dog bites, cat scratches, animal necropsies, and a bite from a pet kinkajou (Harris 2011).

Diagnosis

The ease of diagnosis of blastomycosis is variable. Aspirates of draining tracts, enlarged lymph nodes, or vitreous when posterior segment disease is present, may all yield organisms. MiraVista™ *Blastomyces* antigen EIA is the diagnostic test of choice if the organisms are not seen cytologically. *Blastomyces* antigen can be detected in the urine in 93.5% of cases of blastomycosis in dogs using this test compared to using AGID (17.4%) and enzyme-linked immunosorbent assay (76%) (Spector 2008).

Further study supports the use of antigen testing. Urine *Blastomyces* antigen testing has a high degree of sensitivity for active disease both at the time of diagnosis (100%) and during treatment (at least 82%), and moderate sensitivity for clinical relapse (71%). Urine antigen results can be weakly positive (<1 ng/mL) in dogs that are in clinical remission at the time of drug discontinuation, and this finding is not necessarily predictive of relapse. *Blastomyces* urinary antigen concentrations should be monitored, at a minimum, at the time of diagnosis and when treatment discontinuation is being considered, as well as at any time during treatment when clinical efficacy is in doubt. The urine test should be repeated in any dogs with clinical suspicion of relapse, especially if *Blastomyces* organisms cannot yet be detected (Foy 2014). A human study found lower sensitivities than reported canine blastomycosis with only 76% of patients having antigenuria (Frost 2015).

In order to evaluate the sensitivity and specificity of an enzyme immunoassay (EIA) for antibodies to a recombinant Blastomyces adhesin-1 repeat antigen (rBAD-1), serum and urine samples were collected from dogs with blastomycosis, histoplasmosis, or nonfungal pulmonary disease and from healthy control dogs living in a blastomycosis-endemic area. Serum was tested for antibodies against *B. dermatitidis* with the rBAD-1 antibody EIA and an A-antigen antibody agar gel immunodiffusion (AGID) assay. Serum and urine were tested for *B dermatitidis* antigen with a quantitative EIA. Sensitivity of the quantitative antigen EIA was 100% in serum and urine samples from dogs with blastomycosis, with specificity of 95% in urine samples from dogs with nonfungal pulmonary disease and 100% in urine samples from healthy dogs. Sensitivity of the rBAD-1 antibody EIA (95%) was significantly greater than that of the A-antigen antibody AGID assay (65%). Specificity of the antibody EIA was 88% in dogs with histoplasmosis, 95% in healthy dogs, and 100% in dogs with nonfungal pulmonary disease. This antibody EIA may assist in distinguishing histoplasmosis from blastomycosis (Mourning 2015).

Histologically, no parts of the eye are spared in blastomycosis. The most common features in affected eyes are choroiditis and retinal detachment. Pyogranulomatous inflammation is the most commonly observed response in both the anterior segment and the posterior segment. Lens rupture is seen in about 50% of enucleated canine eyes. Choroidal inflammation is more severe in the nontapetal than in the tapetal choroid. Additional histologic findings include retinal detachment, choroiditis, retinal degeneration, vitreal protein and or cells, protein and/or cells in the anterior chamber, optic nerve inflammation, preiridial fibrovascular membranes, and changes associated with secondary glaucoma. *Blastomyces* organisms are observed primarily in the choroid; rarely, organisms are seen in the anterior segment and retina (Hendrix 2004).

Treatment

Itraconazole is considered to be the treatment of choice and the mainstay of therapy and is clinically very effective in dogs with blastomycosis (Legendre 1996; Mazepa 2011). The recommended starting dose is 5 mg/kg orally twice a day for 5 days, followed by once-a-day administration for approximately 90 days (Legendre 2006). Urine antigen levels are seen to

decrease with itraconazole treatment and can be used to monitor disease resolution. A bioequivalence comparison of generic, compounded, and the reference formulation of itraconazole was done in normal dogs. Pharmacokinetic data for a generic formulation of itraconazole were similar enough to the reference formulation that therapeutic concentrations could be achieved. Compounded itraconazole produced such low plasma concentrations, it is unlikely to be effective; therefore, compounded itraconazole should not be used in dogs (Mawby 2014). Fluconazole is less effective and requires a longer treatment time than itraconazole, but the cost is less (Mazepa 2011). Dogs should be monitored for hepatotoxicity with both drugs. Parenteral amphotericin B is also effective, but renal toxicity may be problematic with this drug.

A genetically engineered, live-attenuated strain of *B dermatitidis* lacking the major virulence factor BAD-1 successfully vaccinates against lethal experimental infection in mice. Thus, the live-attenuated vaccine against blastomycosis may be forthcoming (Wuthrich 2011).

Prognosis for vision varies with respect to location and extent of inflammation within the eye and response to therapy. There is a positive response to treatment with itraconazole in 76% of dogs with posterior segment disease alone, 18% with anterior uveitis, and 13% with endophthalmitis (Brooks 1991). Many eyes that do not respond to treatment, continue to have uveitis, and develop glaucoma are seen to have thriving *Blastomyces dermatitidis* organisms in the eye and/or may have lens rupture secondary to the severe inflammatory response (Hendrix 2004). These findings may explain why the inflammation is intractable in many dogs. Another study evaluated the use of oral prednisone with itraconazole or fluconazole in an attempt to preserve vision. The prednisone dosage ranged from 0.2 mg/kg/day to 1.4 mg/kg/day, and the mean duration was 3 months. The prednisone did not appear to adversely affect survival rate, and all eyes with mild or moderate lesions and half of the dogs with severely-affected eyes were visual at their last recorded recheck examination (Finn 2007).

Cryptococcosis
Cryptococcus is a saprophytic, round, yeast-like fungus, 3.5–7 µm in diameter that has the ability to form a large 1–30-µm thick heteropolysaccharide capsule. The organism may or may not produce a capsule when growing on artificial media or when growing naturally in the environment, but it always produces a capsule in tissues. It reproduces by forming one or two buds, blastoconidia, that are connected to the parent cell by a narrow isthmus. The buds may break off when small and, therefore, the yeast population varies in size. Unlike other dimorphic fungi, the yeast form of *Cryptococcus* is always found under normal laboratory conditions and in infected tissues.

Cryptococcus neoformans is the organism most commonly involved with animal infections seen in the temperate regions of the world. *C. neoformans* is worldwide in distribution, and in addition to people, infects a variety of domestic and wild animals. Much work has been done evaluating the serotypes of cryptococcosis due to its importance in humans with the AIDS virus.

C. gattii was previously associated only with the bark and leaf litter of eucalyptus trees. However, it has now been isolated from native trees in the Pacific Northwest where the incidence of disease from *C. gattii* appears to be increasing in humans and animals. Incidence is also increasing in California. Generally, *C. neoformans* is associated with immunocompromised individuals and *C. gattii* with non-immunocompromised. The species have different molecular types. History of a single feline case suggests that the incubation period for *C. gattii* can be >8 years (Castrodale 2013).

In contrast to other systemic mycoses, the prevalence of cryptococcosis in cats is equal to or greater than that of dogs. The exact mode of infection is unknown, but the most likely route is through inhalation of airborne organisms. In the environment, *Cryptococcus* is unencapsulated and is thus much smaller and has an increased chance for aerosolization and inhalation. The organisms are then deposited in the upper respiratory tract, causing nasal granulomas. 7–14% of animals may have asymptomatic colonization of the nasal passages. The production of a thick capsule and abundant release of glycoprotein into the circulation are hallmarks of virulence. Once the capsule develops in the respiratory tract, it interferes with antigen presentation, subsequent immune response, and elimination. Cryptococcal infection can disseminate hematogenously, often to the central nervous system, or extend locally from the nasal cavity. Vertical transmission has occurred in humans, horses, and a porpoise with organisms in the placenta and amniotic fluid (Norman 2011).

Establishment and spread of infection in the host are highly dependent on immunity. The yearly incidence of cryptococcosis in non-HIV persons varies from 0.4–1.3 cases per 100,000 people. Incidence in HIV-infected patients decreased from 23–66 cases per 1,000 persons in 1992 to 2–7 cases per 1,000 largely due to the availability of highly active antiretroviral therapy. It has been the leading cause of death in HIV-infected individuals with a mortality of 30–60 %. The mortality rate in transplant patients is even higher (20–100%) (Centers for Disease Control). While the presence of underlying disease has been evaluated in dogs and cats, very few dogs and cats with cryptococcosis are shown to have immunosuppressive disease.

The complement system and phagocytic effector cells are the major players in the non-specific host immune response to *Cryptococcus*. The two major functions of the complement system during cryptococcosis infection are to stimulate the chemotaxis of phagocytic effector cells and enhance the uptake of cryptococcal cells by these phagocytes. The cryptococcal polysaccharide capsule is a well-known factor required for the pathogen's virulence, e.g. by inhibiting phagocytosis. *C. neoformans* mutants with a capsule-deficient phenotype are avirulent in mice. Several studies with encapsulated and non-encapsulated *C. neoformans* strains also showed a difference in complement activation dependent on capsulation.

Phagocytosis is triggered by direct recognition of the yeast or by receptor-mediated recognition via complement or antibodies. Conserved structures such as the components of the cryptococcal capsule can be directly recognized by pattern recognition receptors. The importance of macrophages in cryptococcal infections has become increasingly obvious in the last decade. Research has revealed an intriguing interaction between the phagocytic effectors and yeast cells that revealed *C. neoformans* as an intracellular parasite. *Cryptococcus* has a unique method to manipulate host macrophages. After phagocytosis, *C. neoformans* can survive and proliferate within these infected host cells, eventually leading to host cell lysis. Additionally, the yeast can exit macrophages without killing the host cell, thus avoiding a local inflammatory response, and the yeast can be laterally transferred from one macrophage to another (Voelz 2010).

