Diagnosing *Encephalitozoon cuniculi* infection in rabbits

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*Encephalitozoonosis* is a significant disease of domestic rabbits, with its seroprevalence internationally recognised. *Encephalitozoon cuniculi* is widely distributed among different mammalian species, such as rodents, foxes, non-human primates, dogs, cats, pigs, cows, horses and exotic carnivores (Canning and Lom, 1986). Infection has also been reported in birds (Poonacha et al, 1985), although it is primarily seen in rabbits.

Farm and laboratory rabbits have been known as target species for a long time, but according to a UK survey (Keeble and Shaw, 2006), healthy domestic rabbits also show specific antibodies against this parasite in their blood, with a prevalence of 52%.

![Figure 1](image)

Figure 1. A domestic rabbit with severe head tilt secondary to *Encephalitozoon cuniculi* infection.

Its importance is also due to increasing reports of human microsporidial infections. Although human infections have been described to be caused by the same strain isolated in rabbits, a direct zoonotic connection has yet to be found and it has been postulated human infections are mainly of
environmental origin via contaminated water sources or other infected humans or animals (Keeble, 2014).

Several species of *Encephalitozoon*, including *E. cuniculi*, can be serious opportunistic pathogens in immunocompromised individuals, such as those affected by human immunodeficiency virus, undergoing organ transplant or on immunosuppressive medications. The contact between pet owners and susceptible pet species could, therefore, increase the risk of exposure in humans (Keeble, 2014).

However, it is still not clear which animal species play a major role as reservoirs of infection. Rabbits are known to be common carriers, but a study identified microsporidial DNA in a large proportion of droppings from pigeons in the Netherlands. *E. cuniculi* was the third most common microsporidial species identified (Bart et al, 2008). Whether this resulted from true infection is still not clear, but considering the close contact, in urban situations, between humans and pigeons, it is highly likely inhalation of spores might occur, leading to an increased risk of human exposure.

**Life cycle and pathogenesis**

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<th>Table 1. Reasons for difficult clinical diagnosis</th>
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<td>Serology titres do not correlate with clinical signs and degree of parasitism (positive serology is evidence of infection, but not predictive or indicative of clinical signs)</td>
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*(Latney et al, 2014)*

**Table 1.** Reasons for difficult clinical diagnosis.
Encephalitozoon cuniculi is considered a protozoan parasite belonging to the phylum Microsporidia, which comprises more than 1,200 species of ubiquitous, spore-forming, obligate intracellular parasites that infect almost all animal phyla (Didier and Weiss, 2006; Keeling and Fast, 2002). Three different E cuniculi strains have been identified (Didier et al, 1995), but research has pointed out how microsporidia retain fungal elements and are, therefore, considered ancestral relatives of zygomycetes (Keeling et al, 2000; Bohne et al, 2011).

The spore is the infective form, resistant to environmental changes and able to survive up to four weeks at 22°C in dry conditions (Keeble, 2014). E cuniculi has a direct life cycle, with both horizontal and vertical (transplacental) transmission. In rabbits, the common routes of natural horizontal infection are via the small intestine (the spores are shed into the urine of infected rabbits and infection usually occurs via ingestion of urine contaminated food and water) and respiratory tract (inhalation of spores). Experimental routes of transmission also include traumatic transmucosal, intravenous, intrathecal and rectal infection (Latney et al, 2014). Tracheal and transplacental routes of infection have also been reported, but appear rare (Harcourt-Brown, 2002).

Target organs are primarily the CNS, kidneys and eyes, but the liver, lungs and myocardium may also be involved. In these organs the parasite’s damage can cause chronic inflammation and granulomas. When the infection overwhelms the rabbit’s immune system, clinical signs eventually manifest (Harcourt-Brown and Holloway, 2003; Varga, 2014). Symptoms and clinical signs associated with CNS disease, renal and ocular disorders have been reported in pet rabbits (Keeble, 2014; Varga, 2014; Latney et al, 2014; Figures 1 and 2).

