Encephalitozoon cuniculi – Biology, Diagnostics, and Treatment – Q&A

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Abstract: The goals of this master class are to review clinical cases that illustrate the challenges in the diagnosis and treatment of *E. cuniculi*. This proceeding provides the fundaments in areas relevant to this disease and was adapted from the ExoticsCon 2017 Proceedings.

Key words: *Encephalitozoon cuniculi*, rabbit, microsporidia

Introduction - Biology

Encephalitozoon cuniculi is a mammalian microsporidian pathogen with worldwide distribution.The microsporidia possess unique features that make them difficult to classify into a specific phylum. Microsporidia were originally thought to lack mitochondria and thus considered to be very basal eukaryotes. However, small and highly reduced mitochondria, that some researchers have used the term mitosomes to describe, have been identified at the cell organelle level and are considered to be an important differentiating characteristic of the microsporidia. The discovery of a gene for this mitochondrial-type chaperone combined with phylogenetic analysis of multiple gene sequences support a relationship between the microsporida and atypical fungi with extreme host cell dependency.[1-3] In addition, microsporidial spores retain fungal elements, including fungal proteins such as tubulins, trehalose and chitin.[3] The exact branching position within the fungal tree has not been completely clarified, however, gene sequencing supports a relationship with the ascomycete and basidiomycete clade.[1]

Microsporidia produce environmentally resistant spores and must parasitize living cells in order to survive. Microsporidia have a specific and unique method of infecting host cells that involves a specialized extrusion apparatus known as the polar filament or tube. This coiled tube is joined to an anchoring disc at the apical part of the spore and serves as a key diagnostic structure used for identification. The polar tube is employed as an invasive apparatus where a change in pH or osmotic pressure results in an explosive uncoiling of the polar tube with subsequent discharge of infectious sporoplasm either directly into the host cell or after host cell phagocytosis of the spore. In the first method of infection, polar tube uncoiling results in direct invagination of the host cell plasma membrane giving rise to transfer of sporoplasm via creation of a parsitophorous vacuole (PV) where *E. cuniculi* spends its entire inracellular life cycle.[3] Alternatively, this transfer of nucleus-containing sporoplasm may occur after host cell phagocytosis and subsequent polar tube discharge resulting in uptake of sporoplasm by

host-cell phagosomes.[4] Some scientists have suggested that germination out of phagosomes is limited and does not significantly contribute to *E. cuniculi* infection.[3]

Life cycle and pathogenesis

E. cuniculi has a direct life cycle and is capable of both horizontal and vertical (transplacental) transmission. In rabbits, postnatal transmission often occurs within 6 weeks from an infected dam or contact with other infected animals.[5] Spores, measuring 1.5 x 2.5 microns are either ingested or inhaled as 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 1 month after infection and are excreted in large numbers up to 2 months post-infection.[6] *E. cuniculi* spores can survive outside the host for up to 6 weeks at 72F (22C). Shedding of spores is essentially terminated by 3 months post-infection, while others suggest that once infected the host rabbit may shed spores intermittently throughout its life. Following germination via the polar tube extrusion process previously described, the microsporidia undergo a proliferative phase during merogony. Simple structured meronts replicate by binary or multiple division within host cell parasitophorous vacuoles where they are found attached and closely aligned with the PV membrane. Initial target organs for infection include those with high blood flow such as the lungs, liver, and kidney[7], with infection of nervous tissue occurring later in the course of the disease. Differentiation of meronts into sporonts and later into mature spores (sporogony), results in the development of the distinctive polar filament and a rigid spore wall. With time the PV or pseudocyst becomes over-crowded and ruptures resulting in spore release. This cell rupture may result in an inflammatory response with most immunocompetent rabbits developing chronic, subclinical infections in a balanced host-parasite relationship. Granulomatous lesions primarily affecting the brain, kidney, or eyes (lens) may result and are responsible for the most common clinical signs of disease associated with *E. cuniculi* infection. A wide range of other tissues may show morphological lesions, however, in general, these histological alterations are not associated with clinical disease.

There is some debate on the pathophysiology of ocular infections. Wolfer suggested that *E. cuniculi* penetrates the lens during intrauterine development when the lens capsule is thin or absent, and when there is a higher vascular supply.[8] Rupture of the lens capsule releases lens proteins which initiates and inflammatory response, and typical phacoclastic uveitis. More recent research has demonstrated via immunohistochemical staining the presence of organisms in many ocular structures (mostly sclera, cornea and periocular connective tissues, and rarely retina, iris and lens) in adult immunocompetent young laboratory rabbits experimentally infected with *E. cuniculi* spores by gavage tube.[9] Rabbits were followed for up to 8 weeks; in all cases, there were no demonstrated ocular lesions, and histopathology did not demonstrate inflammation. Another study collected ocular structures from newborn kits that died within 10 days of age; these were offspring experimentally infected dams.[10] Fifty-four percent showed evidence of *E. cuniculi* within ocular tissues.

Encephalitozoonosis seems to be a widespread disease in rabbits with reports of infection found in 50-75% of conventional rabbit colonies.[11] Immunocompetency may falter as the host animal ages allowing for an increase in clinical disease in infected pet rabbits. Neurologic disease is the most common manifestation and is most frequently associated with vestibular disease with infected rabbits presenting with varying degrees of head tilt, nystagmus, ataxia, circling and rolling. Renal disease and ophthalmologic pathology have also been associated with clinical encephalitozoonosis. Renal signs may include increased thirst, incontinence and varying degrees of renal insufficiency or failure and ophthalmic disease is most associated with cataracts that may lead to phacoclastic uveitis if disease-associated lens rupture occurs.

