PROTOZOAL DISEASE IN THE EXOTIC COMPANION MAMMAL

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ENCEPHALITOZOONOSIS

Encephalitzoon cuniculi is a microsporidium, obligate intracellular protozoan parasite. Postnatal transmission often occurs within six weeks from an infected dam or contact with other infected animals.[1] A spore, ingested or inhaled, is the infectious stage of *E.cuniculi* with oral ingestion of spores from infected rabbit urine being the most common source of infection. Spores can be found in the urine one month after infection and are excreted in large numbers up to 2 months post infection.[2] *E.cuniculi* spores can survive outside the host for up to 6 weeks at 72° F (22° C). Shedding of spores is essentially terminated by 3 months post infection, but reports of intermittent shedding can be found in the literature. The spore possesses a polar filament, which it extrudes into host intestinal mucosa cells, injecting spore contents and initiating infection.

Multiplication of the *E.cuniculi* organism takes place in host alimentary cell vacuoles, with eventual cell rupture and spore invasion of the reticuloendothelial system and systemic circulation by infected macrophages. Initial target organs include those with high blood flow such as the lungs, liver and kidney, with infection of nervous tissue occurring later in the course of the disease. Further organism multiplication occurs via ordinary fission or schizogony within vacuoles or pseudocysts (schizonts) found in reticuloendothelial cells of target organs. Spores eventually develop and with time, the pseudocyst becomes over-crowded and ruptures. Cell rupture is associated with a chronic inflammatory response and most immunocompetent rabbits develop chronic, subclinical infections in a balanced host-parasite relationship associated with granulomatous lesions primarily affecting the brain, kidney or lens.

Encephalitzoonosis seems to be a widespread disease in rabbits with reports of infection found in 50-75% of conventional rabbit colonies.[1] It is important to note that rabbits with suspected or confirmed infection are more likely to be seropositive than clinically normal rabbits as shown by three separate studies (Table 1). Neurological disease is the most common manifestation of *E. cuniculi* in rabbits. Head tilt, usually an indication of vestibular dysfunction, can be central (cerebellum, brain stem) or peripheral (inner ear), and was the most common clinical sign noted in a retrospective study of rabbits with neurologic disease.[3] Rabbits with vestibular disease may also show varying degrees of ataxia, torticollis, and nystagmus (Table 1). Ataxia, paresis (the second most common sign seen in affected rabbits), and paralysis can also be caused by central (brain or spinal cord) or peripheral nerve disease. Subtle or overt behavioral changes, such as hyperesthesia, may be caused by central or peripheral disease, while seizures and rolling indicate brain lesions. On histopathologic review most animals show CNS perivascular inflammatory infiltrates and less frequently, granulomas. Renal signs associated with infection may include increased thirst, incontinence and varying degrees of renal insufficiency or failure and ophthalmic disease is most commonly associated with cataracts that may lead to phacoclastic uveitis if disease-associated lens rupture occurs.

As *E. cuniculi* organisms spread to various organs, antibodies develop and encapsulation occurs, thus limiting tissue damage and spore excretion. Antibodies become detectable 3 to 4 weeks after infection, with maximum titers occurring 6 to 9 weeks post-infection. A healthy immune system prevents the organisms from multiplying but the spores remain viable. Immunosuppression, as a result of illness, stress or aging may result in overt disease many years after initial infection. Currently, clinical means of diagnosing definitive antemortem encephalitozoonosis depend on clinical signs along with antibody assays and measurement of inflammatory proteins. The acute phase response is a key part of the innate immune system and acute phase proteins represent the core of the early response to stimuli such as trauma, infection, neoplasia and autoimmune disease. The goal of a successful acute phase response is to promote healing and return the body to homeostasis. One acute phase protein, C-reactive protein (CRP), was found to be increased nearly 10-fold in rabbits showing neurologic signs as the result of suspect or confirmed infection with *E. cuniculi.*[4] Several methods for detecting antibodies against *E. cuniculi* are available from commercial laboratories in the United States (Table 2). A positive titer with detection of antibodies does not differentiate between rabbits with an active infection, a latent infection, or rabbits that developed an antibody response and are no longer infected, and therefore, positive results indicate exposure to the organism but do not confirm *E. cuniculi* as a cause of disease. Follow-up samples may clarify equivocal results where early stage infection antibody levels will be considerably higher in the 3-4 week follow-up sample. Previous studies profiling the serostatus of rabbits have found a higher incidence of seropositive results in *E. cuniculi* suspect rabbits versus clinically normal rabbits (Table 1). Serologic testing for *E. cuniculi* infection is muddled by the high prevalence of IgG antibody in clinically normal animals. The Avian and Wildlife Laboratory at the University of Miami, Miller School of Medicine under the direction of Dr. Carolyn Cray has demonstrated higher IgG and IgM titers, as well as CRP levels, in *E. cuniculi* suspect rabbits showing neurologic signs and offers a viable approach to diagnosis of this disease.[5] The same response was not seen in rabbits showing only renal or ophthalmic clinical signs.