The production of melanin-like compounds has been demonstrated in cryptococcal cell walls in tissue. In vitro melanization has been associated with resistance against host effector cells, oxidants, microbicidal peptides, and amphotericin B. Other fungi may employ melanin as a virulence factor for infection of animals or plants, suggesting that melanin synthesis may be a common strategy used by fungal pathogens to subvert host defenses (Casadevall 2000).

Disease in Dogs
Typically infection involves young, adult dogs. Doberman Pinschers, Great Danes, and American Cocker Spaniels seem to be over-represented. Central nervous system signs are typically multifocal and attributable to meningitis or encephalomyelitis and may include head tilt, nystagmus, facial paralysis, ataxia, mild paresis to complete paralysis, depressed reflexes, circling, and seizures.

The most common ocular abnormalities are extensive granulomatous chorioretinitis, retinal hemorrhage, papilledema, retinal detachment and optic neuritis associated with dilated pupils, and blindness. Anterior uveitis and a retrobulbar abscess with lysis of the orbital bones have also been described. Rarely, anterior uveitis and retinal detachment may occur without the presence of granulomas. The eye is involved secondary to hematogenous spread or extension from the brain along the optic nerve. One study found that overall 45% of infected dogs had ocular signs with the great majority of them associated with the posterior segment (Trivedi 2011). Cutaneous involvement has been found in dogs but is much less likely than in cats.

Disease in Cats
In cats cryptococcosis typically causes upper respiratory signs, hard subcutaneous nodules over the bridge of the nose, lymphadenomegaly, and ulcerative or proliferative lesions in the oral cavity. Thoracic radiographs are often normal, although small nodular lesions may be present. Cutaneous lesions, including papules or nodules varying from 1–10 mm in diameter are also common in cats and occur in 40–50% of infected cats. Neurologic signs depend on the lesion location in the central nervous system. Ocular abnormalities occur in some cats and include blindness due to exudative retinal detachment, granulomatous chorioretinitis, panophthalmitis, anterior uveitis, and optic neuritis. Optic neuritis is associated with central nervous system inflammation. Adnexal cryptococcosis is not unusual, and lesions have been seen both within the third eyelid and the palpebral conjunctiva. Lesions may cause hyperemia and roughened proliferative areas on the conjunctiva with focal white incrustations and subepithelial deposits. Cytology of conjunctival scrapings is diagnostic. Overall, 32% of cats with cryptococcosis had ocular signs with the great majority of them associated with the posterior segment (Trivedi 2011). Pedigree cats were almost three times more likely to be diagnosed with cryptococcosis than domestic cats in one study (McGill 2009).

Disease in Other Species
Disseminated cryptococcosis has been described in a Moluccan Cockatoo that had bilateral retinal detachments and then developed exophthalmia secondary to disease in the sinuses. Bilateral chorioretinitis has been diagnosed in ferrets with *C. gattii* (Morera 2011, Ropstad 2011). The owners and fellow ferret were asymptomatic carriers (Morera 2011).

Diagnosis
Cryptococcosis is diagnosed on the basis of clinical signs; identification or culture of the organism in ocular or other tissue aspirates, including cerebrospinal fluid (CSF); results of histopathologic examination; and results of serologic testing. The latex cryptococcal agglutination test is considered to be the most reliable serologic test because it can detect the presence of cryptococcal antigen in body fluids including serum, urine, CSF, aqueous humor, and vitreous. The magnitude of antigen titers tends to correlate with severity of disease and response to therapy. In cats this test has a sensitivity of 95–98% and a specificity of 100%. The test has not been evaluated in many dogs. This test may be negative in focal infections. CSF serology may be more sensitive for central nervous system cryptococcosis and is preferred to serum serology in animals with neurologic

signs. In one study, in the few animals that were negative for antigen, the organism was readily identified from aspirates or histopathology (Trivedi 2011). Species differentiation from nine cats revealed seven were *C. gattii* and two were *C. neoformans*. Of eight dogs, six had *C. neoformans* and two had *C. gattii.*

Pathologic lesions associated with cryptococcosis can vary from a gelatinous mass, consisting of numerous organisms with minimal inflammation, to a severe granulomatous reaction. In those rare cases with severe inflammation, organisms are typically scarce. In the eye, lesions are predominantly in the optic nerve, retina, and choroid. As with other mycoses, even when anterior uveitis is present, organisms are rarely demonstrated in the anterior uvea. The disseminated form is found more often in dogs than cats. In dogs, lesions are commonly found in the respiratory tract, the kidneys, lymph nodes, spleen, liver, thyroid gland, adrenal glands, pancreas, bone, gastrointestinal tract, muscle, myocardium, prostate gland, heart valve, and tonsils. In cats, granulomas are typically found in affected tissues. The organisms appear as poorly stained pleomorphic yeasts surrounded by a wide capsular halo with a "soap bubble" appearance on H&E. The organisms are typically numerous and vary in size and shape.

Azole antifungals and amphotericin B are the most commonly used antifungal drugs to treat canine cryptococcosis; however, strains are resistant to antifungal drugs, especially fluconazole. Cautious
use of glucocorticoids in critically affected dogs with central nervous system presentations can improve outcome (Vorathavorn 2013).

Coccidioidomycosis

Coccidioides immitis and *C. posadasii* are found only in the mycelial phase in a specific ecological region, the Lower Sonoran Life Zone. Geographically, this region is within the southwestern United States, Mexico, and Central and South America, including Guatemala, Honduras, Columbia, Venezuela, Paraguay, and Argentina. Sandy, alkaline soil, high environmental temperatures, low annual rainfall, and low elevation characterize this zone. Almost all cases are diagnosed within this region; however, occasionally stray cases have a history of residence or travel within this area. This disease is known as San Joaquin Valley fever, valley fever, or desert fever. During periods of increased temperature and minimal rainfall, coccidioides lies dormant in the mycelial phase, below the soil surface. Periods of heavy rainfall cause mycelial proliferation, extension to the surface, and maturation to arthrospores. Infection occurs most often during dry weather following heavy rainfall, when arthrospores are easily dispersed (Graupmann-Kuzma 2008).

The major route of infection is by inhalation. Arthroconidia are thick walled, barrel-shaped, rectangular, multinucleate, 2–4 µm wide, and 3–10 µm long. Very few (less than 10) arthroconidia must be inhaled to produce disease. The incubation period from inhalation is 1–3 weeks. Recovery from the initial infection in people results in lifelong immunity, but resistance to infection in animals is uncertain. Dissemination involves the reproductive cycle of spherules to endospores to new spherules. If the disease disseminates, the organs that are usually affected are bones, eyes, heart, pericardium, testicles, brain, spinal cord, and visceral organs (primarily the spleen, liver, and kidneys).

A strong cell-mediated immune response is required for resolution of coccidioidomycosis and protection of the host against dissemination of the infection beyond the lungs. Antibodies are made by most hosts but have not been shown to have a significant role in clearance of infection, although antibody production is useful diagnostically as a marker of infection (Graupmann-Kuzma 2008). Antigen testing in serum and urine appears to be an insensitive method of diagnosis (Kirsch 2012).

Disease in Dogs

Clinical findings in dogs with disseminated disease include chronic dry or moist cough, persistent or fluctuating fever, anorexia, weight loss, depression and weakness, lameness, localized peripheral lymphadenomegaly, and draining skin lesions. The most common sign of disseminated disease is lameness secondary to osteomyelitis. Clinical signs of central nervous system involvement include seizures, ataxia, behavioral changes, and coma. Dogs with neck and back pain or paralysis may have vertebral osteomyelitis. Coccidioidal infection of the heart or pericardium in dogs can lead to heart failure, arrhythmia, syncope, and sudden death. Ocular coccidioidomycosis causes signs of conjunctivitis, keratitis, uveitis, secondary glaucoma, retinal detachment, granulomatous panuveitis, posterior hyphema, chorioretinitis, and orbital cellulitis. Most cases are unilateral.

Young adult, large-breed, outdoor, working, and sporting dogs have an increased risk of developing coccidioidomycosis. In endemic areas, dogs that are outdoors during the day or are walked in the desert are more likely to become infected compared to dogs kept indoors (Graupmann-Kuzma 2008).

Disease in Cats

In cats, skin lesions are the most common clinical sign. But fever, inappetence, bone lesions, respiratory disease, and weight loss are also commonly found. Clinical ophthalmologic abnormalities are usually bilateral and include hyperemic, conjunctival masses, fluid-filled periorbital swellings, anterior uveitis, granulomatous chorioretinitis, and retinal detachments.

Cats are diagnosed using a combination of clinical findings, serology, and viewing of *Coccidioides* spherules by either aspiration cytology or biopsy. Active anterior uveitis and periocular swelling resolve with treatment. Chorioretinal granulomas, although persistent, significantly decrease in size (Tofflemire 2010). Neither feline leukemia virus nor feline immunodeficiency virus appears to predispose to feline coccidioidomycosis (Graupmann-Kuzma 2008).

Disease in Other Species

Coccidioidomycosis has been diagnosed in a ring-tailed lemur. Necropsy showed numerous granulomas within the retina with organisms throughout the choroid and within the granulomas. Non-domestic animals rarely show ocular signs with coccidioidomycosis, as was shown in two other studies looking at horses and llamas. A recent report, however, describes a llama with keratouveitis and systemic disease secondary to *C. posadasii;* no treatment was done and the llama was euthanized (Coster 2010). A rhinoceros from Texas died from this disease. In another case, a chimpanzee was undergoing treatment with an experimental triazole for coccidioidomycosis when she was diagnosed with severe conjunctivitis in the right eye. Subsequently, she developed a coccidioidal granuloma of the ventral conjunctiva and anterior uvea (Hoffman 2007). A koala from the San Diego Zoo was diagnosed post-mortem, and ocular lesions were noted seen but not described (Burgdorf-Moisuk 2012).