***E. cuniculi* in other mammals and humans**

E. cuniculi is the most commonly reported microsporidian of nonhuman mammals.[12] Other than rabbits, symptomatic infection appears to be rare. Three strains of E. cuniculi have been characterized: Strain I

(Karyotypes A,B,C) reported in rabbits, mice and humans, Strain II (Karyotype F) in blue foxes and mice and Strain III (Karyotypes D,E) isolated from domestic dogs and humans.[12] Unclassified strains have been reported in a wide host range of mammals including rats, guinea pigs, hamsters, horses, cows, mink and nonhuman primates.[12] Several reports have identified *E. cuniculi* in asymptomatic laboratory rats and guinea pigs based on histopathologic identification of the organism in the CNS on necropsy and/or serology.[13-15] A new *E. cuniculi* isolate, identified in free-ranging rats, was characterised as strain II based on the rDNA internal spacer sequence.[16] Western blot analysis of this isolate revealed slight differences to other strain II isolates originating from laboratory mice and farmed blue foxes. This new isolate caused disseminated infection in liver and lungs upon oral innoculation of Brown Norway rats and was successfully transmitted to sentinel rats.[16] *E. cuniculi* is also zoonotic particularly in immunocompromised humans where human immunodeficiency virus (HIV) infection has progressed to Acquired Immunodeficiency Disease Syndrome (AIDS).[17]

Clinical Presentation

There are three main manifestations of clinical disease associated with *E. cuniculi* infection. These include renal, ophthalmic and central nervous system symptoms.[18-21] Clinical signs of vestibular disease include circling, falling to one side, rolling, head tilt, and nystagmus. Vestibular disease may be central or peripheral in location and may be caused by bacterial infection, inflammation or other process. Vestibular signs may range in severity from mild to extreme, may respond to minimal treatment and resolve within a week, or may last for many weeks or months.

Renal lesions are often an incidental finding, or if severe, the rabbit patient may present with clinical signs related to renal dysfunction such as polyuria, polydipsia, or weight loss/cachexia.[15-18] Pre-anesthesia bloodwork in young rabbits for evaluation prior to elective surgery may reveal renal values that are abnormally elevated.

Ophthalmic symptoms in rabbits with *E. cuniculi* include cataract formation and phacoclastic uveitis from in utero infection.[18-21] Neurological symptoms that can develop in rabbits infected with *E. cuniculi* include heat tilt, ataxia and/or vestibular symptoms.[18-21] Because these symptoms can also occur with other conditions such as otitis media/interna, the clinician has to consider other conditions and utilize diagnostic tools to determine if the neurological symptoms are related to *E. cuniculi* infection or not. Other differential diagnoses for head tilt and ataxia in pet rabbits include infection (bacterial, toxoplasmosis, viral), neoplasia such as lymphoma, toxicological conditions, and congenital abnormalities.

There is no gender predilection for *E. cuniculi* infection in rabbits. In practice (T. Ritzman, personal communication, March 2022), dwarf breeds of rabbits are more commonly affected. Ocular lesions such as phacoclastic uveitis is often seen in young rabbits whereas central nervous system symptoms may occur more often in older animals. Most rabbits with *E. cuniculi* are asymptomatic and the extent to which *E. cuniculi* infection in rabbits causes clinical signs is controversial. The severity of the clinical signs does not always have a direct correlation to the severity of the lesions inside the patient. Physical examination findings of infected rabbits can include changes with any of the previously mentioned organ systems (renal, neurological, ocular). There is also potential for cardiac and hepatic involvement with *E. cuniculi* infection. Risk factors for the disease in rabbits include immunosuppression, which can predispose the animal to clinical disease, stress, poor diet, chemotherapy, and glucocorticoid therapy.[18-21]

Client Reassurance

Neurological signs are often acute in nature. When rabbit owners see their pet rolling uncontrollably, they usually call the hospital in a panic, and are concerned that the rabbit has had a stroke. The receptionist should recommend the client place the pet in a secure carrier with a rolled towel on each side and bring the rabbit in right away for an emergency exam. During the exam it is important to educate the owner as to what to expect. The nystagmus and torticollis are present primarily during the active phase of the illness, and when the nystagmus improves or resolves, then the rabbit is considered to be improving. Prognosis varies depending on the underlying cause of the vestibular disease as well as how well the client can care for the rabbit during its recovery. The severity of clinical signs is not correlated with prognosis and there is no medical reason to euthanize these rabbits. Affected rabbits often appear to be anxious in the first 24-48 hours, but as they start to feel better, they adapt to the situation and cope well with the vestibular signs. Rabbits can adapt and learn to live with significant vestibular signs for several weeks or months. In some patients, clinical signs will acutely resolve, and rabbits can live normal or nearly normal lives thereafter. Owners should however be warned that, once infected, future recrudescence of the parasite is possible, with associated clinical signs.

Diagnostics (non-serological)

Antemortem diagnosis of *E. cuniculi* is often presumed and based on signalment, clinical signs, and exclusion of other disease, positive antibody titers, and response to treatment. Definitive diagnosis in the live rabbit is problematic, since a positive antibody titer indicates exposure and is not definitive for active infection. Definitive diagnosis requires identification of the organism and characteristic inflammation in affected tissues. This result is usually obtained at the time of post-mortem examination with histopathology of the affected tissues. Some inflammatory lesions can be found in clinically normal rabbits at time of necropsy, which are supportive of subclinical disease related to *E. cuniculi* infection. Post-mortem diagnosis is difficult as spores are infrequently detected by routine histological exam although detection of *E. cuniculi* in tissues can be aided by immunohistochemistry.[20,21]

Hematology to evaluate complete blood cell count (CBC) and biochemistry profile can be unremarkable in affected patients or may reflect non-specific changes such as increased packed cell volume (PCV) with dehydration secondary to renal disease or anorexia. The biochemistry profile may be unremarkable or may show increased renal values such as elevated blood urea nitrogen (BUN) and creatinine. It should be noted that these and other complicating abnormalities seen in bloodwork may affect the clinician's decision on which medications can be administered. Urine sedimentation analysis can be a useful diagnostic test in infected rabbits that are actively shedding the spores in their urine. A Gram's stain of the urine sediment from an infected rabbit may reveal the spores. It can be challenging to diagnose from this test since rabbits infected with *E. cuniculi* usually shed the spores in their urine for up to three weeks following infection and the spores are not always present in the urine during the later clinical signs of the disease.