In the absence of controlled studies it is difficult to assess the efficacy of therapeutic agents against *E. cuniculi* as latent infections occur and some clinical cases may improve spontaneously without treatment, presumably as a result of the host's immune response.[2] In addition, clinical signs may not be associated with presence of the protozoa itself, but rather with the inflammatory response that persists after the organism has been eliminated. Treatment protocols for rabbits showing clinical signs suspicious for *E. cuniculi* infection have been based on fundamental principles of therapy

for granulomatous inflammation, on studies demonstrating efficacy against human encephalitozoon infections, and on in vitro susceptibility studies of *E. cuniculi* organisms to various pharmacologic agents. However, universal agreement is lacking on how to effectively treat this disease in rabbits. Several benzimidazole derivatives including albendazole (30 mg/kg q24h for 30 days), oxibendazole (30 mg/kg PO q24h for 7-14 days, then 15 mg/kg PO q24h for 30-60 days) and fenbendazole (20 mg/kg PO q24h for 30) have all been used to treat presumptive *E.cuniculi* infections in rabbits based on their anti-inflammatory actions and their in-vitro anti-protozoal activity including bioenergetic disruptions of membranes and microtubular (tubulin) inhibition. A retrospective study reviewed clinical and histologic findings associated with suspected benzimidazole toxicosis in rabbits that had a history of treatment with a benzimidazole drug and concurrent evidence of histologic lesions (bone marrow aplasia or hypoplasia and intestinal tract crypt epithelial necrosis) or clinical signs (enteritis, coagulopathy or sepsis) consistent with benzimidazole toxicosis.[6] Albendazole was used in 10, fenbendazole in 2, and oxibendazole in 1 of 13 rabbits included in this study. Affected rabbits presented with clinical signs ranging from acute lethargy and death, to inappetence, lethargy, pale mucous membranes and hemorrhage. This report provides evidence that benzimidazoles should be used judiciously in rabbits at published doses, and not without warning owners of potential risks. Therapeutic blood sample monitoring is warranted during treatment. Overall treatment success of encephalitozoonosis is based on resolution or improvement in clinical signs. Some clinicians advocate the administration of one dose of a short acting corticosteroid (ie, dexamethasone, 0.1 mg/kg SC) to control infection-associated inflammation when neurologic signs appear acutely. Cleaning and sanitation are essential to limit transmission. Most disinfectants are effective at inactivating spores, including quaternary ammonium compounds, amphoteric surfactants, phenolic derivatives, alcohols, iodophors, and hydrogen peroxide. *E. cuniculi* has shown zoonotic potential especially in immunocompromised humans such as transplant recipients, those infected with human immunodeficiency virus (HIV) or the elderly, and gives relevance to knowing the serologic status of many pet rabbits.

COCCIDIOSIS

Ferrets, rabbits, and less commonly guinea pigs may all manifest with intestinal coccidiosis, It is considered rare in the rat and chinchilla. Husbandry and individual patient immune status play a large roll in manifestation of clinical signs; most commonly diarrhea and varying degrees of dehydration and anorexia depending on severity of the infestation. Treatment is the same for all species and involves improved husbandry and environmental sanitation along with sulfa antibiotics.

FERRET

Sledge DG, et al. Outbreaks of severe enteric disease associated with *Eimeria furonis* infection in ferrets (*Mustela putorius furo*) of 3 densely populated groups. JAVMA. 2011;123 (12): 1584-1588.

From June 2005 to December 2009, three separate ferret breeding/shelter facilities experienced outbreaks of severe diarrhea with high morbidity and mortality. All 3 facilities were dynamic with new ferrets being introduced on a regular basis. Clinical signs of enteric disease were similar in all 3 groups with diarrhea ranging from beige, pasty and gelatinous to dark black and tarry being the most commonly reported abnormality. Fecal samples from affected ferrets in all three groups were examined by direct smear technique and fecal flotation. Multiple testing of pooled diarrheic samples in one group sporadically identified coccidial oocysts in low numbers. Similar testing in the other two groups failed to identify any coccidia oocysts. Necropsy and histopathology of tissues from deceased ferrets in all 3 groups showed lesions most consistently in the intestines. Intracytoplasmic coccidia, often in high numbers and representing multiple life stages, were identified in the superficial mucosal epithelial cells of intestinal villi in the jejunum and ileum from ferrets in all three outbreak groups. Speciation of the coccidia was based on morphology of the sporulated oocysts and by PCR amplification of a fragment of the gene encoding the SSUrRNA of *Eimeria* spp. Testing for other pathogens including coronavirus associated with epizootic catarrhal enteritis, rotavirus, influenza virus and parvovirus associated with Aleutian disease were all negative.

