



## **ENCEPHALITOOZON CUNICULI INFECTION IN DOMESTIC RABBITS**

ELISABETTA MANCINELLI, DVM, MRCVS, CERTZOOMED

ECZM RESIDENT IN SMALL COMPANION EXOTIC MAMMAL MEDICINE AND SURGERY

Great Western Exotic Vets

Unit 10 Berkshire House, County Park,

Shrivenham Road, Swindon, SN1 2NR

[www.gwexotics.com](http://www.gwexotics.com)

Encephalozoonosis is a very common infection in domestic rabbits. This parasite is widely distributed amongst different mammalian species such as rodents, foxes, non-human primates, dogs, cats, pigs, cows, horses and exotic carnivores [3]. The infection has also been reported in birds [18] but it is primarily seen in rabbits. Farm and laboratory rabbits have been known for a long time as target species but according to a recent survey [15] healthy domestic rabbits also show specific antibodies against this parasite in their blood with a prevalence of 52%. Its' importance is also due to the increasing reports of human microsporidial infections over the past years. At present transfer of infection has been reported to happen from humans to rabbits. Although infections in humans have been described to be caused by the same strain isolated in rabbits, a direct zoonotic connection has not been found yet and it has been postulated that human infections are mainly of environmental origin via contaminated water sources or other infected humans [14]. Several species of *Encephalitozoon*, including *E.cuniculi*, can be serious opportunistic pathogens in immunocompromised individuals such as those affected by HIV, on immunosuppressive medications or undergoing organ transplant. The contact between pet owners and susceptible animal species could therefore increase the risk of exposure in humans [14]. However at present it is still not clear which animal species play a major role as reservoir of infection. Rabbits are known to be common carriers but a study recently identified microsporidial DNA in a large proportion of droppings from pigeons in the Netherlands. *E.cuniculi* was the third most common microsporidial species identified [1]. Whether this was a result of a true infection is still not clear but considering the close contact, in urban situations, between humans and pigeons, it is highly likely that inhalation of spores might occur leading to an increased risk of human exposure.

*E.cuniculi* is a protozoal parasite of mammals belonging to the phylum *Microspora*, genus *Microsporidia*, which comprises over 1,200 species of ubiquitous spore-forming obligate intracellular parasites. Three different *E.cuniculi* strains have been identified so far [6]. The only rabbit strain is Strain I whereas Strain II and III have been isolated from rodents, dogs and humans respectively. Despite being eukaryotic organisms Microsporidia lack many typical organelles such as mitochondria and Golgi apparatus [10].

The spore is the infective form of the parasite, resistant to environmental changes and able to survive up to four weeks at 22°C in dry conditions [14]. The spores are shed into the urine of infected rabbits and infection usually occurs via ingestion of urine contaminated food and water. Tracheal and transplacental routes of infection have also been reported as possible, but appear much less frequent [10].

The spores have a particular organelle called polar tubule which is extruded during the infection phase to transfer the sporoplasm from the spore into the host cell. Inside the host cell spores multiply and mature, eventually causing rupture of the cell and release of the spores which can then infect other cells. The reticuloendothelial cells are frequently infected and responsible for the distribution of the parasite throughout the body [11].

Target organs are primarily

- the central nervous system,
- the kidneys and the eyes
- but the liver and heart may also be involved.

In these organs the parasite's damage can cause chronic inflammation and granulomas [10]. When the infection overwhelms the rabbit's immune system, clinical signs eventually manifest. Many factors are involved in the development of overt clinical disease: the immune status of the rabbit, the route of infection and the strain of parasite involved. These contribute to the fact that not necessarily all the infected rabbits will show clinical signs of disease: carriers and asymptomatic infections are extremely common [14]. If, during its life, for any reason, the infected rabbit becomes immunosuppressed than symptoms associated with granulomatous encephalitis and nephritis may eventually manifest.

According to a recent study neurological signs are the most common clinical presentation of encephalitozoonosis in pet rabbits [16] and the most frequent neurological signs are associated with vestibular disease, as reported by others previously [11].

- head tilt
- ataxia
- circling and rolling
- nodding or swaying at rest
- nystagmus

are frequent findings indicative of vestibular pathology.