Diagnosis

Coccidioidomycosis should be considered in any dog or cat that has been potentially exposed during the previous 3 years and is presented with chronic illness, respiratory signs, lameness, lymphadenopathy, non-healing cutaneous lesions, or neurological, ocular, or cardiac abnormalities. Clinicopathologically, hyperglobulinemia and hypoalbuminemia are common. Radiography of the bones and thorax may be helpful to reveal osteomyelitis or a diffuse interstitial pattern in the lungs, respectively.

Cytologically, unstained preparations under reduced light reveal 10–80 µm round, double-walled structures with endospores. Wright stain, Papanicolaou's, and periodic acid-Schiff (PAS) stains are most helpful. Not all spherules will contain recognizable endospores, and smaller spherules may have crumpled, transparent walls. Histopathology specimens, using H & E or PAS are also helpful for diagnosis, and the fungus may be isolated. Serologic testing can also be done. The tube precipitin test measures IgM antibody levels; IgM both appears and disappears early in the course of disease. The complement fixation test measures IgG antibody, which persists longer. The complement fixation titer is indicative of the severity of infection. A titer of >1:32 is indicative of disseminated disease. It is possible to have one test come out positive and the other negative, depending on the stage of infection; thus, the two tests should be performed in parallel. Latex agglutination and ELISA are now employed by some laboratories. AGID is specific but relatively insensitive and should be done following ELISA and/or latex particle agglutination, which are good screening tests because they are very sensitive, but they have false positives.

Ocular disease caused by *Coccidioides immitis* tends to be more suppurative, destructive, and more prone to progress to panophthalmitis than other mycoses. Anterior uveal involvement may be more common with *Coccidioides.*

Itraconazole and fluconazole are the most commonly used and efficacious drugs for treatment.

Histoplasmosis

There are several varieties of *Histoplasma. capsulatum*. The variety *capsulatum* causes American histoplasmosis and is known to infect small wild mammals like rodents, as well as dogs and cats; it causes localized disease in the skin, as well as disseminated disease involving all organs. The variety *duboisii* is distinguished from others by the production of larger (up to 20 µm in diameter) lemon-shaped yeast cells in tissues; it is acquired in Central and West Africa between the latitudes 15°N and 10°S. It has been described in humans and baboons only and is isolated from the soil. The variety *farciminosum* (an Old World horse pathogen) known to cause epizootic lymphangitis was first described in 1873, when yeast cells sized 3 µm were discovered in tissue samples of infected horses.

H. capsulatum is widely distributed in soil. In the United States, major endemic areas are associated with the Ohio, Missouri, and Mississippi River valleys, but histoplasmosis has been identified in most of North and South America. The disease was recently described in a cat from Italy (Mavropoulou 2011). Similarly to *Blastomyces*, a mycelial stage is present in the soil, and once in the pulmonary system, the fungi convert to a budding yeast phase. The organisms in the yeast phase are small at 2–4 µm.

At ambient temperatures, *H. capsulatum* grows as a mold, preferably on bat and bird guano. The fungus produces macro- and infectious microconidia. At temperatures above 35 °C, the fungus changes into its parasitic stage, forming oval yeast sized 2–5 µm. Histoplasmosis is probably acquired by inhalation of microconidia that are small enough to reach the lower respiratory tract. Incubation after exposure is approximately 12–16 days. The microconidia convert to the yeast phase in the lung and reproduce by budding. The yeast organisms are phagocytized by cells of the host's mononuclear phagocyte system

and undergo further intracellular replication. Lymphatic and hematogenous dissemination may then occur.

Disease in Dogs

In the dog, clinical signs of disseminated histoplasmosis are most often referable to the gastrointestinal tract or liver. Pyrexia, malaise, and coughing also occur. Reports of ocular signs are relatively rare but usually include pyogranulomatous chorioretinitis, anterior uveitis, conjunctivitis, blepharitis, and optic neuritis. Experimentally, ocular lesions are seen in 66% of cases.

Disease in Cats

Cats are similarly susceptible hosts as dogs to *H. capsulatum*. Disease usually develops in young cats (<4 years of age). Most cats develop disseminated disease and have clinical signs of depression, weakness, weight loss, fever, anorexia, bone lesions, and pale mucous membranes. Less frequent clinical signs include vomiting, diarrhea, blindness, and lameness. Prevalence of clinical evidence of pulmonary disease varies between studies. Visceral lymphadenomegaly, splenomegaly, and hepatomegaly are also seen. Ocular signs include conjunctivitis, granulomatous blepharitis, granulomatous chorioretinitis, retinal detachment, and optic neuritis. Anemia and hypoalbuminemia are common laboratory abnormalities.

Itraconazole is commonly used for treatment. Median duration of the antifungal treatment was 5 months for cats that survived to discharge in one study, and overall survival at time of discharge for cats was 55% (Aulakh 2012). Cats treated with fluconazole have a similar mortality and recrudescence rates to those treated with itraconazole (Reinhart 2012).

In a retrospective study, results of a urine antigen assay were compared with standard diagnostic methods in cats with clinical signs suggestive of histoplasmosis. Antigenuria was detected in 17/18 cats with a histopathologic or cytopathologic diagnosis of histoplasmosis (Cook 2012).

Disease in Horses

H. capsulatum was diagnosed in a horse with ulcerative keratitis that responded to topical fluconazole (Richter 2003). *H. farciminosum* causes epizootic lymphangitis in Africa, the Middle East, India, and the Far East. Ocular signs begin as serous then purulent ocular discharge with blepharitis and dacryocystitis. Nodules on the conjunctiva ulcerate. Severe diffuse swelling of the eyelids with palpebral granulomas occurs, and the horses may not be able to open their eyes. Pneumonia, abortion, and disseminated disease also occur. This is a reportable disease.

Disease in Other Species

Histosplasmosis has also been diagnosed in snow leopards in Mexico, a Bengal tiger in Wisconsin, and a wild European hedgehog in northern Germany (Jacobsen 2011). Systemic disease with a granulomatous plaque on the eyelid and oral candidiasis in an eclectus parrot has also been seen (Quist 2011).

Histology (using GMS) and culture are used for diagnosis. Serology using AGID is very specific but only about 80% sensitive. Because of the cross reactivity in the urine antigen test for *Blastomyces*, this test is also recommended for use in diagnosing histoplasmosis. The cross reactivity is not seen as a problem because the preferred treatment for both mycoses is itraconazole (Spector 2008).

However, a study was done to evaluate the sensitivity and specificity of a 3rd generation antigen enzyme immunoassay (EIA) on urine samples for the diagnosis of histoplasmosis in dogs. Cases for which urine samples were submitted for Histoplasma antigen testing were reviewed and compared to the gold standard of finding Histoplasma organisms or an alternative diagnosis on cytology or histopathology. Seventeen cases were considered true positives based on identification of the organism, and 41 cases were considered true negatives with an alternative definitive diagnosis. Two cases were considered false negatives, and there were no false positives. Sensitivity was 89.47% and the negative predictive value was 95.35%. Specificity and the positive predictive value were both 100%. The Histoplasma antigen EIA test demonstrated high specificity and sensitivity for the diagnosis of histoplasmosis in dogs (Cunningham 2015).

Splenic changes seen on ultrasound are consistent, as shown in a retrospective review of cats with histosplasmosis. Splenomegaly was documented in all cases (15/15), and a hypoechoic appearance of the spleen was documented in 14/15 of cases. The spleen was diffusely and uniformly affected in 14/15 (six homogenous and eight with a subtle mottled appearance) and had discrete nodules in 1/15 cats (Atiee 2013).

The body's reaction to *Histoplasma capsulatum* is quite varied from other systemic mycoses. Typically, we see a diffuse granulomatous and lymphocytic choroiditis with little suppuration and without much of the destruction that characterizes blastomycosis and coccidioidomycosis. Organisms are usually very numerous and visible as small spherical bodies within the cytoplasm of macrophages.

Aspergillus spp.

Aspergillus spp. are ubiquitous in the environment. *Aspergillus fumigatus* is the most common species associated with disease in the dog. *Aspergillus* spp. are 3–5 µm in diameter. They have characteristic repeated, dichotomous branching with uniform directional orientation, hyphae branching at an approximate 45° angle from the main hyphae, and presence of septae. Aspergillosis has been reported in dogs, cats, sheep, cattle, alpaca, horses, rabbits, and humans. In most species, except for the dog, *Aspergillus* is usually confined to the pulmonary system. Inhalation of microconidia appears to be the primary infectious route, accounting for the high incidence of respiratory tract infections. A high percentage of human ophthalmic infections are related to immunosuppression and spread from adjacent sinus infections.

Aspergillus felis, a novel heterothallic species in *Aspergillus* section Fumigati has been isolated from three host species with invasive aspergillosis, including a human patient with chronic invasive pulmonary aspergillosis, domestic cats with invasive fungal rhinosinusitis, and a dog with disseminated invasive aspergillosis. All of the cats presented with unilateral exophthalmia and current or historical nasal discharge. Disease in all host species was often refractory to aggressive antifungal therapeutic regimens. Phenotypic analyses show that *A. felis* can be distinguished from the related species *A. viridinutans* by its ability to grow at 45°C and from *A. fumigatus* by its inability to grow at 50°C.

Disease in Dogs

Clinical signs may include pelvic limb weakness, ataxia, paralysis, pyrexia, diskospondylitis, decreased appetite, lameness, PU/PD, and gastrointestinal signs. Ocular signs include swollen irides, cells within the vitreous, chorioretinitis, and retinal detachments. The most frequently reported sign of infection in the dog is nasal cavity disease. Orbital aspergillosis secondary to invasion from the sinus has been diagnosed in a dog. Disseminated aspergillosis seems to occur most commonly in the German shepherd dog.