PCR testing of urine and cerebrospinal fluid were not found to be reliable with only 30% and 0% positive results in confirmed/suspect rabbits, respectively.[22] However, PCR was found to be useful in the detection of DNA in the brain, kidney, and eye.[22]

In summary, antemortem diagnosis of encephalitozoonosis and treatment in the rabbit patient can be challenging. The veterinary clinician should consider the constellation of medical history, clinical symptoms, as well as diagnostic testing to determine if there is a high suspicion of *E. cuniculi* infection in the patient.

Serodiagnostics

Seroprevalence is high worldwide – with some variation – but generally exceeds 50%.[23-28] In previous work by the Avian & Wildlife Laboratory, 79% of *E. cuniculi* suspect rabbits were IgG seropositive vs. 41% of non-suspect rabbits.[25]

Antibody detection can be performed via various methods but in the U.S., it is primarily determined by ELISA techniques. As many rabbits are seropositive for *E. cuniculi* even in the absence of clinical signs, testing at a single screening dilution for positive or negative results can be problematic. The addition of IgM testing has aided in the diagnosis of this infectious process. Jeklova and colleagues reported the highest seropositive rate for IgM and IgG was found in rabbits with neurological clinical signs (n=500).[27] In a study in the U.S., IgM titers were found to be nearly 3-fold higher and IgG titers were found to be at least 2-fold higher in rabbits with presumptive infection.[29] The most obvious application of serology is the definition of a true negative rabbit; negative IgM and IgG results are very rarely observed in infected rabbits.[29] Current first significant titers for the ELISA at the Avian & Wildlife Laboratory remain as IgM 1:64 and IgG 1:512.

Quantitation of C reactive protein (CRP) has been reported to be a valuable adjunct test. A significant 10-fold increase in CRP was observed in rabbits with presumed infection.[30] While CRP is not specific for *E. cuniculi* infection, the presence of elevated levels does enhance the positive predictive value of the serodiagnostic titers.

A quantitative immunoblot for testing the anti-*E. cuniculi* antibodies in serum[31,32] and mass spectrometry iTRAQ® protocol was used to compare blood proteome of diseased rabbits with the one of controls[33,34].The serology assay showed high diagnostic performance for *E. cuniculi* infection in rabbits, with 88.4% and 84.3% sensitivity for IgG and IgM detection, and 98.8% and 98.8% specificity for IgG and IgM, respectively.[32] It provided a more accurate analysis of the humoral response than with conventional ELISA testing ($P<0.0001$). The most antigenic bands observed on immunoblotting were identified as corresponding to seven *E. cuniculi* proteins: polar tube protein 3, polar tube protein 2, and for the first time reported, heat shock related 70kDa protein, polysaccharide deacetylase domain-containing protein, zinc finger protein, spore wall and anchoring disk complex protein EnP1, and translation elongation factor 1 alpha.

Recent work in rabbits has demonstrated that a TH1 response with a dominant IFN-ɣ expression in the lymphoid system and a TH2 response in the intestine.[35] The authors propose this may allow E. cuniculi to persist in the host. This was further supported in a second model examining the percentage of IL-4 positive cells in the brain of infected rabbits.[36] Interestingly, work in mouse macrophages has demonstrated that infection resulted in a polarization of these cells to a phenotype that also further promoted survival and multiplication of *E. cuniculi*.[37] Future work must determine the essential immune response needed to combat *E. cuniculi* and understand any immune altering properties and pathophysiology of the infectious agent and resultant disease.[38]

Imaging

Many diagnostic imaging techniques may be employed for the diagnosis of *E. cuniculi* in pet rabbits, primarily to rule out other conditions, rather than to obtain a definitive diagnosis for this disease. In suspect *E. cuniculi* cases the gold standard is to perform computed tomography (CT) imaging or magnetic resonance (MR) imaging, or both, to fully assess the central nervous and musculoskeletal systems. If these are not available, then radiography and ultrasonography may be useful for identifying differential diagnoses of this condition.

CT imaging

CT is useful for ruling out middle ear disease[39], as a main differential diagnosis for *E. cuniculi*, in cases presenting with head tilt, ataxia and vestibular disease. It should be noted, however, that concurrent infection with *E. cuniculi* and middle ear disease may occur. CT is also useful for evaluation of the skull, vertebral column, long bones, and joints, to rule out other causes of ataxia. The technique may require the animal to be anaesthetized, which poses an increased risk in severely affected animals, however conscious CT imaging is routinely performed with good success (E. Keeble, personal communication, March 2022).[40] Intravenous contrast agent is useful for highlighting areas of inflammation and differentiation of abscesses versus neoplasms.[41]

MR imaging

Magnetic resonance imaging can be used to identify central nervous system soft tissue abnormalities in fine detail, such as neoplasms, abscesses, or granuloma. Normal brain anatomy has been described using this modality in rabbits.[42] A general anesthetic is required, which may be a concern in debilitated or severely affected animals. In *E. cuniculi* cases, lesions are found within the cerebrum most commonly, followed by the leptomeninges and more rarely involving the cerebellum, central vestibular system, and spinal cord.[19] The position and extent of any observed lesions however may not correspond to the severity and range of clinical signs observed, further hindering the clinical diagnosis.[43] A human case infected with *E. cuniculi* showed no pathology on initial CT exam. However repeat CT and MR imaging 10 days later revealed a subdural abscess which was positive for *E. cuniculi* spores on aspirate. The patient was well by this stage with almost no neurological signs, despite extensive lesions on MR imaging.[44] MR imaging findings in *E. cuniculi* infected rabbits have not been described in the literature to the authors' knowledge. CT and MRI are useful for assessing the anatomical position and extent of any observed lesions and may help determine a prognosis and treatment plan.[41]

Other imaging techniques

Radiography and ultrasonography are useful in the differential diagnosis of disease which could present with similar clinical signs, such as otitis media, spinal lesions, osteoarthritis and renal disease.[19,45] Radiographic assessment of kidney size, shape and position (average kidney size is 1.4-2.2 times the length of the 2nd lumbar vertebra).[46] Excretory urography is useful for evaluation of renal function and sub-gross anatomy.[47] Ultrasonographic evaluation of the kidneys for renal size, shape and the relative ratio of cortex to medulla in suspect *E. cuniculi* cases is recommended. (Average kidney size in rabbits < 2kg weight is: 22-36mm length, 10.5-17mm width, 12-20mm height). With *E. cuniculi* infection kidneys may be small and irregular due to chronic damage and there is typically a loss of boundary between the cortex and medulla or cortical thinning.