Other neurological signs such as

- paresis or paralysis of one or both hind legs
- seizures
- behavioural changes

are also commonly encountered in practice [16]. The degree of torticollis is considered an important prognostic factor in rabbits showing clinical signs of vestibular disease. In many cases, unfortunately, it is very difficult to distinguish between peripheral and central

vestibular disease only on the basis of a neurological examination. In a few cases vertical or positional nystagmus, cranial nerve deficits, depressed mentation, evidence of cerebellar or postural damage are also present, aiding in the interpretation of the neurological findings. A full neurological examination should be performed in all these cases, including evaluation of cranial nerve function, postural reaction, spinal reflexes and sensory assessment. A thorough examination can be difficult to perform in rabbits and careful interpretation of results is mandatory because rabbits are prey species, they tend to react differently from dogs and cats in a stressful situation, for example by freezing [14]. The main differential diagnosis, when a rabbit is presented with neurological signs such as head tilt, include: meningoencephalitis, primarily caused by *Pasteurella multocida*; less commonly *Toxoplasma gondii* infection and rarely by listeriosis. Viral infections (such as *Herpes simplex 1*), vascular pathology, degenerative disease and neoplasia have also been reported [16] but are less common. In the USA, Baylisascaris species are the well known cause of cerebrospinal nematodiasis which can cause neurological signs similar to *E. cuniculi*. Otitis media/interna, mainly caused by *P. multocida*, remains the main differential for the presence of vestibular disease in domestic rabbits [10]. Spinal trauma resulting in vertebral dislocations or fracture, intervertebral disc disease, inherited congenital abnormalities (e.g. splay leg), spondylosis, osteoarthritis and toxins such as lead, also need to be considered in the list of possible causes of neurological syndrome in rabbits [14].

Rabbits suffering from chronic renal failure often present with non specific clinical signs such as lethargy, weight loss, reduced appetite leading to anorexia. Polyuria, polydipsia and urinary incontinence may or may not be present [11; 16] and urine scalding of the perineal skin is also commonly seen. Contrasting literature results report a similar incidence of *E. cuniculi*, with positive results in 10% of rabbits with kidney failure [16; 11] in comparison to a previous study which reported it in 31% of seropositive rabbits [7]. A high percentage of cases also show ocular lesions such as cataracts, hypopyon, phacoclastic uveitis or even blindness. These lesions are reported to be typically monolateral with occasional bilateral involvement described [11; 16] and generally seen in younger rabbits compared to those presented for renal failure [9]. Eye disease may be initially treated with local and systemic formulations. Phacoemulsification for lens removal might be indicated in some cases but when the disease is advanced and prognosis is poor then enucleation might be the best treatment option.

The impact that the different clinical presentations of *E. cuniculi* infection can have on the quality of life of serologically positive rabbits varies depending whether neurological, renal or ocular disorders are present, alone or in combination. Generally vestibular neurological disorders seem to affect less the general condition of seropositive rabbits when compared to those affected by renal disease and non-vestibular neurological syndrome which normally present with secondary problems such as reduced appetite. Furthermore it was observed that renal failure was a reason for euthanasia in the majority of cases compared to a survival rate of 50% of rabbits showing neurological signs [11; 16].

These findings underlie the difficulty in reaching an appropriate diagnosis of active encephalitozoonosis in the live rabbit and outline the importance of considering other causes of disease as responsible for the clinical signs observed. A correct and prompt diagnosis in vivo would be important for specific therapy, accurate prognosis and allow consideration of possible zoonotic risks. Unfortunately in the majority of cases a definitive diagnosis can be reached only post mortem because histopathology is required to conclusively identify the parasite or its spores.

In the UK an ELISA antibody assay is available for measurement of serum antibody level (IgG) to *E.cuniculi* [2] and it is now considered the standard method to support the in vivo diagnosis. When a rabbit is first infected, antibodies start to rise after 3-4 weeks and at least 4 weeks before histopathological changes are visible in the kidney or the parasite is excreted in the urine. Histopathological changes in the brain are generally seen much later, usually over 8 weeks after antibodies are detectable. Antibodies are passively transmitted from an infected dam to her offspring which can show antibodies until they are 4 weeks old. After maternal antibodies wane they become susceptible to natural infection and, if infected then, after an initial seronegative period seroconversion occurs at 8-10 weeks [11]. The detection of antibodies in the blood can be a useful tool in the diagnosis of encephalitozoonosis but it cannot distinguish between an active, early, reactivated or chronic infection. IgG are therefore merely considered indication of exposure. When the test is negative though, in a rabbit showing clinical signs of disease, we can definitively rule out *E. cuniculi* as the cause of the disease. By performing a second test on a blood sample collected at least 4 weeks later from the previous one we can try to identify an active infection by demonstrating a rising antibody titre. It is important to remember that rabbits may show a considerable individual variation in their immune response with some rabbits showing persistently high antibody levels for years even in absence of clinical signs and others becoming seronegative soon after initial infection. Simultaneous testing of IgM, which is more indicative of a current active infection, in combination with IgG testing could therefore give a better indication of the infective status of the affected rabbit [12]. Unfortunately IgM testing is not yet available in UK.