Disease in Cats

Cats with sino-orbital aspergillosis typically have a history of sneezing and nasal discharge, although it may have resolved by presentation. Many also have exophthalmia or presence of a mass or ulcer in the pterygopalatine fossa. Retrobulbar and nasopharyngeal masses are characterized by necrosis and well-vascularized granulomatous inflammation. Granulomas contain areas of necrosis with abundant PAS-positive fungal hyphae.

Fourteen of 16 isolates of *A. fumigatus* or *Neosartorya* spp. (formerly classified as Aspergillus) were resistant to fluconazole. Only isolates of *Neosartorya* spp. showed resistance to itraconazole, voriconazole, and/or posaconazole. All three isolates of *A. fumigatus* tested were susceptible to voriconazole and posaconazole. No isolates were resistant to amphotericin B. Two cats that had surgical exenteration of the right orbit subsequently became blind in the left eye. In one case, there was mycotic involvement of the optic chiasm. In the other case, a retrobulbar mass effaced the left optic nerve. Cats with sino-orbital aspergillosis tend to have a very poor prognosis even with medical and surgical therapy (Barrs 2012).

Currently the treatment of choice is a combination of systemic antifungal treatment (itraconazole, fluconazole, amphotericin B, posaconazole, voriconazole or terbinafine) in combination with local therapy, using clotrimazole or enilconazole intranasal infusions under general anesthesia. Voriconazole has been associated with neurological effects in cats and is very expensive (ataxia, paraplegia and CN defects) so safety studies are indicated (Hartmann 2013). Itraconazole and voriconazole cross-resistance was common in vitro (Barrs 2013). Reports are somewhat conflicting about the efficacy of itraconazole. Posaconazole has been evaluated and appears to be promising with prolonged treatment but relapses are common (Corrigan 2015).

Aspergillus udagawae and *A. viridinutans* have also both been associated with orbital aspergillosis (Kano 2013).

Disease in Other Species

An alpaca was described that was blind with retinal detachments, retinal hemorrhages, and optic neuritis. Necropsy revealed pulmonary and renal lesions. Organisms were found in the affected tissues.

Aspergillous is known to cause air sac disease in birds, but it has also been associated with nervous and ophthalmic complications one week after an acute episode of aspergillosis in a turkey flock. Cloudiness of the eye with severe conjunctivitis was associated with paralysis in broiler breeders. Aspergillus blepharitis, keratitis and keratoconjunctivitis (turbid discharge, cloudy cornea, and cheesy yellow exudates within the conjunctival sac) have been reported in numerous species (Seyedmousavi 2015).

Diagnosis

Gross findings on necropsy in dogs may reveal white nodular lesions in the myocardium, skeletal muscles, diaphragm, kidneys, liver, and spleen. Ocular findings may include lymphocytes, macrophages, fibrin, and aggregates of septate hyphae adjacent to the lens capsule. In addition, the hyphae may penetrate the lens capsule. Organisms can be seen in the vitreous,

retina, and choroid. The organisms are PAS positive, typically linear with 5–15 segments, and have occasional focal bulges, club formation, and branching. Since the posterior segment is often the most severely affected part of the eye, vitreocentesis has the highest probability of obtaining material for cytology and culture.

Because cellular immunity is important to the host response against *Aspergillus*, humoral antibodies may not be detectable. Systemic aspergillosis is often associated with debilitation or immunologic suppression from improper housing, stress, primary disease, or prolonged antibiotic or corticosteroid usage. Diagnosis of *Aspergillus* is made on the basis of identification and culture of urine sediment, serum, synovial fluid, vitreous, lymph node, CSF, or intervertebral disc centesis specimens. Vitreocentesis may reveal septate hyphae and small round, spore-like structures. One report described a blood and urine galactomannan (GM) antigen assay that was found to have a sensitivity and specificity for serum of 92 and 86%, respectively, and for urine of 88 and 92%, respectively. False negatives were seen only in dogs with localized pulmonary aspergillosis. Use of a cutoff GM index of 1.5 increased specificity to 93% for both serum and urine without loss of sensitivity for diagnosis of disseminated infection. High-level false positives (> 1.5) occurred in dogs with other systemic mycoses and those treated with Plasmalyte (Garcia 2012).

Miscellaneous Fungi
Ocular *Pseudallescheria boydii* was reported in a German shepherd and has been reported in equine keratomycosis.

Rhizopus is an opportunistic fungus. There was one report of a calf with disseminated *Rhizopus* infection. It had bilateral ocular lesions, including endophthalmitis and intumescent immature cataracts. Histologic ocular abnormalities included vasculitis, suppurative keratitis, anterior uveitis and chorioretinitis, and intumescent immature cataracts. Hyphae were noted in all ocular tissues (Vasconcelos 1995).

Candida albicans is a dimorphic fungus that may cause either localized or generalized disease. Local proliferation in wounds or mucosal surfaces is the first step in the spread of infection, and it generally occurs in immunosuppressed or debilitated animals. Corneal and conjunctival infections and panuveitis have been reported in cats and horses and seen in other species. Histopathology reveals pyogranulomatous inflammation with organisms.

Methods for Fungi Diagnosis
Direct examination can be made by scraping surface lesions of the eyelids, conjunctiva, and cornea and direct staining the smears from aspirated specimens. The presence of fungi can be diagnosed, but identification of filamentous fungi needs to be done by culture or PCR. Histopathology of deep corneal biopsies or enucleated specimens also allows demonstration of fungal organisms. Histologic sections stained with Giemsa stain, PAS, or methenamine silver are sensitive.

Sabouraud's dextrose agar is the medium most commonly used for culturing ocular fungi. Typically, fungi grow within 4 days, but as many as 30 days should be allowed. Definitive identification is often made on the basis of morphologic structure of the organism, including the direct microscopic appearance of the fungus, clinical specimen, morphology of the colony and type of pigmentation, microscopic appearance of fruiting heads and spores for mold colonies, and the morphology of the yeast and type of budding.

ALGAL DISEASE
Protothecosis
Prototheca are achlorophyllous algae. *Prototheca zopfii* and *Prototheca wickerhamii* are known pathogens. Virulence varies between the species, and *zopfii* is most commonly isolated from dogs. Cells are spherical to oval in shape and range from 1–13 µm in diameter. The hyaline cell wall is approximately 0.5 µm thick. The cytoplasm is granular and basophilic, and the nucleus is small and centrally located. Reproduction is through endosporulation with cleavage of the parent cell, resulting in the release of 2–20 or more endospores. Empty hyaline shells may be visible among intact cells within lesions.

Prototheca is an environmental contaminant found in water systems and soil and is the only plant known to cause infections in humans and animals. However, it is only minimally pathogenic and does not spread between hosts. The disease has been reported worldwide. In North America, the disease is primarily in the southeastern United States.

Immunosuppression, especially if cell-mediated immunity, favors establishment of infection. Another mechanism for infection is the inability of the host's PMNs to specifically destroy *Prototheca* after phagocytosis. It has been shown that various Prototheca species form biofilms composed of surface-attached cells in all growth phases linked together by matrix containing DNA and polysaccharides. These biofilms caused decreased release of IL-6 by mononuclear immune cells and responded differently to treatment with antimicrobials. Prototheca biofilms may contribute to the chronic and hard-to-treat character of these algal infections. (Kwiecinski 2015) In humans, infection is usually cutaneous, subcutaneous, or bursal. In many dogs, evidence of chronic colitis with multisystemic dissemination is reported.

Disease in Dogs

The primary clinical sign is usually hemorrhagic diarrhea or colitis; however, dogs may present with blindness as the initial symptom. The colon is the probable site of primary infection and entry for disseminated disease. There is an apparent predisposition for the Collie breed and females. A number of organ systems, including the lymphatic, renal, and pulmonary, are commonly involved. Intermittent bloody diarrhea, weight loss, and debility are clinical findings. In addition, renal failure has been diagnosed, as well as chronic ulcerative skin lesions. Central nervous system and ocular involvement are also common and are exhibited with severe depression, deafness, ataxia, circling, paresis, and blindness. Ocular signs include anterior uveitis, secondary glaucoma, chorioretinitis, and retinal detachment.

Disease in Cats

Prototheca in the cat appears to be limited to cutaneous disease.

Diagnosis

Identifying organisms on Wright's stain can be done on specimens obtained by vitreocentesis, cerebral spinal fluid tap, or colonic scrapings. Additionally, Gormori's methenamine silver or PAS will also show organisms on histopathology. *Prototheca* sp. are extracellular, round to oval organisms with thin, unstained walls. Larger cells may contain endospores. *Prototheca* is also grown readily in laboratory media. Therapy with itraconazole has been attempted but has been unsuccessful long term.

Histologic lesions are identical to those seen with blastomycosis, with the exception of the different organisms. The algae are free or within macrophages. They are spherical to oval, from 2–20 μm, and have a refractile cell wall that stains with PAS or GMS. Multiple daughter cells form and are enclosed within a single cell wall (Mercedes sign). There is no budding.

PARASITES
Protozoal Diseases
Toxoplasmosis

Toxoplasmosis affects most warm-blooded animals. Infection is caused by the obligate intracellular protozoal parasite *Toxoplasma gondii*.

The cat is the only known definitive host for the parasite. The life cycle is divided into intestinal and tissue phases. The intestinal or coccidian phase is limited entirely to the cat. The asexual phase includes formation of tachyzoites and bradyzoites. Tachyzoites are crescent-shaped, 3 x 7 μm, and represent the pathogenic propagative form of the parasite, which is present during active infectious disease. Bradyzoites are comma-shaped, 2 x 7 μm, and found in chronic disease.