In conclusion, while it is not possible to make a definitive diagnosis of *E. cuniculi* using imaging modalities, they are very useful in determining the differential diagnoses.

Treatment

Treatment for *E. cuniculi* infection in the rabbit patient is usually provided on an outpatient basis unless the patient has severe disease. The goal of treatment is to reduce inflammation and prevent formation of spores.[18-21] Many rabbits with encephalitozoonosis improve with supportive medical care and good nutrition. An important aspect of treatment of this infection is fluid support to ensure the patient is normally hydrated. Because of the risk of renal disease in these patients, dehydration can exacerbate already existing renal problems. Rabbits with neurological symptoms affecting their mobility and movement usually benefit from cage confinement. Special caging can include padded cage, or a smaller, one level cage which allows for safer movement in rabbits with head tilts, ataxia, or vestibular symptoms. It is important, however, to encourage regular exercise and movement once a patient has improved with their neurological function.

Diet plays an important role in the maintenance of health for *E. cuniculi* infected rabbits. The patient needs to continue to eat during illness. The clinician should consider whether their patients with neurological symptoms might benefit from anti-nausea medication. Anorexia can occur in *E. cuniculi* infected rabbits and patients that will not eat readily on their own will need to be syringe fed gruel such as Critical Care for Herbivores (Oxbow Pet Products, Murdock, NE) or Emeraid Herbivore (Lafeber Company, Cornell, IL). A general guideline for syringe feeding amount would be 10-15 ml/kg orally every 6-8 hours. High carbohydrate or high fat foods should be avoided and are contraindicated for the rabbit patient. Fresh water should always be offered. If the patient is having difficulty using a water bottle a different method for providing the water should be considered.

Medications

Disease treatment: The two main differentials for vestibulitis in rabbits are otitis media and *E. cuniculi* - induced inflammation. Therefore, recommended treatments (S. Kanfer, personal communication, March 2022) include an antibiotic and an anti-inflammatory. Careful selection of a rabbit appropriate antibiotic should be chosen as antibiotic therapy in hind gut fermenters has to be selected for the unique physiology of these patients to avoid antibiotic induced dysbiosis. Where possible responsible antibiotic stewardship dictates the antibiotic choice is based on culture and sensitivity results. Examples of systemic antibiotic medications considered appropriate in lagomorphs include Enrofloxacin (10mg/kg PO q12h; Diamondback Drugs Compounding Pharmacy, Scottsdale, AZ, USA) or trimethoprim-sulfa (30mg/kg PO q12h). Meloxicam is an effective and safe anti-inflammatory (0.3 – 0.5mg/kg PO q12h; up to 1mg/kg q24h; Meloxidyl, Ceva Animal Health LLC, Lenexa, KS, USA) but this drug is contra-indicated in animals with impaired renal function or dehydration.[48] Steroid therapy can be associated with a high risk of intestinal and liver problems in rabbits, and is well known to cause immunosuppression in rabbits, therefore should be avoided unless truly necessary.[49] There is a recommendation using meloxicam past resolution of signs (S. Kanfer, personal communication, March 2022).

Benzimidazole anthelmintics are effective against *E. cuniculi* in vitro and have been shown to prevent experimental infection in rabbits. Efficacy in rabbits with clinical signs is unknown. Anecdotal reports suggest a response to treatment, but many rabbits with neurological symptoms often improve over time with supportive care alone. Long-term treatment of 30-60 day duration is recommended, as treatment only serves to prevent replication of the organism, rather than actually eliminate it.[18-21] Published treatments for *E. cuniculi* infection include fenbendazole as the most commonly used and effective medication.[18-21] The current recommendations are fenbendazole (20mg/kg PO q24hrs; Panacur, Intervet Inc, Madison, NJ, USA) [50] or oxibendazole (15mg/kg PO q24hrs; Anthelcide EQ, Zoetis Inc, Kalamazoo, MI, USA).

There have been reports of rabbits developing acute bone marrow failure and death associated with benzimidazole therapy,[51] most commonly occurring at the 30-day mark, but also experienced at the 14-day mark as well. If benzimidazole therapy is used, it is prudent to evaluate a CBC prior to initiating therapy as well as 7-10 days into therapy to rule out any signs of bone marrow suppression. The bone marrow suppressive effects are anecdotally reversible in some rabbits if therapy is stopped at the first sign of pancytopenia (J. Graham, personal communication, March 2022). Since the benzimidazole medication is not a cure, it does not clear the organism from the rabbit's system, and has such a severe adverse effect, use benzimidazole medications with caution.[51] Some practitioners choose to use benzimidazoles only in rabbits that have severe symptoms that are unresponsive to other medications, and to use them for no more than one to two weeks at a time, then to pulse dose for one to two-week periods for a short period of time (S. Kanfer, personal communication, March 2022). Monitoring complete blood cell count weekly is recommended, but the bone marrow failure may be per acute and occur too quickly to identify in time. Treatment of bone marrow failure requires emergency blood transfusions, epoeitin alfa (100U/kg SQ q

2days; Procrit, Janssen Products, Horsham, PA, USA) and filgrastim (5mcg/kg SQ q12hrs; Neupogen, Amgen, Thousand Oaks, CA, USA), and has only been successful in one rabbit.[51]

Gastrointestinal support:
As mentioned previously, rabbits with vestibulitis may initially be anxious because they cannot control their own movements, may be physically unable to grab food because they cannot stop rolling or balance themselves, or may feel nauseated. They require supportive care to keep them hydrated and fed. In most cases crystalloid fluids administered subcutaneously once or twice a day is sufficient for hydration (25-50ml/kg/day SQ q 12-24hrs). Most rabbits will quickly learn how to eat on their own despite the challenges, but some rabbits will need to be syringe fed. Rabbits with torticollis find it easier to eat greens and more challenging to eat pellets. These rabbits also appear to prefer greens over hay, and greens contain a higher water content which helps combat dehydration. Therefore, it is recommended to feed large amounts of green leafy vegetables such as lettuces and parsley, so that the rabbit never runs out. This is temporary, while the rabbit is suffering from the vestibular disease. For rabbits that spend time lying on their side or back, it is recommended to place a large pile of greens on top of the rabbit's face, so as they eat the greens, gravity causes the additional greens to drop down for them to eat.