Polymerase chain reaction (PCR) has been long used in human medicine to detect even small amount of *E.cuniculi* DNA in patient's urine and sputum [5]. PCR is now available for detection of *Encephalitozoon* DNA in rabbit's urine and cerebrospinal fluid. However it can be negative in clinically ill patients and it is not necessarily correlated with the severity of the disease [4] therefore at present PCR is not routinely performed. It also has to be considered that spores are shed in the urine of infected rabbits from 3-5 weeks post seroconversion and only sporadically so their detection appears to be not always possible. Considering that spores may be present in urine of symptomatic and asymptomatic rabbits, detection of spores in urine cannot be used as a valid method for diagnosis of clinically manifest encephalitozoonosis. In the same study [4] PCR methods of detections were found to be more sensitive when performed on samples from eyes with phacoclastic uveitis possibly due to the higher spore concentration in lens material.

CSF examination could provide further information, either via cytological examination or PCR. CSF sampling is considered technically feasible, although with certain risks, in

domestic rabbits and increased concentration of protein and lymphomonocytic pleocytosis have been shown to occur in rabbits with neurological disorders caused by *E.cuniculi* infection. The analysis of CSF could support a clinical diagnosis of encephalitozoonosis but any other viral, immune-mediated or protozoan encephalitis and CNS lymphoma may induce the same cytological changes [12]. Urinary protein: creatinine ratio cannot be used as a diagnostic test in the diagnosis of this disease as it has been found not to vary between *E.cuniculi* positive and negative rabbits [19].

On post mortem examination visible changes may be evident in target organs, especially the kidney and brain. Histopathology remains the most common method for post mortem identification of granulomatous meningoencephalitis and chronic interstitial nephritis caused by *E.cuniculi* infection. Lesions in the kidney and brain are usually found about four and eight week's respectively after initial seroconversion [4]. Availability of more sophisticated laparoscopic equipment could allow biopsy collection via endoscopy of tissue samples to be submitted for histopathological examination. This will permit a more accurate diagnosis in vivo and a more specific treatment.

Therapy aims at reducing inflammation and blocking spore formation. Anti-inflammatory drugs such as steroids can be given in the acute phase of disease to suppress the granulomatous inflammation, with careful consideration of the rabbit's extreme sensibility to their side effects. Non-steroidal anti-inflammatory drugs, such as meloxicam, can also be safely used. Many in vitro studies have been carried out on the use of benzimidazole drugs in rabbits [8]. These drugs are microtubule inhibitors therefore block the extrusion of the polar filament preventing cell infection. Albendazole, used to treat infections in humans, has been reported to cause bone marrow suppression and liver failure in rabbits [17]. Fenbendazole is, at present, the drug of choice as it has been shown to be effective in reducing clinical signs in an already established infection and to prevent it in exposed animals, when administered at 20mg/kg once daily for 28 days [21], coupled with frequent environmental disinfection. Broad spectrum antibiotics can be prescribed when necessary to reduce the risk of secondary infections. Fluid therapy and assisted feeding are mandatory to correct dehydration and for appropriate intensive care. Response to treatment may vary depending on whether the infection is acute or chronic and the level of tissue damage sustained. Many rabbits, despite treatment, do not improve clinically because the changes and damage that have already taken place in many organs cannot be reversed.

More recent research has attempted reclassification of microsporidia as fungi rather than protozoa because they contain chitin and trehalose. In vitro studies have therefore been carried out to evaluate the susceptibility of the parasite to Polyoxin D and Nikkomycin which are chitin synthetase inhibitors [20]. The author is not aware of any in vivo studies carried out on rabbits at present.

Encephalitozoon cuniculi, as with other microsporidia, is widespread amongst mammals and rabbits in particular. This explains how difficult is to prevent establishment of infection. *E.cuniculi* free colonies can be created but it will be time consuming and expensive. What is recommended at present is that rabbits should be tested, isolated if positive and treated.

Seroconversion usually occurs before renal shedding so in contact rabbits could be tested to identify infected animals even before the parasite is excreted, in an outbreak. These animals should be isolated and treated as well. These might not be practically possible for pet rabbits so administering prophylactic fenbendazole to reduce the likelihood of infection, by observing good hygiene practices and performing routine disinfection to reduce urinary contamination might be of extreme importance.

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