Cats become infected by ingesting bradyzoite cysts from feces and infected intermediate hosts, such as rodents or birds. After being ingested, bradyzoites rapidly transform to tachyzoites, penetrate into the cat's intestinal mucosa, and undergo an intra-epithelial cycle of sexual proliferation that results in development and shedding of oocysts. Oocysts detach from the intestinal epithelium and are shed in the feces. After primary infection, cats can shed millions of fecal oocysts, each of which is 11 - 14 μm by 9–11 μm in size and contain two sporocysts. In the external environment, the cysts undergo sporulation within 1–3 days and then become infectious.

Tissue damage results from both intracellular growth of the organism and the host immunologic responses. The type and severity of disease depends on the number of organisms, strain of *T. gondii*, organs infected, and immune status and response of the host. An intact cell-mediated immune system is extremely important for resisting *T. gondii* infection.

In cases of ocular inflammation associated with *Toxoplasma* sp., cellular immune memory to uveal antigens may perpetuate the inflammatory disease as damage to uveal or retinal cells stimulate lymphocytes and plasma cells, which then may initiate production of autoantibodies and further contribute to uveitis.

T-cell-mediated immunity appears to play the major role in resistance against *T. gondii*, but infection with *T. gondii* also elicits humoral immunity. IgM, IgG, IgA, and IgE are produced against both the membrane and intracellular proteins. Specific antibody in the presence of complement lyses extracellular tachyzoites.

In humans, most cases of ocular toxoplasmosis are thought to be reactivations of congenitally acquired infection. The appearance of the retinitis is considered pathognomonic – an active white elevated area of necrotizing retinochoroiditis near a pigmented scar. Lesions are commonly located centrally near the macula. Anterior uveitis occurs infrequently, and when it does, it is considered to be a hypersensitivity reaction without organisms present.

<u>Disease in Cats</u>

Clinical signs depend on the number of tachyzoites released, the ability of the immune system to limit tachyzoite spread, and the organs damaged by the tachyzoites. Because adult immunocompetent animals control tachyzoite spread efficiently, toxoplasmosis is usually a subclinical illness. However, in kittens, tachyzoites may spread systemically and cause interstitial pneumonia, myocarditis, hepatic necrosis, meningoencephalomyelitis, chorioretinitis, lymphadenopathy, and myositis. Corresponding clinical signs include fever, diarrhea, cough, dyspnea, icterus, seizures, and death. Finally, immunocompromised adult cats are susceptible to developing acute generalized toxoplasmosis.

The appearance of ocular lesions in cats with systemic illness or those experimentally infected include multifocal chorioretinitis, manifested as dark gray hyporeflective areas within the tapetal fundus, and multifocal grey to white infiltrates in the nontapetal fundus. Anterior uveitis may also occur, and the tachyzoites have been identified in the iris and ciliary body in cats with widespread systemic involvement. Additionally, optic neuritis and blindness may result. Experimental infections in the cat have resulted primarily in chorioretinal lesions, but retrospective studies of spontaneous ocular toxoplasmosis indicate that multifocal granulomatous panuveitis is the prevalent ocular manifestation.

The role of toxoplasmosis in causing anterior uveitis in cats without systemic illness is controversial. Chorioretinitis is the most common ocular sign in humans; yet in cats, the most common sign appears to be non-granulomatous anterior uveitis. Proponents of this theory that toxoplasmosis causes anterior uveitis in cats cite increased serum antibody levels to *T. gondii* compared to cats without uveitis, aqueous humor antibody levels that suggest local production, and improvement in some cats on anti-toxoplasmic therapy alone. Those disbelieving of this theory cite the facts that histologic evidence of the organism in the eye is lacking, and that many clinicians report that treatment with anti-toxoplasmic drugs in cats with anterior uveitis does not appear to improve or resolve signs.

<u>Disease in Dogs</u>

Dogs may be infected by ingesting sporulated oocysts, cat excreta, tissue cysts in infected meats, or a transport host, or by congenital transmission. Infection is usually sub-clinical. Clinical signs can include neuromuscular, respiratory, or gastrointestinal disease. Ocular toxoplasmosis has been reported infrequently in dogs. Anterior uveitis, chorioretinitis, and vitritis can be seen. Hyperplasia of the ciliary epithelium and a pseudocyst of ciliary epithelium without inflammation have also been reported. Additional findings may include optic neuritis, scleritis, extraocular myositis, and keratoconjunctivitis. Even though signs of anterior uveitis may not be seen clinically, histologically inflammation of the iris may be present. Clinical systemic disease in dogs with toxoplasmosis is relatively rare, and it is usually associated with other disease such as canine distemper virus.

Diagnosis

The laboratory diagnosis of *T. gondii* infection includes parasite isolation, histologic identification, and serologic evaluation. Results of serologic evaluation must be scrutinized, and animals suspected to have *T. gondii* must be tested for IgM and IgG. One confounding factor is that the serologic test for IgM is not very specific and, therefore, an animal with any kind of strong immune response can have an elevated IgM that is non-specific for *T. gondii*. Therefore, a convalescent titer 4–6 weeks later is important to the diagnosis of disease. Histologically, granulomatous and non-granulomatous inflammation is possible. PCR and serology have been compared using aqueous humor and serum in cats with uveitis, and several more cats were positive based on PCR than on serology (Powell 2010).

Comparison of levels of aqueous humor *T. gondii* antibodies to serum antibodies (Goldmann-Witmer coefficient or C value) gives information regarding ocular antibody production, but it still does not prove ocular toxoplasmosis even if the C value is high. *T. gondii*-specific antibodies may be produced in the eye in response to other factors. Typically a C value >8 is accepted as evidence of local antibody production.

C value for any antibody class is calculated as follows:
IgM C value = (*T. gondii* IgM – aq/serum)/(total IgM – serum/aq)

Clindamycin is the drug of choice for treating toxoplasmosis.

Neosporosis
Neospora caninum is a coccidian protozoal parasite similar to *T. gondii*. The disease is found worldwide. Dogs are the definitive and intermediate host of this protozoan. The sexual cycle of *N. caninum* is completed in the gastrointestinal tract of dogs following ingestion of tachyzoites, and oocysts are shed in the feces. Oocysts that sporulate in the environment are then infective. After being ingested by an intermediate host, the oocysts release sporozoites in the gut. Sporozoites change to tachyzoites, which then spread throughout the body, leading to tissue cyst or bradyzoite formation. Transmission of neosporosis by the transplacental route also occurs and, therefore, most reports involve neonatal infection.

N. caninum has been determined to infect dogs, cat, cattle, sheep, horses, goats, moose, wolves, bison, caribou, musk ox, llamas, alpacas, and deer. It is a major cause of abortion in cattle. Wild mammals may be a reservoir for infection in cattle. The mode of transmission most studied is transplacental. Tachyzoites and tissue cyst bradyzoites occur as in toxoplasmosis.

Disease in Dogs
Clinical disease is most often reflective of neuromuscular disease in dogs; however, other clinical signs of polymyositis, myocarditis, hepatitis, and dermatitis may manifest following infection. Ocular lesions are seen most often in those patients exhibiting central nervous system manifestations and include blindness, chorioretinitis, anterior uveitis, optic neuritis, Horner's syndrome, and extraocular myositis.

Disease in Other Species
Ocular infection of *Neospora* in calves can cause exophthalmia.

Diagnosis
Diagnosis is made by demonstration of the tachyzoites, FA, immunohistochemical stains, and PCR. Treatment includes trimethoprim sulfa and pyramethamine and clindamycin.

Encephalitozoon cuniculi
Encephalitozoon cuniculi is an obligate intracellular *Microsporidium*. The organism has a wide host distribution, but primarily affects rabbits. *E. cuniculi* is also considered as an opportunistic pathogen in immunocompromised humans. In rabbits, the eyes, central nervous system, and kidneys are predilection sites. *E. cuniculi* is a eukaryotic organism that sporulates, and the spores that survive in the environment are thought to be the transmissible agents. The infective spores are shed in the urine of affected rabbits and provide the most likely route of exposure for other animals. Experimentally documented infection has occurred in rabbits via intravenous, intracerebral, oral, nasal, and rectal routes. Documented evidence

of vertical transmission of *E. cuniculi* in rabbits also exists. Currently the most viable theory for the phacoclastic uveitis form of the disease is vertical transmission due to the disease occurring in young animals and the appearance that the uveitis develops after lens rupture. It is thought that the transmission may occur during the first 3rd of gestation after the lens placode is formed, but before the lens capsule develops. Dwarf rabbits, such as the Jersey wooly breed and New Zealand white rabbit, may be more susceptible to clinical disease.

Disease in Rabbits

Most rabbits with phacoclastic uveitis have cataracts, whitish/yellowish intraocular masses (granulomas) on the iris, and anterior uveitis. The rabbits typically have blepharospasm, marked conjunctival and episcleral hyperemia, aqueous flare, miosis, and iridal swelling and hyperemia. Cataracts may or may not be visible. Ocular lesions are unilateral. Other common lesions in rabbits include granulomatous encephalitis and nephritis. Less commonly hepatitis, myocarditis, and enteritis are seen. The most typical disease pattern, however, is a subclinical, chronic, persistent infection.

The majority of the rabbits with neurological signs show signs of vestibular disease such as ataxia, circling, rotation, and nystagmus. Other signs observed are paresis or paralysis, seizures, nodding or swaying when resting, behavioral changes, and facial nerve paralysis. Rabbits with signs of kidney failure show non-specific symptoms such as lethargy, anorexia, or weight loss. Azotemia occurs secondary to the renal disease.