Anti-vestibular medication:
Meclizine is recommended to help decrease the vestibular symptoms and nausea. This medication may also have a calming effect with mild sedation (12.5-25mg per rabbit PO q12hrs; Motion-Time, Time-Cap Labs Inc, Farmingdale, NY, USA). More recently Maropitant has been recommended and can be used instead of or in addition to meclizine (1mg/kg SQ q24hrs; Cerenia, Zoetis Inc, Kalamazoo, MI, USA).[52,53] For rabbits that are continuously and severely rolling, Midazolam is extremely helpful and relatively safe (0.3mg/kg SQ q4-6hrs prn; Akorn Inc, Lake Forest, IL, USA). The dose can be titrated to effect. If the rabbit is too sedate, give 0.1mg/kg. If the rabbit starts to roll again after a few hours and the owner wants it to sleep through the night, give 0.5mg/kg. Midazolam works nicely with the vestibular rabbits because it is very safe, can stimulate appetite, aids in relaxation and decreasing anxiety, the dose is small, and owners can easily be taught to give the injection with an insulin syringe. As the rabbit's vestibular symptoms improve, the rabbit may be switched over to oral diazepam to decrease the incidence of rolling (1mg per rabbit PO q12hrs; Teva Pharmaceuticals USA Inc, North Wales, PA, USA). Benzodiazepines are a frequent treatment for dizziness and vertigo in humans. Many of these benzodiazepine drugs are controlled medications and this state and federal laws will guide the practitioner on how this class of medication can be prescribed and dispensed.

Ophthalmic care:
Rabbits with vestibular disease may develop corneal ulcerations, sometimes of the down eye, sometimes of the up eye. It is recommended to start ophthalmic ointment at the first sign of vestibular disease and rolling, to prevent eye injury. You can start with artificial tears (OU q8hrs; Henry Schein, Dublin, OH, USA), but often you will need to change to an antibiotic ointment after a corneal ulcer develops. Flurbiprofen ophthalmic solution 0.03% (Bausch & Lomb, Valent Pharmaceuticals North America, Bridgewater, NJ, USA) can help reduce ocular inflammation. The use of systemic nonsteroidal anti-inflammatory therapy should be used with caution in patients with renal disease. Collagen shields may also be considered for treatment of chronic corneal ulcers in pet rabbits.[54]

Corticosteroid use:
Corticosteroid use with *E. cuniculi* treatment is controversial. Rabbits are very sensitive to the immunosuppressive effects of corticosteroids. Its use in the rabbit patient may worsen clinical signs of *E. cuniculi* infection or subclinical bacterial infection. Because of this caution should be utilized when considering this form of treatment in rabbits.

Alternative treatments

Once the nystagmus resolves, then that is the cue that the dizziness and inflammation has reduced, and the active disease is improving. At the onset of signs, you can start neck massage, acupuncture, and cold laser therapy. But it is very difficult to perform physical therapy of the neck when the rabbits have nystagmus, are actively dizzy and rolling. After the nystagmus resolves, you can start physical therapy such as turning the head opposite the tilt, encouraging the rabbit to walk, and rubbing the ear on the opposite side of the head tilt. The clinician will have to balance the potential stress of handling and the patient's condition prior to considering whether physical manipulations are appropriate.

Physical supportive care

The rabbits with mild vestibular symptoms that are not rolling can usually be sent home with the owner to care for and administer medications. Most of these cases will not get worse and will improve over a couple of weeks. But the purpose of this paper is to address the rabbits that are rolling uncontrollably. When hospitalizing vestibular rabbits in the veterinary clinic, the tendency is to place them in a regular sized cage using a rolled towel on either side, or a rolled towel in a circle, like a bed. Usually, the rabbits still end up rolling and flailing several times throughout the day, which is stressful for the rabbits and staff, and can lead to the rabbit becoming injured. The following describes a technique to keep these rabbits more comfortable and secure. It is recommended that they stay in a large top opening carrier or small cage, with rolled up towels or blankets on each side, creating a "deep canyon" for them to stay in. It is important that the padding extends high up on the sides. This gives them support to lean against, and a light pressure to help decrease the rolling. There is also a towel on the bottom of the carrier that should be changed at least twice a day. Be careful that the rabbit does not overheat. The rabbit will still roll, and will still end up upside down at times, but it is lessened, and they seem to feel more secure. The rabbit should always have a large amount of greens and may be offered pellets and hay. Periodically the rabbit should be offered a bowl or syringe of water, but it should not have a water bowl or bottle left in the carrier as that is likely to cause an injury when the rabbit rolls.

The rabbit should stay in the carrier primarily, and if it is placed in a less supportive area it will likely roll uncontrollably and become stressed. The rabbit can have its medications administered while it is sitting inside the carrier, or it can be carefully lifted out, but the owner should be shown how to restrain a dizzy rabbit to prevent it from rolling when being lifted or moved. Lift the rabbit's body with one hand and use your other hand to hold the head in line with the body. Quickly bring the rabbit's body close to your body for additional support. Then place all four feet down on a towel on a table while still holding onto its body and head, and do not let go or the rabbit will roll. If the rabbit is laying upside down, it can still receive oral medications and syringe feeding, just go slowly.

If the rabbit is bonded to another rabbit, the carrier can be placed inside the other rabbit's area, and they can have visits. The vestibular rabbit should stay in this carrier until it is no longer rolling, or the rolling is significantly decreased. As the rolling lessens, the padding can be decreased, and the rabbit can have more therapy time outside the carrier.

Rabbits that are rolling uncontrollably do not have a guarded prognosis. Most of these rabbits do improve and respond to treatment. But nursing them is very labor intensive and can take two weeks or longer, and most clients are not experienced enough, plus they usually must go to work every day. In addition, most clients are very upset by seeing their rabbits in this condition, even if the rabbits are doing well. It is recommended that these rabbits be hospitalized during the severe rolling phase, but that may take one to three weeks of care. If the owner cannot afford for these rabbits to be hospitalized, another alternative is that an experienced technician or trusted rabbit rescuer can board the rabbit. Euthanasia is also an option where owners are unable to provide home care or afford the cost of hospitalization.