Diagnosis

Figure 2. Ocular disease may be seen in rabbits infected with Encephalitozoon cuniculi.

The life cycle of E cuniculi and pathogenesis of associated infection allow E cuniculi to escape a
serological titre correlation to clinical disease severity, evade humoral-mediated immunity in its rabbit host, remain impervious to therapeutic attempts to reduce sporont-mediated inflammation and escape therapeutic attempts to eliminate germination in chronically infected patients (Latney et al, 2014; Table 1). These are the reasons why an accurate in-vivo diagnosis of encephalitozoonosis in pet rabbits is often challenging and frustrating.

Moreover, although extensive literature review reveals the lesions caused by E cuniculi are commonly found histologically in the CNS, these lesions are not consistent and don’t correlate with the reported clinical signs (Latney et al, 2014). However, because this parasite has been recognised as potentially zoonotic, it would be extremely important to recognise its true prevalence and make an appropriate in-vivo diagnosis in pet rabbits so possible risks to their owners could be identified. It cannot be overstated that an appropriate diagnosis also implies ruling out differential causes of disease. Several studies have evaluated different tests with variable results.

**Serology**

When a rabbit is first infected, antibodies start to rise after three to four weeks and at least four 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 more than eight weeks after antibodies are detectable (Cox and Gallichio, 1978). Antibodies are also passively transmitted from an infected dam to its offspring, which can show antibodies until approximately four weeks.

After maternal antibodies wane, they become susceptible to natural infection. If infected at this point, after an initial seronegative period, seroconversion occurs at eight to 10 weeks (Harcourt-Brown and Holloway, 2003).
Table 2. Interpretation of serology titres

<table>
<thead>
<tr>
<th>Titer result</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>Single negative IgG titre in a healthy animal</td>
<td>No exposure, recent exposure, very young animal, immunosuppressed animal</td>
<td>Retest in three weeks to confirm non-exposure</td>
</tr>
<tr>
<td>Single positive IgG titre in a healthy animal</td>
<td>Early infection (within three weeks post-exposure), chronic infection and/or previous infection and recovery from <em>E cuniculi</em>, or reinfection</td>
<td>Consider testing IgM</td>
</tr>
<tr>
<td>Both IgM and IgG elevated in animal with or without clinical signs</td>
<td>Clinically healthy rabbits (subclinical acute infection), acute <em>E cuniculi</em> infection, rabbits with clinical signs of disease not associated with <em>E cuniculi</em> infection</td>
<td>Treat with fenbendazole and retest after four weeks and if still elevated then repeat treatment</td>
</tr>
<tr>
<td>IgM elevated IgG negative</td>
<td>Acute infection (fewer than 30 days post-infection)</td>
<td>Start treatment, but retest in four weeks when IgG should be elevated</td>
</tr>
<tr>
<td>IgG elevated IgM negative</td>
<td>Chronic infection, exposure</td>
<td>If no clinical signs present then monitor the titres in case IgM becomes elevated, indicating acute re-infection or reactivation. If clinical signs present, start treatment</td>
</tr>
<tr>
<td>IgG and IgM negative in an animal with clinical signs</td>
<td>Rules out <em>E cuniculi</em> as cause of disease</td>
<td>Revise diagnosis</td>
</tr>
</tbody>
</table>

(Modified from Keeble, 2011 and 2014; Latney et al, 2014)

Table 2. Interpretation of serology titres.

The detection of circulating antibodies by using ELISA, indirect fluorescent antibody technique and carbon immunoassay methods can be a useful tool in the diagnosis of encephalitozoonosis (Csokai et al, 2009a and b; Table 2 helps with interpretation of serology titres). However, the measurement of serum IgG to *E cuniculi* cannot distinguish between active, early, reactivated or chronic infection (Boot et al, 2000; Csokai et al, 2009b). IgG are therefore merely considered indication of exposure or infection status.