Definitive diagnosis of encephalitozoonosis in the living animal can be difficult. Histological examination and demonstration of the organism is usually required to confirm infection. Tentative diagnosis of encephalitozoonosis in rabbits is routinely made on clinical signs in combination with serological testing. Serological testing is the commonly used method of diagnosing *E. cuniculi* infections. Nevertheless, the presence of antibodies indicates only chronic infection and does not confirm the organism as a causative agent of disease. The titers are expected to be higher than 1:40. Examination of body fluids (urine, CSF) by using trichrome staining or PCR may yield an ante-mortem diagnosis of encephalitozoonosis, but these tests are not yet available as routine diagnostic techniques.

Rabbits with signs of phacoclastic uveitis must undergo surgical removal of the lens via phacoemulsification. Topical steroids have little to no effect. All seropositive rabbits with supposed encephalitozoonosis are usually treated with fenbendazole orally once daily (20 mg/kg) for 4 weeks. Additionally, rabbits showing neurological signs may receive enrofloxacin or oxytetracycline. Initially, antibiotic therapy may be complemented with corticosteroid treatment.

Typically, rabbits with an evidence of a phacoclastic uveitis do not show other symptoms and survive. About half the rabbits that exhibit neurological signs recover, but the majority of rabbits with kidney failure die.

Histologically, an intraocular, locally extensive pyogranulomatous infiltration that encases a wide anterior lens capsule break is commonly detected. The major feature in affected eyes is rupture of the anterior lens capsule and the presence of neutrophils within the lens cortex. There is a zonal pattern to the inflammation, with neutrophils in the lens cortex, surrounded by a layer of foamy macrophages with an outer ring of fibrous tissue containing lymphocytes and plasma cells. Inflammation is centered entirely on the break in the lens capsule. The anterior uveal tract itself is relatively spared. Organisms are found only within liquefied lens cortex and are usually in close approximation to the anterior lens epithelium. The microsporidia are apparent with silver stain and are Gram- positive and acid-fast. Immunohistochemically, spores reacting with anti-*E. cuniculi* occasionally within macrophages and lens epithelial cells, have been seen. (Wolfer 1996)

Other lesions are most frequently recognized in the brain and kidney, and consist of focal nonsuppurative granulomatous meningoencephalitis and focal to segmental interstitial nephritis with variable degrees of fibrosis when systemic disease is present (Künzel 2008).

Disease in Cats

E. cuniculi has been described in 11 cats with focal anterior cortical or mature cataract and secondary uveitis. Median age was 3.5 years. Most were affected bilaterally. Favorable outcomes followed phacoemulsification and treatment with fenbendazole. All cats had a positive antibody titer for *E. cuniculi,* and DNA was detected by PCR in 18/19 lenses and in 10/19 aqueous samples. Spores were detected via histology (acid fast trichrome) in lens material subjacent to the capsule and in lens epithelium (Benz 2011).

Disease in Other Species

Cataract formation secondary to *E. cuniculi* has also been reported in the Blue fox; it is thought to be infected in utero. Cataracts have also been seen in infected mink. Humans can develop keratoconjunctivitis, keratitis, uveitis, and endophthalmitis.

Parasitic flagellates
Trypanosomiasis

Trypanosomiasis is caused by the hemoflagellate protozoan, *Trypanosoma* sp. Most diseases are transmitted by the tsetse fly. Infected flies inoculate trypanosomes into the skin of animals. After the trypanosomes grow for a few days and cause localized swellings, they enter the lymph nodes, then the bloodstream, where they divide rapidly by binary fission. Depending on the species, the endothelial cells or other tissues are invaded and damaged.

Immune complexes are made that cause inflammation, which contributes to the signs and lesions of the disease. When appropriate antibodies are made against the surface-coat glycoproteins, the trypanosomes die. However, trypanosomes have multiple genes that code for different surface-coat glycoproteins that are not vulnerable to the immune response, which results in persistence of the organism. The number of antigenic types of glycoprotein that can be made is unknown, but exceeds several hundred. This antigenic variation has prevented development of a vaccine and permits reinfections when animals are exposed to a new antigenic type.

Disease in Dogs and Cats

Trypanosomiasis brucei in the dog and cat can cause corneal opacification, blepharitis, conjunctivitis, and keratitis. Ciliary body cysts may form that contain organisms. Parasitemia in peripheral blood smears and aqueous fluid confirms the infection. The disease occurs in Africa.

Trypanosoma evansi causes keratitis, conjunctivitis, blepharitis, anterior uveitis, and endophthalmitis in dogs. It is often considered separately from other trypanosomiasis because it is transmitted by biting flies. It occurs in North Africa, the Middle East, Asia, the Far East, and Central and South America. Experimentally in cats, corneal edema and vascularization have been seen. Anterior chambers may contain fibrin and an inflammatory infiltrate of neutrophils, lymphocytes, and plasma cells. Similar infiltrations are present in the iris, ciliary body, limbus, and conjunctiva. *T. evansi* trypomastigotes can be observed in smears made from the aqueous humor (Da Silva 2010).

Disease in Livestock

Livestock trypanosomiasis mainly affects animals in sub-Saharan Africa, Asia, and South America. Relapsing hypopyon, bilateral epiphora, photophobia, mucous exudation, and mild keratitis may occur. Eyelid edema develops late in the course of disease and is part of the extensive subcutaneous edema. Histologic examination reveals trypanosomes in the fibrin of the anterior chamber and anterior uvea, along with a mononuclear inflammatory infiltrate. The retina and choroid have perivascular mononuclear cuffing, and optic neuritis is common. Extraocular muscles often have intense mononuclear inflammation with organisms. In goats experimentally infected with *T. evansi* (Morales et al., 2006), ocular lesions include corneal ulceration and chorioretinitis. Aqueocentesis may demonstrate trypanosomes.

Salmonid cryptobiosis

Salmonid cryptobiosis is caused by the hemoflagellate *Cryptobia salmositica,* which is transmitted by leeches (*Piscicola salmositica*). Clinical signs include anorexia, exophthalmia, splenomegaly, hepatomegaly, general edema, and abdominal distention with ascites. Clinical pathology reveals a microcytic and hypochromic anemia, and red cells give a positive antiglobulin reaction. This is seen on the west coast of North America.

Leishmaniasis

Visceral leishmaniasis is most commonly caused by the flagellate organism *Leishmania infantum*. The disease is endemic along the Mediterranean shore and in parts of east Africa, India, and Central and South America. Reports suggest that there are now endemic areas in the United States. This is not surprising since domestic and wild members of Canidae serve as reservoir hosts, and the intermediate host, a sandfly (*Phlebotomus* spp.), is also found in the United States.

Disease in Dogs

Ocular findings include blepharitis with simple or granulomatous conjunctivitis; a grayish-white, fibrous appearance to the nictitating membrane; keratoconjunctivitis, uveitis, rubeosis iridis, scleritis, superficial or deep keratitis, anterior uveitis, KCS, secondary glaucoma, retinitis, and endophthalmitis. Ocular disease may be the only clinical abnormality. The anterior segment is usually more severely involved than the posterior segment, and signs are typically bilateral. Ocular manifestations occur in up to 81% of affected dogs. *L. infantum* infection is highly prevalent in endemic areas.

Systemic signs include lymphadenopathy, splenomegaly, hepatomegaly, renal failure, anemia, thrombocytopenia, muscular weakness, cachexia, abnormal locomotion, and varying non-pruritic dermatologic conditions.

Histopathologically, there is vasculitis with intense inflammatory zones with lymphocyte infiltrates, plasma cells, and macrophages. Amastigotes are seen in the ciliary processes, ciliary body, limbus, lacrimal duct, and histiocytes. The diagnosis is confirmed on the basis of finding the organism, i.e. amastigotes that are round to oval and 1.5 to 5 µm in size in tissue

aspirates. Additionally, the organism can be found on histopathologic or immunoperoxidase evaluation of cutaneous or organ biopsy specimens. PCR, ELISA, and IFA can also be done.

The use of PCR from conjunctival swabs and conjunctival biopsies may be helpful in the diagnosis of leishmaniasis. Experimentally infected dogs are positive by conjunctival PCR at 45 days of infection before seroconversion. Dogs with higher parasite loads in the meibomian glands and lacrimal glands were more likely to have ocular signs of KCS (Larajo 2011).

kDNA PCR/hybridization and quantitative real-time PCR (qPCR) targeting the DNA polymerase gene of *L. infantum* has been used for diagnosis and assessment of parasite load in clinical samples. The highest parasite burdens were detected in skin regardless of the presence of clinical signs. The qPCR results emphasized the role of dogs, particularly asymptomatic dogs, as reservoirs because of the high cutaneous parasite loads. These results may help to explain the maintenance of high transmission rates and numbers of cases in endemic urban regions (de Almeida Ferreira 2012).

Treatment of canine leishmaniasis with allopurinol leads to a significant improvement in clinical signs but does not eliminate the *Leishmania* organisms.

<div align="center">Disease in Cats</div>

Ocular lesions include conjunctivitis, blepharitis, and/or keratitis. Histologically, infiltrates are composed of sheets of large foamy macrophages, with some multinucleated giant cells. A high number of amastigotes are found within the macrophages and giant cells. The depth and extent of the infiltration varies tremendously (Navarro 2010).

<div align="center">**Parasitic Infections**</div>
<div align="center">**Habronemiasis**</div>

Habronema is an infection of the conjunctiva and eyelid of horses of primarily historical significance; however, it may be remerging due to developing resistance to ivermectin. Habronema is typically a parasite of the equine stomach. The species of importance are *Draschia megastoma*, *Habronema muscae*, and *Habronema microstoma*. The adult organisms range from 13–25 mm long. Normally, the full life cycle occurs in the stomach. When flies ingest the larvae, the larvae migrate to the head of the fly. When the fly alights on a warm, moist surface such as a muzzle, the larvae change hosts. Typically, those larvae that are swallowed complete their life cycle and do not cause problems to the host. The larvae that transfer to the conjunctiva or the area of the medial canthus where moisture is constant reach the end of their life cycles. These larvae can cause persistent cutaneous granulomas referred to as cutaneous habronemiasis.