Pathology

The lesions, gross and histologic, of *Encephalitozoon cuniculi* generally follow the route of infection and organ predilection. The severity of the lesions depends on the immunocompetence of the host rabbits with many cases being clinically silent. After ingestion, *E. cuniculi* organisms invade the intestinal epithelium and are engulfed by macrophages. From here the infected macrophages disseminate throughout the body.[55] The kidneys, lungs, and liver are the first organs to be targeted due to their high blood flow. There is a predilection for the kidneys, brain, and ocular structures.[56,57] Approximately one month after exposure the spores can be found excreted intermittently in the urine.[56]

Gross pathology

For the most part, there are few significant gross lesions that would suggest an infection specifically with *E. cuniculi*. In acute infections, lesions may be present in the kidneys and these are of irregular, dark red, depressed foci throughout the cortex. The chronic lesions of the kidney are of irregular pitted areas on the surface representing renal fibrosis.

Histopathology

Histology findings due to the infection are primarily of granulomatous lesions. These are typically evident within the interstitium of the lung, kidney, liver, heart, and brain. Within the lung, there may be a focal to diffuse interstitial pneumonia. Hepatic lesions will also be focal granulomatous inflammatory infiltrates usually associated with periportal lymphocytic infiltration. Similar focal lymphocytic infiltrates may be present throughout the myocardium. Within the kidney, the early lesions of the infection will be a focal to segmental granulomatous interstitial nephritis associated with renal tubular epithelial necrosis and a mononuclear cellular infiltration. These lesions will usually be present at all levels of the renal tubule with minimal involvement of the glomeruli. The spores may be identified within parasitophorous vacuoles in the tubular epithelium, as well as free within tubular exudates.[58]

In the central nervous system, the lesions do not occur until at least a month post exposure. These will be focal nonsuppurative granulomatous meningoencephalomyelitis. There will be foci of astrogliosis and perivascular lymphocytic infiltrations. At this point, it may be possible to differentially stain using a Gram or Giemsa stain and spores may be highlighted within the granulomatous inflammatory foci (within parasitophorous vacuoles in the neutrophil and endothelium).[57]

In some rabbits, *E. cuniculi* infections have been associated with a phacoclastic uveitis as well as cataract formation. The lesions appear to be more frequently identified within dwarf rabbits, who also frequently have a meningoencephalomyelitis.[59] Histologically, there will be a keratitis with rupture of the lens capsule and inflammatory infiltrates that include heterophils as well as macrophages and multinucleate giant cells. Lymphocytic plasmacytic infiltrates are more commonly identified in the iris and ciliary body.[56]

Chronic lesions are of a fibrosing interstitial nephritis in older rabbits, suggesting chronic and/or persistent infections. These are of interstitial fibrosis, loss of the parenchyma, and a granulomatous cellular inflammation. Myocardial fibrosis described infrequently in aging rabbits is also suspected to be associated with *E. cuniculi* infections as a sequela to the early myocarditis.[56] Microbes are seldom identified associated with these lesions, although immunohistochemistry may prove helpful.[56-58]

For acute lesions, it may be possible to identify the spores by using a Gram stain (Brown & Brenn stain on histologic samples).[56] These spores are poorly staining on hematoxylin and eosin stain. The structures are Gram-positive, and they are approximately 1.5 x 2.5 µ in size. Spores may be found within the epithelial cells, in macrophages, and/or free within collecting tubules of the kidney, inflammatory foci of

the brain, and within the lens cortex, lens epithelium, and inflammatory material in cases of phacoclastic uveitis.[56,57]

Commonly, the diagnosis of the disease, especially without visualization of the microorganisms, is based upon the characteristic inflammatory lesions as well as the tissues that are involved (kidney, brain, and eye).[58] Differential diagnoses for rabbits with the neurologic clinical signs should include otitis interna, toxoplasmosis (both of which may occur concurrently with *E. cuniculi* infection), and *Baylisascaris* migration.

Summary

The course of vestibular disease in rabbits and how this relates to the underlying disease process is still undergoing research. Otitis media can be challenging to diagnose, even with bulla radiographs or a CT scan. Affected rabbits may or may not have recurrent disease. Most cases are single rabbits in a household of rabbits, but more than one rabbit may have vestibular disease in some households.

The *E. cuniculi* titers in many of the rabbits with vestibular disease will vary given the high seropositive status in rabbits. Serodiagnostic testing continues to be a stopgap until more specific diagnostic testing becomes available and the pathophysiology of *E. cuniculi* infection is further defined. The use of a strong clinical evaluation and adjunct diagnostics (serodiagnostic and other) is key to patient evaluation.

Rabbits that are severely rolling can be rehabilitated with patience, medical treatment, and supportive care over a period of several weeks to months. Although many rabbits improve, some will not respond to treatment and die following refusal to eat and weight loss despite supportive care measures. Vestibular disease in rabbits generally appears to have an acute active phase with profound neurologic signs, followed by reduction of inflammation, nystagmus, and dizziness. The ultimate outcome for surviving rabbits can range from a mild residual head tilt to chronic balance issues.