It is also important to remember 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 (Keeble, 2011). Differing responses may also be influenced by *E cuniculi* exposure load (Latney et al, 2014).
Simultaneous screening of IgM, which is more indicative of active infection (IgM titres will be elevated from day 0 to 35 post-exposure and can persist for up to 18 weeks in rabbits), in combination with IgG, gives a better indication of the infective status of the affected rabbit (Jeklova et al, 2010a and b).

Positive serology is strong evidence of infection, but conventional titres are not necessarily correlated with the degree of parasitism, nor are they predictive or indicative of clinical signs of disease (Latney et al, 2014). Second, seroconversion does not result in a protective response or immunity for the patient (Latney et al, 2014). In fact, all immune competent animals exposed to *E cuniculi* develop antibodies that may persist for the life of the host; however, they do not offer protection against reinfection (Didier et al, 2000).

If the humoral immune response alone is not protective, the cell-mediated immunity is instead considered essential to eliminate the parasite. In humans, rabbits, non-human primate and rodent studies of microsporidial infections, it has been shown humoral-mediated responses to *E cuniculi* do not provide immunity (Valencakova and Halanova, 2012). *E cuniculi* is an intracellular parasite and T-cell mediated cytolytic processes that aim to eliminate infected cells from the host, appear to be most important in conferring resistance (Valencakova and Halanova, 2012).

Therefore, the humoral response of the production of antibodies (from B-cells) alone is not sufficient to prevent re-infection; it only indicates the rabbit has a persistent *E cuniculi* infection. Thus, a rabbit can be infected with *E cuniculi* more than once in its lifetime. This point is very important to remember when trying to determine whether an animal has been exposed to E cuniculi by assessing a patient’s serological reactivity against E cuniculi.

**PCR**

**Figure 3.** Multiple irregular, dark red, depressed, subcapsular foci on the surface of the renal cortices are often visible on postmortem examination and are considered indicative of
encephalitozoonosis.

PCR has long been used in human medicine to detect even small amounts of *E cuniculi* DNA in patients’ urine and sputum (De Groote et al, 1995). PCR and real-time PCR can be used for detection of Encephalitozoon DNA in a rabbit’s urine and cerebrospinal fluid (Zietek et al, 2014; Jass et al, 2008). However, Cox et al (1979) demonstrated urine spore excretion progressively declines and ceases in acutely infected rabbits at 98 days post-infection.

Urine excretion and elimination rates will need to be studied in chronically infected rabbits to ascertain the true value of PCR urine spore detection. Spores are shed in the urine of infected rabbits from three to five weeks post-seroconversion and only sporadically, so their detection appears to not always be possible and not necessarily correlated with the severity of the disease (Csokai et al, 2009a).

PCR methods were found to be more sensitive and specific when performed on samples from eyes with phacoclastic uveitis, possibly due to the higher spore concentration in lens material (Csokai et al, 2009a). PCR spore detection in cerebrospinal fluid samples has demonstrated variable sensitivity (Jass et al, 2008).

At present it appears PCR is an unreliable diagnostic test for in-vivo detection of *E cuniculi* when performed on samples from body fluids and tissues, other than in the ocular contents of rabbits with phacoclastic uveitis (Csokai et al, 2009a; Kunzel and Joachim, 2010). When interpreting PCR results, it is important to note PCR spore detection will rely heavily on stage of infection (acute, latent or reinfection) and on dose-dependent spore burden within the patient. PCR spore detection in cerebrospinal fluid or urine samples in seropositive animals could therefore result in false-negative results (Kunzel et al, 2008).

**Cerebrospinal fluid analysis**

Cerebrospinal fluid (CSF) examination could provide further information, either via cytological examination or PCR. CSF sampling is considered technically feasible, though 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 (Jass et al, 2008).

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 (Jass et al, 2008).

**Urinary protein to creatinine ratio**

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 (Reusch et al, 2009).

**Electrophoresis**

Data presented by Cray et al (2009) suggested gamma globulin elevation on protein electrophoresis alongside increased IgG ELISA titres may aid in the antemortem diagnosis of clinical encephalitozoonosis.