The typical cutaneous habronemiasis lesions are characterized by an initial rapid production of granulation tissue that does not resolve during fly season. Raised, ulcerative granulomas, often containing characteristic yellow, plaque-like "sulfur granules" 1–2 mm in diameter tend to form near the medial canthus, and the larvae can be seen. Pruritus is intense, and secondary injury may result in a horse's effort to find relief.

Habronema conjunctivitis is usually in the form of an ulcerated nodule containing caseo-calcareous foci situated near the medial canthus. These nodules tend to abrade the cornea unless they are removed surgically. Ivermectin is the treatment of choice for habronemiasis; however, fenbendazole is also effective.

Histologically, there are multiple coalescing eosinophilic granulomas surrounding live or dead larvae. Mast cells are not commonly seen, but when they are these lesions need to be differentiated from cutaneous mast cell tumors.

Habronemiasis was diagnosed in an adult dromedary camel (*Camelus dromedarius*) that presented with a non-healing, severely pruritic, ulcerative fibrotic plaque located at the medial canthus. Histological examination of surgical biopsies identified degenerating nematode larvae within eosinophilic granulomas. Treatment involved repeated debridement of the lesion, injectable ivermectin and anti-inflammatory therapies, and injectable and topical antibiotics (Myers 2010).

<div align="center">**Ocular Nematodiasis**</div>

Intraocular nematodiasis is reported infrequently in domestic animals. Ocular nematodiasis includes two distinct conditions, namely ocular filariasis and ocular larval migrans.

<div align="center">**Ocular filariasis**</div>

Ocular filariasis due to aberrant migration of immature *Dirofilaria immitis* occurs in dogs and humans. The condition occurs in dogs with and without concurrent microfilaremia. Ocular involvement with *D. immitis* is thought to arise as a result of aberrant migration of fourth-stage larvae from the subconjunctival space into the eye, with subsequent development into immature adults or fifth-stage larvae. Approximately half of the infected dogs do not have microfilaremia or are occult. The German Shepherd dog may be predisposed.

Ocular involvement is unilateral, and the worm is most commonly found in the anterior chamber. Anterior uveitis is a consistent ocular manifestation, and ocular discomfort is often exasperated by examination of the affected eye with light, as this stimulates parasitic movement. With this disease, corneal edema may be severe and hinder examination of deeper intraocular structures. Uveitis and mild to severe corneal opacity are the predominant signs. Uveitis is commonly attributed to direct mechanical trauma or reaction to metabolic waste products of the parasite. Antigen-antibody complex formation may be an additional factor in the uveitis when severe corneal scarring and pigmentation occur after removal of the parasite. Typically, one 5–10-cm filaria is seen undulating in the anterior chamber; it may migrate freely between the anterior and posterior chambers and vitreous.

The prognosis is favorable with anti-inflammatory therapy and manual removal of the filaria. Delay in surgical removal may increase the likelihood of posterior segment migration, and with continued inflammation, enucleation may be required. Pre-surgical adulticide therapy is not advised, as a severe inflammatory reaction to the dead filaria may cause intractable uveitis. Microfilaricide administration can cause increased activity of the filaria and transient exacerbation of clinical signs.

Angiostrongylus vasorum, a *Metastrongylus* nematode that infects the pulmonary artery and right ventricle, has also been found in the anterior chamber of dogs. This parasite is primarily found in Europe. Migrating larvae may become aberrant and may be found in the eye. Severe granulomatous uveitis and secondary glaucoma have been observed with chronic disease. Additionally, infection may manifest in acute cases as a free nematode in the anterior chamber.

Ocular larval migrans

Ocular larval migrans (OLM) generally refers to aberrant ocular migration of *Toxocara* spp. or *Baylisascaris* sp. *T. canis* is of public health significance, as the nematode causes OLM and visceral larval migrans (VLM) in children. With *Toxocara* and *Baylisascaris,* the L3 form that typically migrates to the lung from the stomach aberrantly migrates to the eye. *Toxocara* seems to have a propensity for the eye, whereas *Baylisascaris* seems to be neurotrophic. In dogs and humans, OLM due to *Toxocara* spp. is characterized by inflammation primarily of the retina and vitreous. Ophthalmoscopy reveals areas of hyperreflectivity, hyperpigmentation, and vascular attenuation or small, solitary focal granulomas in the posterior segment. Additionally, OLM has been reported to cause orbital cellulitis. Merle dogs with environmental exposure, or those that are fed raw meat, demonstrate asymmetrical retinal atrophy, focal granulomatous formation, and occasionally intraocular larvae. Anterior uveal involvement is rare.

Onchocerciasis

In the United States, cases of canine onchocercosis have been reported since the 90s in California, Utah and Arizona but nematodes were not identified at species level. In onchocerciasis, microfilariae are widely distributed in the dermis. The black fly deposits larvae into the host, which then mature to adults. The microfilariae from the adults cause disease. Previously there was debate as to whether the organism that affects dogs and cats is *Onchocerca lienalis* or *Onchocerca lupi*. PCR and DNA sequencing of the mitochondrial cytochrome oxidase I (cox1) and NADH hydrogenase 5 (nd5) genes has demonstrated >99% similarity between the sequences obtained from canine ocular tissues previously diagnosed with onchocerciasis, and the sequences were most similar to *O. lupi* (>99% similarity) (Labelle 2012 and others). Additionally, the following circumstantial evidence: i) the site of the infection (i.e., filarioids were detected in the ocular region while *O. lienalis* localizes to the gastro-splenic ligament area of cattle); ii) the failure of experimental infection of dogs with *O lienalis*; iii) the occurrence of gravid female nematodes in the patients above suggests that dogs are most likely the primary/proper host for those parasites, as is the case for *O. lupi* (Otranto 2015). Canine ocular onchocerciasis has been reported in dogs from multiple places, including Germany, Greece, Hungary, Portugal, and the Western United States. *Onchocerca* uses *Wolbachia* as endosymbionts; attacking the *Wolbachia* may be useful in treatment.

Disease in Dogs

Clinically there are two forms of ocular onchocerciasis: acute and chronic. Acute onchocerciasis is characterized by conjunctivitis, epiphora, photophobia, chemosis, and periorbital swelling, and in some cases, parts of the parasite are observed on the conjunctival surface or other periocular tissues. In chronic cases, parasite-containing granulomatous nodules are found on various parts of the eye (i.e., eyelids, nictitans, conjunctiva, and sclera). Onchocerciasis primarily causes pea- to bean-sized masses in the conjunctiva, nictitans, and sclera. However, it may also cause anterior and posterior uveitis, peri-orbital swelling, exophthalmos, excessive lacrimation, discharge, discomfort, photophobia, conjunctival congestion, protrusion of nictitating membrane, granuloma formation, and localized corneal edema. If *O. lupi* adults develop in the retrobulbar space of the eye one may need to rely on the detection of microfilariae in skin snips and may also need to use imaging tools (i.e., ultrasound scans, computed tomography)(Otranto 2015).

Surgical removal may be curative, but medical therapy is often needed as well (Gardiner 1993; Komnenou 2003). Post-operative medical therapy includes prednisolone 0.5 mg/kg PO BID for at least 3 to 4 weeks and doxycycline 5 mg/kg PO BID for at least 6 to 8 weeks. Additionally, 2.5 mg/kg of melarsomine is given IM twice within 24 hours to 1 week after

surgery, followed by ivermectin 50 µg/kg SC and melarsomine 1 month after surgery. Where melarsomine is not available, ivermectin can be effective at 200 µg/kg PO or SQ as a single dose 1 week after surgery and then repeated at 6 months (Komnenou, personal communication 2011).

Another regimen that is said to be the only treatment with reputed efficacy for ocular onchocercosis is surgical removal of the parasitic nodule followed by post-operative therapeutic administration of melarsomine (2.5 mg/kg intramuscularly, daily for two days), ivermectin (50 µg/kg subcutaneously, one month after the initial treatment), topical antibiotics and systemic prednisolone. The above pharmaceutical treatment has been successfully applied to control the relapse of nodules up to more than one year of follow-up. However, although Wolbachia endosymbionts were detected in *O. lupi* any chemoprophylactic combination of tetracyclines with macrocyclic lactones has never been tested (Otranto 2015).

Today, the skin-snip for the detection of skin-dwelling microfilariae is the most sensitive procedure for the diagnosis in asymptomatic dogs. This tool should be used for screening dogs relocated from endemic areas, such as New Mexico since the incubation period of *O. lupi* infection is usually long. Commercial kits used for screening *D. immitis* infection are not able to detect cross-reactions with *O. lupi*.

Disease in Horses
Onchocerca cervicalis adults are found in the nuchal ligament of horses. Small (<1 mm), raised, white nodules in the pigmented conjunctiva adjacent to the temporal limbus are pathognomonic of *Onchocerca* infection. Depigmentation of the bulbar conjunctiva in this area also frequently occurs. Other lesions of onchocerciasis involve the cornea; these include edema and punctate or streaking opacities of the stroma, superficial erosions, and a wedge-shaped sclerosing keratitis emanating from the temporal limbus. Intraocular structures also may be affected by microfilariae of *Onchocerca* sp. There may be an association with the microfilariae and equine recurrent uveitis.