References

1. Didier ES, Weiss LM. Microsporidiosis: current status. Curr Opin Infect Dis. 2006; 19(5): 485-492.
2. Katinka MD, Duprat S, Cornillot E, et al. Genome sequence and gene compaction of the eukaryote parasite *Encephalitozoon cuniculi.* Nature3 2001; 414:450-453.
3. Bohne W, Bottcher K, Grob U. The parasitophorous vacuole of *Encephalitozoon cuniculi*: Biogenesis characteristics of the host cell-pathogen interface. Intnl J Med Micro. 2011; 301: 395-399.
4. Franzen C, Muller A, Hartmann P, et al. Cell invasion and intracellular fate of *Encephalitozoon cuniculi* (Microsporidia). Parasitology. 2005; 130: 285-292.
5. Hunt C. Radiographic interpretation of the vertebral column. In*:* BSAVA Manual of Rabbit Surgery, Dentistry and Imaging (Harcourt-Brown F, Chitty J, Eds.), Gloucester, UK, BSAVA Publications, 2013: 76-83.
6. Franssen FF, Lumeij JT, van Knapen F. Susceptibility of *Encephalitozoon cuniculi* to several drugs *in vitro*. Antimicrob Agents Chemother. 1995; 39: 1265-1268.
7. Künzel F. *E. cuniculi* in rabbits and cats: Aetiological relevance, clinical manifestation and diagnostic challenges. Habilitation Thesis. 2016; 1-139.
8. Wolfer J, Grahn B, Wilcock B, Percy D. Phacoclastic uveitis in the rabbit. Prog Vet Comp Ophthalmol. 1993; 3: 92-97.
9. Jeklova E, Leva L, Matiasovic J, et al. Characterization of humoral and cell-mediated immunity in rabbits orally infected with *Encephalitozoon cuniculi*. Vet Res. 2020; 51: 79.

10. Ozkan O, Karagoz A, Kocak N. First molecular evidence of ocular transmission of Encephalitozoonosis during the intrauterine period in rabbits. Parasitol Intl. 2019; 71: 1-4.
11. Lyngset A. A survey of serum antibodies to *Encephalitozoon cuniculi* in breeding rabbits and their young. Lab Anim Sci. 1980; 30: 558-561.
12. Didier ES, Didier PJ, Snowden KF, et al. Microspordiosis in mammals. Microbes and Infection. 2000; 2: 709-712.
13. Illanes OG, Tiffani-Castiglioni E, Edwards JF, et al. Spontaneous encephalitozoonosis in an experimental group of guinea pigs. J Vet Diagn Invest. 1993; 5: 649-651.
14. Boot R Van Knapen F, Kruijt BC. Serological evidence for *Encephalitozoon cuniculi* infection (nosemiasis) in gnotobiotic guinea pigs. Lab Anim. 1988; 22: 337-342.
15. Majeeb SK, Zubaidy AJ. Histopathological lesions associated with *Encephalitozoon cuniculi* (nosematosis) infection in a colony of Wistar rats. Lab Animal. 1982; 16: 244-247.
16. Muller-Doblies, Herzog K, Tanner I, et al. First Isolation and characterization of *Encephalitozoon cuniculi* from a free-ranging rat (*Rattus norvegicus*). Vet Parasitol. 2002; 22; 107(4): 279-285.
17. Didier ES, Maddry JA, Brindley PJ, et al. Therapeutic strategies for human microsporidia infections. Exper Rev Antiinfect Ther. 2005; 3: 419-434.
18. Deeb BJ, Carpenter JW. Neurologic and musculoskeletal disease. In: Quesenberry KE, Carpenter JW, eds. Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery, 2nd ed. Philadelphia: WB Saunders, 2004: 194-210.
19. Harcourt-Brown, F M & Holloway, HK R (2003). *Encephalitozoon cuniculi* in pet rabbits. Veterinary Record. 152; 427–431.
20. Harcourt-Brown F. Neurological and locomotor diseases. In: Harcourt-Brown F ed. Textbook of Rabbit Medicine. Oxford. Butterworth-Heinemann, 2002: 307-323.
21. Oglesbee B. Encephalitozoonosis. In. Oglesbee B ed. Blackwell's Five-Minute Veterinary Consult: Small Mammal, 2nd Edition, Wiley-Blackwell, 2011: 400-402.
22. Csokai J, Joachim A, Gruber A, Tichy A, Pakozdy A, Kunzel F. Diagnostic markers for encephalitozoonosis in pet rabbits. Vet Parasitol. 2009; 163(1-2): 18-26.
23. Künzel F, Joachim A. Encephalitozoonosis in rabbits. Parasitol Res. 2010; 106:299-309.
24. Keeble E. Encephalitozoonosis in rabbits –what we do and don't know. In Pract. 2011; 33:426-435.
25. Vergneau-Grosset C, Larrat S. Microsporidosis in vertebrate companion animals. J Fungi. 2016; 2(1), 3.
26. Cray C, Arcia G, Schneider R, et al. Evaluation of the usefulness of an ELISA and protein electrophoresis in the diagnosis of *Encephalitozoon cuniculi* infection in rabbits. Am J Vet Res. 2009; 70-478-482.
27. Jeklova E, Jekl V, Kovarcik K, et al. Usefulness of detection of specific IgM and IgG antibodies for the diagnosis of clinical encephalitozoonosis in pet rabbits. Vet Para. 2010; 170: 143-148.
28. Hein J, Flock U, Sauter-Louis C, Hartmann K. *Encephalitozoon cuniculi* in rabbits in Germany: prevalence and sensitivity of antibody testing. Vet Rec. 2014; 174:350.
29. Cray C, McKenny S, Perritt E, Arheart KL. Utility of IgM titers with IgG and CRP quantitation in the diagnosis of suspected *Encephalitozoon cuniculi* infection in rabbits. J Exot Pet Med. 2017; 24: 356-360.
30. Cray C, Rodriguez M, Fernandez Y. Acute phase protein levels in rabbits with suspected *Encephalitozoon cuniculi* infection. J Exot Pet Med. 2013; 22: 280-286.
31. Künzel F, Peschke R, Tichy A, Joachim A. Comparison of an indirect fluorescent antibody test with Western blot for the detection of serum antibodies against *Encephalitozoon cuniculi* in cats. Parasitol Res. 2014; 113(12): 4457–62.
32. Desoubeaux G, Pantin A, Peschke R, Joachim A, Cray C. Application of Western blot analysis for the diagnosis of *Encephalitozoon cuniculi* infection in rabbits: example of a quantitative approach. Parasitol Res. 2017 ; 116(2): 743–50.