Infection of the intestinal tract of ferrets may occur from numerous species of coccidia, including *Isospora laidlawi, Isospora eversmanni, Eimeria ictidea, Eimeria vision* and *Eimeria furonis*. Of these, *E furonis* is most commonly reported and has been generally thought to cause subclinical infections. To the authors knowledge this is the first report of outbreaks of severe enteric disease associated with this organism in multiple ferrets. This series of outbreaks suggests that the initial diagnosis, management of spread and treatment of infected ferrets can be challenging.

RABBIT

- Intestinal coccidia (*Eimeria* spp) may result in diarrhea and is more common in juvenile or immunocompromised rabbits. Remains a major disease problem mostly in commercial rabbitries. *E. intestinalis* and *E. flavescens* are considered most pathologic. Oocysts require 1 or more days to sporulate at room temperature before they are infective. When ingested, sporulated oocysts (sporocysts) release sporozoites , which invade enterocytes and multiply by schizogony. Dx: fecal flotation; exact identification is not essential in practice as therapeutic options are the same for each species. [don't confuse nonpathogenic GI yeast (*Saccharomyces guttulatus*) with coccidia or suspect as pathogen]
- Hepatic coccidiosis- *Eimeria stiedae* Sporozoites can be found in liver within 48 hours post ingestion of sporulated oocysts. Migrate to liver via lymphatics or hematogenous→ invade epithelial cells of the bile ducts and schizogony begins. Following gametogeny, oocysts are formed, released into the bile ducts, and passed into the intestines. Infections may be clinical or subclinical. Weanling rabbits most often affected. Pathology; weight loss, diarrhea, ascites, icterus. Liver

periportal mixed inflammatory cell infiltration with bile duct epithelial hyperplasia and ductal dilation. Grossly seen as yellow to gray raised circumscribed lesions.

GUINEA PIG

- Eimeria caviae in the intestinal coccidia of guinea pigs. It is usually non-pathogenic but occasionally causes colitis, watery diarrhea and death, esp. in weanlings.[7]
- Renal coccidiosis from *Klosiella cobayae* has been reported in the guinea pig but is an uncommon finding. Clinical signs are normally absent and the diagnosis is usually based on necropsy and identification of the schizogonous stage in glomerular capillaries, or more commonly schizonts or sporocysts in the cytoplasm of renal tubule epithelial cells. A nonsuppurative inflammatory infiltrate of the renal tubules has also been noted. Infected animals clear when housed on wire floored cages, which prevents contact with infective urine. Sulfadimethoxine and trimethoprim-sulfa may also be effective in treating this renal parasitism.

CRYPTOSPORIDIOSIS

Cryptosporidium spp are protozoa that inhabit the respiratory and intestinal epithelium[8] and have been identified in over 150 mammalian hosts including rabbits, guinea pigs, mice and ferrets. Transmission is through ingestion of sporulated oocysts from contaminated water or food.[8] Clinical signs include intractable diarrhea, anorexia, weight loss, fever, CNS-associated neurologic signs and death. Rabbits are likely susceptible to infection with *Cryptospridium* rabbit genotype, *C parvum* and *C meleagridis.*[9] *Cryptosporidium wrairi* is a major cause of small intestinal disease in guinea pigs, especially juveniles, weanlings and immunosuppressed animals.[7] Diagnosis is made by identifying the small oocysts in the feces through direct examination or centrifugation, often with the help of acid-fast or fluorescent antibody staining. PCR for *Cryptosporidium* is offered by various veterinary laboratories. Many species of *Cryptosporidium* may serve as human pathogens and thus have zoonotic potential. Practices to reduce zoonotic transmission include thorough hand washing and preventing animal fecal contamination of water supplies.

TOXOPLASMOSIS

Toxoplasmosis gondii is an obligate intracellular parasite. T gondii can potentially infect any mammalian host, including rabbits, ferrets, guinea pigs and the chinchilla. After ingestion, sporulated oocysts rupture in the intestinal tract and release sporozoites which enter and multiply in the intestinal epithelium and associated lymph nodes to produce tachyzoites. Tachyzoites spread to other tissues of the body where they continue replication. Infection with Toxoplasma oocysts most likely occurs through environmental contamination of food supply with cat feces or in the case of carnivore's ingestion of toxoplasma encysted raw meat. Affected animals may manifest with a variety of clinical signs including anorexia and lethargy and neurologic signs of ataxia, head tremors, blindness and limb weakness resulting from nonsuppurative meningoencephalitis.