Histopathologically, nodules are a pyogranulomatous or granulomatous reaction with eosinophils associated with the adult worms. Lymphoplasmacytic uveitis, pre-iridal fibrovascular membranes, and evidence of secondary glaucoma are also seen. Microfilariae are seen in the uteri of females and the surrounding tissues and can be isolated from skin biopsy specimens.

No treatment is effective against the adults. Ivermectin (200 µg/kg) and moxidectin (400 µg/kg) are efficacious against microfilariae. Nodules can be surgically removed.

Disease in Cats
Orbital *O. lupi* has been diagnosed in two cats from the western United States, and both were FeLV positive and had signs of glaucoma or facial nerve paralysis. Both cats had posterior episcleral parasites seen histologically. Cause of glaucoma was not identified. There was no suspicion of onchocerciasis prior to histopathology. Parasites were gravid, indicating patent infection (Labelle 2011).

Disease in Humans
River blindness (*Onchocerciasis volvulus*) is spread by the bite of the black fly. It causes years of suffering with potential blindness and severe skin itching so that social life, work, education, and socio-economic development are affected. Some 18 million people worldwide are infected and 80 million more are at risk; 500,000 are blind. Most infected people live in Africa. Two large public health campaigns currently operate worldwide with goals of either elimination or control of this parasite. The cornerstone of these campaigns is the mass distribution of ivermectin, delivered semi-annually or annually, and donated in perpetuity for this cause by Merck. When ivermectin is delivered in a long-term, sustained fashion to large percentages of at-risk populations (e.g., >85% is the goal in Latin America), dermal microfilarial levels fall and new eye lesions and transmission are prevented.

Ophthalmomyiasis
Ophthalmomyiasis refers to aberrant ocular migration of fly larvae, most commonly of the order Diptera, including *Cuterebra, Chrysomya, Dermatobia*, the sheep nasal botfly (*Oestrus ovis*), and the cattle warble (*Hypoderma bovis*). Each species has a typical host such as rodents, lagomorphs, or ungulates. However, atypical hosts are often accidently parasitized.

Ophthalmomyiasis is named according to the location of aberrantly migrated larvae. Ophthalmomyiasis externa refers to larvae in the orbital and adnexal tissues. Ophthalmomyiasis interna anterior refers to larvae in the anterior chamber. Ophthalmomyiasis interna posterior refers to the larvae in the posterior segment. Most species have been reported to cause human ophthalmomyiasis, but in the few reports in dogs and cats, the offending species has always been *Cuterebra*.

Transmission occurs when the eggs are deposited in the environment, and body heat of the passing host triggers the first-instar to hatch and transfer to the host. The point of entry of the fly larvae is unknown, but it is postulated that fly larvae

cross the conjunctival surfaces. The larvae migrate for days and eventually localize in the subcutaneous tissue. Here they make a pore through which to breathe and void excrement. In 30 days, they exit the host and pupate.

Most ophthalmomyiasis is noted as an incidental finding in the chronic stages, although it can be found in the acute stages with anterior uveitis or panuveitis. Direct viewing of the larvae in the anterior or posterior segments has been reported, but more commonly wandering tracks are seen in the ocular fundus.

Ophthalmomyiasis interna posterior has been reported most often in dogs, cats, and humans. The characteristic lesions are road-map-like subretinal tracts that may be active or inactive. Active disease may be associated with uveitis, retinal detachment, and hemorrhage. The larva may be visible in active infections; larvae have been identified in the anterior segment with concurrent uveitis. Increased numbers of migratory tracts in the retina may be seen daily in active infections.

Inactive infections require no therapy, whereas anti-inflammatory therapy is indicated in active disease. Organophosphates may be administered in an attempt to kill the larva, but dead larva may exacerbate inflammation. The larva may also be observed for spontaneous departure. Physical removal of the larvae from the anterior chamber is recommended. Laser therapy or killing the larvae while in the eye may cause severe inflammation that destroys the eye.

Cuterebra spp.

Migration of *Cuterebra* larva in the subcutaneous tissues of the cat is common, and the eyelids, conjunctiva, or cornea may be affected. Additionally, larvae have been found within the feline and canine orbit. Intracameral cuterebra larvae have been found in cats, causing severe panophthalmitis. Cuterebra larva can directly penetrate the sclera, causing severe anterior uveitis characterized by large fibrin clots. Blindness typically occurs. Cuterebra larvae have also been found in the vitreous. Larva have been demonstrated histologically with coagulation necrosis and hemorrhage of the optic nerve, retina, and choroid, as well as anterior uveitis. Cerebral cuterebriasis has also been reported as a cause of blindness in cats. A recent report described a cuterebra at the lateral canthus with posterior extension into the orbit in a puppy (Crumley 2011). Symblepharon resulted from ophthalmomyiasis in a dog (Delgado 2012). Physical removal of the larvae is indicated when possible. The 3rd instar can be as large as 3 cm long and 1 cm wide. Care must be taken to not crush the larvae because a severe inflammatory response may occur.

Thelaziasis

Thelaziasis, also known as eyeworm infection, is caused by nematodes of the genus *Thelazia* (Spirurida: Thelaziidae), which are transmitted by secretophagous flies into the orbital cavities and surrounding tissues of many species of mammals. *Thelazia* spp are found in cattle, horses, pigs, sheep, goats, deer, water buffalo, camels, rabbits, dogs, cats, and humans. Ocular thelaziasis occurs in the western United States, Europe, and Southeast Asia.

Adult *Thelazia* can be found under the nictitating membrane, and in the conjunctival sac and lacrimal duct. The milky-white worms are approximately 10–14 mm long. The lateral serrations of nematode cuticles are responsible for causing mechanical damage to the conjunctiva and cornea, leading to the lacrimal secretions that are fed on by non-biting Diptera. The first-stage larvae are ingested by flies. After undergoing two molts, third-stage larvae are transferred back to the eye when the fly feeds. Not all vectors in the natural disease have been identified.

Disease in Dogs

Both *T. callipaeda* in Asia and *T. californiensis* in western North America infect the dog. *T. callipaeda* is also found in Europe, with case reports from southern France, Italy, Belgium, and Portugal. It has been seen in both humans and animals. This nematode species infects dogs, cats, foxes, rabbits, and wolves. In affected animals, *T. callipaeda* adult and larval stages may cause mild ocular manifestations (e.g., conjunctivitis, epiphora, and ocular discharge) or more severe manifestations (e.g., keratitis and corneal ulcers). *Phortica variegata* (Diptera: Drosophilidae) is the vector. That there is only a single haplotype of *T. callipaeda* in Europe, irrespective of country of origin and hosts, suggests a close association and a likely co-evolution between the nematode and its vector. An overall infection rate of 39.1% was seen in carcasses of red foxes, wolves, beech martens, brown hares, and wild cats in an area of southern Italy where canine thelaziasis is highly prevalent (Otranto 2009).

T. californiensis affects animals in North America, including dogs, mule deer, and others. The vectors are *Musca* spp. and *Fannia* spp. Infection causes purulent conjunctivitis with blepharospasm, epiphora, conjunctivitis, keratitis, and intense lacrimal secretion. Topical levamisole (2% aqueous solution or ointment) or SQ ivermectin and physical removal of the nematodes are effective treatments. Increased international trade and travel have increased the frequency of diagnosis of parasites in locations previously considered unlikely.

One study compared a spot on formulation and a slow release collar for the prophylactic activity against *T. callipaeda* infection in a population of dogs living in an endemic area in France. The monthly application of the spot on formulation containing 10% imidacloprid and 2.5% moxidectin was shown to be highly effective in preventing *T. callipaeda* whereas the

slow-release collar containing 10% imidacloprid and 4.5% flumethrin did not display any protection against canine thelaziosis (Lechat 2015).

<u>Disease in Horses and Cattle</u>

Horses are infected primarily by *T. lacrymalis*, and cattle mainly by *T. gulosa, T. skrjabini*, and *T. rhodesii.* Different species have varying preferences for the cornea, conjunctival sac, nictitating membrane, lacrimal gland and its ducts, the gland of the nictitating membrane, and the nasolacrimal ducts. Worms may also be found on the periorbital hair or skin during anesthesia or following migration after death of the host.

Asymptomatic infections in both horses and cattle appear to be typical of thelaziasis in North America. Infection may be encountered incidentally during surgery, and reports have indicated a surprisingly high prevalence when a specific search is made at necropsy. However, invasion of the lacrimal gland and excretory ducts may cause inflammation and necrotic exudation. Inflammation of the lacrimal ducts and sac has also been reported in horses. Mild to severe conjunctivitis and blepharitis are common. Also, keratitis, including opacity, ulceration, perforation, and permanent scarring may develop in severe cases, particularly with *T. rhodesii* infections in cattle. Subconjunctival cysts may also develop in cattle.

A clinically feasible technique for reliable detection of adult eyeworms is lacking. Gross inspection of the eyes may reveal the worms and is generally recommended for *T. rhodesii*, commonly found in the conjunctival sac. However, *T. gulosa* and *T. skrjabini* in cattle, and *T. lacrymalis* in horses tend to be more evasive and are less apt to be seen. Topical anesthetics allow for tissue manipulation and are helpful for detection and recovery of worms. Microscopic examination of lacrimal fluids for embryonated eggs or larvae may be attempted.

Parasitic Metazoans

Cysticercosis is a zoonotic disease caused by the larval form of *Taenia solium*, the pork tapeworm. Swine are the natural intermediate host of *T. solium*. The larval stage of *T. solium* may invade the eye and orbit of various species. The typical clinical sign is orbital cysts and ocular cysts. The parasite stimulates a fibrous cyst that is formed by the host, which typically does not have inflammatory cells. Eventually, macrophages along with lymphocytes and plasma cells accumulate around this cyst and eventually surround the parasite. The specific inflammatory response includes CD4, CD8, and IgM lymphocytes.