33. Kumar Y, Liang C, Bo Z, Rajapakse JC, Ooi EE, Tannenbaum SR. Serum proteome and cytokine analysis in a longitudinal cohort of adults with primary dengue infection reveals predictive markers of DHF. PLoS Negl Trop Dis. 2012; 6(11): e1887.
34. Bourassa S, Fournier F, Nehmé B, Kelly I, Tremblay A, Lemelin V, et al. Evaluation of iTRAQ and SWATH-MS for the quantification of proteins associated with insulin resistance in human duodenal biopsy samples. PloS One. 2015; 10(5): e0125934.
35N. Jeklova E, Lena L, Matiasovic J, et al. Characterization of humoral and cell-mediated immunity in rabbits orally infected with *Encephalitozoon cuniculi*. Vet Res. 2020; 51:79.
36N. Davila-Martinez C, Castillo-Velazquez U, Soto-Dominguez A, et al. Immunohistochemical localization of TNF-α and IL-4 in granulomas of immunocompetent and immunosuppressed New Zealand white rabbits infected with *Encephalitozoon cuniculi*. Cytokine. 2020; 130:155055.
37N. Dalboni L, Saraiva A, Konno F, et al. *Encephalizoon cuniculi* takes advantage of efferocytosis to evade the immune response. PLoS ONE. 2021; 16(3):e0247658.
38N. Han Y, Gao H, Xu J, et al. Innate and adaptive immune responses against microsporidia infection in mammals. Front Microbiol. 2020; 11:fmicb.2020.01468.
36. De Matos,R, Ruby J, Van Hatten RA Computed tomographic features of clinical and subclinical middle ear disease in domestic rabbits (*Oryctolagus cuniculus*): 88 cases (2007-2014). JAVMA. 2015; 246(3): 336-343.
37. Richardson J, Longo M, Liuti T, Eatwell, K Computed tomographic grading of middle and external ear disease in conscious domestic rabbits (*Oryctolagus cuniculi*), Vet Rec 2019; 184: 679.
38. Veraa S, Schoemaker N. CT and MRI scanning and interpretation. In: BSAVA Manual of Rabbit Surgery, Dentistry and Imaging, Eds. F. Harcourt-Brown and J.Chitty, Gloucester:BSAVA, 2013:107-114.
39. Müllhaupt D, Augsburger H, Schwarz A, et al. Magnetic resonance imaging anatomy of the rabbit brain at 3 T. Acta Vet Scand. 2015; 57(1): 47.
40. Latney L, Bradley CW, Wyre R. *Encephalitozoon cuniculi* in pet rabbits: diagnosis and optimal management. Vet Med: Resch Rep. 2014; 5: 169-180.
41. Ditrich O, Chrdle A, Sak B, et al. *Encephalitozoon cuniculi* genotype I as a causative agent of brain abscess in an immunocompetent patient. J Clin Micro. 2011; 49(7): 2769–27671.
42. Fisher P, Carpenter J. Neurologic and musculoskeletal diseases. In: Quesenberry KE, Carpenter JW, eds. Ferrets, Rabbits, and Rodents: Clinical Medicine and Surgery, 3rd ed. Philadelphia: WB Saunders, 2012: 245-256.
43. Harcourt-Brown, F. Radiographic signs of renal disease in rabbits. Vet Rec. 2007; 160: 787-794.
44. Porzio P, Pharr JW, Allen AL. Excretory urography by intraosseous injection of contrast media in a rabbit model. Vet Radiol Ultrasound. 2011; 2(3): 238-43.
45. Delk KW, Carpenter JW, KuKanich B, Nietfeld C, Kohles M. Pharmacokinetics of meloxicam administered orally to rabbits (*Oryctolagus cuniculus*) for 29 days. Am J Vet Res. 2014; 75(2): 195-9.
46. Jeklova E, Leva L, Jaglic Z, Faldyna M. Dexamethasone-induced immunosuppression: a rabbit model. Vet Immunol Immunopathol. 2008; 122(3-4): 231-240.
47. Suter C, Müller-Doblies UU, Hatt J-M, Deplazes P. Prevention and treatment of *Encephalitozoon cuniculi* infection in rabbits with fenbendazole. Vet Rec. 2001; 148(15): 478-80.
48. Graham JE, Garner MM, Reavill DR. Benzimidazole toxicosis in rabbits: 13 cases (2003 to 2011). J Exotic Pet Med. 2014; 23(2): 188-195.
49. Ozawa SM, Hawkins MG, Drazenovich TL, Kass PH, Kynch HK. Pharmacokinetics of maropitant citrate in New Zealand White rabbits (*Oryctolagus cuniculus*). Am J Vet Res. 2019; 80(10): 963-968.
50. McGee W, Kendall L, Boscan P, Sadar M. Effect of maropitant, a neurokinin-1 antagonist, on post-operative pain and appetite in rabbits (*Oryctolagus cuniculus*). ICARE Conference Proceedings (London). 2019;350.
51. Eshar D, Wyre NR, Schoster JV. Use of collagen shields for treatment of chronic bilateral corneal ulcers in a pet rabbit. J Small Anim Pract. 2011; 52(7):380-3.

52. Didier ES, Stovall ME, Green LC, Brindley PJ, Sestak K, Didier PJ. Epidemiology of microsporidosis: sources and modes of transmission. Vet Parasitol. 2004; 126-145-166.
53. Cox JC, Hamilton RC, Attwood HD. An investigation of the route and progression of *Encephalitozoon cuniculi* infection in adult rabbits. J Protozool. 1979; 26:260-265.
54. Jeklova E, Leva L, Kummer V, Jekl V, Faldyna M. Immunohistochemical detection of *Encephalitozoon cuniculi* in ocular structures of immunocompetent rabbits. Animals 2019; 9(11)1-7.
55. Leipig M, Matiasek K, Rinder H, Janik D, Emrich D, Baiker K, Hermanns W. Value of histopathology, immunohistochemistry, and real-time polymerase chain reaction in the confirmatory diagnosis of *Encephalitozoon cuniculi* infection in rabbits. J Vet Diagn Invest. 2013; 25(1)16-26.
56. Percy DH, Barthold SW. Rabbit. In: Percy DH, Barthold SW, eds. Pathology of Laboratory Rodents and Rabbits. Third edition, Hoboken NJ: Blackwell Publishing; 2008; pp.290-294.

Figure 1. “Deep canyon” configuration for secure support.