Toxoplasmosis is an uncommon cause of neurologic disease in rabbits and infections are usually subclinical.[3] One study measuring serum antibodies to *Toxoplasma gondii,* by indirect enzyme-linked immunosorbent assay, in domestic rabbits from three rabbit farms in Mexico, demonstrated a seroprevalence in 26.9% of animals tested.[10] Signs of CNS infection may include ataxia, tremors, posterior paresis, paralysis, and tetraplegia. In most species, clinical toxoplasmosis is often associated with immunosuppression, and although the role of the domestic rabbit in the epidemiology in humans has not been established in detail, some studies suggest potential zoonosis.

Toxoplasma may induce granulomatous meningoencephalitis similar to encephalitozoonosis, but foci of necrosis as well as tachyzoites may be found in many organs including skeletal muscle, spleen, heart, lung and lymph nodes.[3] This disease can be differentiated from encephalitozoonosis by serologic testing and histologically by demonstration of *E. cuniculi* spores in brain tissue and immunohistochemical labeling.[3]

Diagnosis is based on clinical signs, serologic techniques for IgG and IGM as well as *T. gondii*–specific antigens in the serum, or histopathology on necropsy. If diagnosed, treat with trimethoprim-sulfa and pyrimethamine or clindamycin or doxycycline. Clindamycin should not be used because it causes gastrointestinal dysbiosis and death in rabbits. *T. gondii* may be transmitted in herbivores congenitally or by ingesting oocysts from infected cat feces. Prevent toxoplasmosis in rabbits by avoiding exposure to outdoor grazing areas, feed or bedding contaminated by cat feces.

MISCELLANEOUS PROTOZOA

Miscellaneous protozoa including *Giardia*, *Trichomonas, Entamoeba* and *Spironucleus* spp have been reported in exotic pet mammals most notably the rodents. Husbandry, most commonly cleanliness, crowding/housing and nutritional deficiencies, as well as individual patient immune status play a large roll in manifestation of clinical signs; most commonly diarrhea and varying degrees of dehydration and anorexia depending on severity of the infestation. Treatment usually involves an 'azole' antiparaciticide such as fenbendazole or metronidazole.

HAMSTER

In one review of Syrian hamsters in 2 large United States commercial breeding facilities used to supply the pet trade a multifactorial underlying etiology for hamster enterocolitis was found in shipped weanlings.[2] The study looked at 15 weanling hamsters, 9 of which were severely affected with enterocolitis and humanely euthanized and necropsied. The remaining 6 appeared clinically normal, with normal fecal consistency, and were hospitalized for several days for monitoring and continued to have normal stools. At the end of this time, these individuals were also euthanized and necropsied. Pathology found in these 15 weanling hamsters included:

- *Clostridium piliforme* (Tyzzer's Disease) was found in all 9 clinically ill hamsters

- *Campylobacter* spp was found in 8 of 9 clinically ill hamsters
- *Clostridium dificile* toxins were found in 2 of 9 clinically ill hamsters
- Numerous protozoa including *giardia, trichomonads, entamoeba, spironucleus muris* were variably detected in all individuals
- Stomachs heavily colonized with the yeast *Torulopis (Candida*) in all hamsters
- All hamsters (healthy and sick) positive for Sendai virus and PVM (pneumonia virus of mice)
- All hamsters (healthy and sick) positive for the tapeworm, *Hymenolepis nana*

Table 1. Percentage of rabbits showing a seropositive status to *E. cuniculi* in 3 separate studies. Note the statistically significant difference between asymptomatic rabbits and those showing neurologic, renal and ocular signs

Signs of disease	Deeb, 2004 [1279 rabbits]	Harcourt-Brown, 2003 [180 rabbits]	Kunzel, 2008 [224 rabbits]
Asymptomatic	49%	37%	35%
Vestibular signs	78%	88%	90%
Paresis, paralysis	63%	71%	44%
Renal signs	61%	86%	72%
Intraocular lesions	75%	100%	84%

Table 2. Laboratories that provide *E. cuniculi* testing in the United States.

Laboratory	Serology	Specimen requirements	Shipping requirements
Sound Diagnostics Woodinville, WA	ELISA	0.25 mL serum	Does not have to be shipped on ice
University of Miami, Avian and Wildlife Laboratory	Serology IgG, IgM, CRP	0.5 mL serum or whole blood	Overnight shipping preferred. Does not have to be shipped on ice
Charles River Labs, Wilmington, MA	Multiplexed fluorometric immunoassay (MFIA) with either enzyme-linked immunosorbent assay ELISA or immunofluorescent antibody (IFA) as confirmation as needed	150ul diluted serum (1 part serum diluted with 4 parts phosphate buffered saline)	Overnight on ice

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