**Acute phase proteins**

![Figure 4](image)

*Figure 4.* This CT image shows marked remodelling of the left temporomandibular joint region and left frontal bone, with marked irregular new bone formation on the intracranial surface. The rabbit was presented for head tilt, *Encephalitozoon cuniculi*-negative and confirmed to have an intracranial abscess on postmortem examination.

The measurement of acute phase proteins has been advocated as an adjunct test in the diagnosis of *E cuniculi* infection in pet rabbits (Cray et al, 2013). In *E cuniculi*-suspected rabbits, C-reactive protein (CRP) appeared severely increased. CRP is an acute phase protein known to increase within 24 hours of an inflammatory insult, so may have value as an adjunct test or aid in the differential diagnosis process, but not as a primary diagnostic test. Furthermore, *E cuniculi* suspected rabbits with ocular and renal disease did not seem to generate CRP levels comparable to those with neurological disease (Cray et al, 2013).

**Postmortem and histopathology**

On postmortem examination visible changes indicative of chronic nephritis, such as fibrosis, subcapsular pitting and adherence of the capsule to the underlying parenchyma, may indicate disease (Csokai et al, 2009b; *Figure 3*). Histopathology may reveal inflammatory lesions suggestive of *E cuniculi* infection, including non-suppurative to granulomatous
meningoencephalomyelitis and chronic interstitial nephritis on haematoxylin and eosin stain of target organs.

Lymphoplasmacytic to granulomatous infiltrates and accompanying fibrosis may be seen in other target organs, such as the liver, lung and heart. Lesions in the kidney and brain are usually found about four and eight weeks, respectively after initial seroconversion (Csokai et al, 2009b). Occasionally spores can be identified, but are best highlighted by a Gram stain. Additional special stains can confirm the presence of mature *E cuniculi* spores, including the periodic acid-Schiff stain, Ziehl-Neelsen acid-fast stain and modified trichrome stains (Latney et al, 2014).

It is important to remember the histologic severity and distribution of lesions associated with *E cuniculi* infection are not directly correlated with the severity of neurological clinical signs or the neuroanatomic localisation of antemortem neurological disease (Kunzel et al, 2008; Latney et al, 2014; Csokai et al, 2009). Immunohistochemistry and nested PCR may help obtain a more definitive diagnosis.

**Kidney biopsy**

Availability of more sophisticated laparoscopic equipment could allow antemortem biopsy collection via endoscopy of tissue samples to be submitted for histopathological examination. This will permit a more accurate diagnosis in-vivo (from nine weeks following infection) and a targeted treatment (Keeble, 2011).

**Ancillary diagnostic tests**

Haematology, biochemistry and routine urinalysis may be helpful to evaluate the general health status of a patient and will certainly be useful to identify and help managing concurrent diseases (for example, renal disease) that may be induced/exacerbated by *E cuniculi*. Full neurological and ophthalmoscopic examinations should always be carried out as they will help the ruling out of other causes of disease. Further diagnostic modalities (including radiography, CT and MRI) may also be helpful to exclude or confirm other differential diagnosis (*Figure 4 and 5*).

**Conclusions**
Clinicians face a diagnostic challenge when trying to correlate serological titres and severity of clinical disease, detect spores in urine of infected animals and confirm neurological disease as definitively due to *E cuniculi* infection in a domestic rabbit with compatible clinical signs. Unfortunately, no single test can provide a definitive, clear diagnosis.

There are four major difficulties the clinician faces.

- Conventional titres are not correlated with the severity of clinical signs.
- Seroconversion does not result in a protective response or immunity for the patient.
- The histologic severity and distribution of lesions associated with *E cuniculi* infection are not directly correlated with the severity of neurological clinical signs or the neuroanatomical localisation of antemortem neurological disease.
- Only a handful of studies are available, therefore it is difficult to draw clear treatment recommendations. Readers should refer to the references for in-depth information.

References


