# *Encephalitozoon cuniculi* in cats

## Overview / Abstract

*Encephalitozoon (E.) cuniculi* is a common obligate intracellular intracellular microsporidian parasite of rabbits (*Oryctolagus cuniculus*), that is increasingly recognised as a pathogen of cats and other mammalian species.

These guidelines aim to review the literature on feline *E cuniculi* infection and provide recommendations on prevention and management.

**Infection in cats:** *E cuniculi* infection should be considered as a differential diagnosis in cases of feline uveitis and cataract formation. It is not significantly associated with either chronic kidney disease or meningoencephalitis. *E cuniculi* infection is more common in stray or feral cats than in pet cats.

**Diagnosis and treatment:** Serological tests for antibody detection in the blood are easy to perform and can be useful for diagnosis, but their specificity is low as antibodies have been found in apparently healthy cats. PCR appears to be more sensitive than histopathology for diagnosis, and is more sensitive when performed on cataractous lenses compared with aqueous humour, although ease of sampling is an obvious limitation. Treatment is with fenbendazole for 3 weeks and phacoemulsification to remove microsporidia from cataractous lenses.

**Zoonotic risk:** *E cuniculi* is a potential zoonotic agent, and there is a particular risk to immunocompromised humans posed by infected rabbits. Albeit infrequent, spore shedding has been identified in cats, so care should be taken around infected cats.

## Introduction

*Encephalitozoon cuniculi* is a common obligate intracellular micro-sporidian parasite of rabbits, which is increasingly recognised as a pathogen of cats and other mammals. These unicellular microsporidia were previously considered ‘primitive’ protozoa; however, more recent insight gained through molecular phylogenetic analysis is indicating that these organisms are not primitive but instead degenerate, and that microsporidia are related to the fungal Kingdom, either as a basal branch of the Fungi or as a sister group (Han and Weiss, 2017). Four species have been identified by PCR and sequencing: strain I is the rabbit strain; strain II: is the mouse strain, strain III is from the dog (Didier et al, 1995) and strain IV is from the cat (Benz et al, 2011 and Benz personal communication) .

In rabbits, *E cuniculi* can infect all organs, but specifically causes chronic kidney and central nervous system disease, (Nast et al., 1996; Harcourt-Brown and Holloway, 2003; Künzel et al., 2008; Valencakova et al., 2008; Csokai et al., 2009; Csokai et al., 2009; Rodriguez-Tovar et al., 2017) as well as cataract formation (Ashton et al., 1976) with lens capsule rupture and phacoclastic uveitis. (Stiles et al., 1997; Felchle and Sigler, 2002; Giordano et al., 2005; Künzel et al., 2008; Csokai et al., 2009; Morsy et al., 2020). Infected rabbits shed spores in urine (Cox and Pye, 1980; Csokai et al., 2009; Abu-Akkada and Oda, 2016; Rodriguez-Tovar et al., 2017) and faeces (Valencakova et al., 2008).

The susceptibility of cats to *E cuniculi* infection was first reported in 1985, in an experimental infection of feline leukaemia virus-infected kittens (Pang and Shadduck, 1985).

## Epidemiology

Kvac et al. (2017) detected *E. cuniculi* spores in the faeces of one pet cat and eight strays among 255 cats sampled in central Europe, and Piekarska et al. (2017) found spores in the faeces of one of 44 Polish cats. No *E. cuniculi* spores were detected in the faeces of 40 and 26 cats in two studies in Iran, although *E. bieneusi* spores were found in the faeces of 3 /40, and 3/26 cats, respectively (Jamshidi et al., 2012; Askari et al., 2015). No *E. cuniculi* spores were found in the faeces of ten Spanish cats tested (Lores et al., 2002).

Halánová et al. (2003) found antibodies to *E. cuniculi* in 17/72 cats in eastern Slovakia using an indirect immunofluorescence antibody test (IFAT). In the same study, anti-*E. cuniculi* antibodies were found in 26/456 (5.7%) human sera samples examined. The highest occurrence of anti-microsporidial antibodies was found in a group of 24 immunocompromised patients: 37.5% (9/24) (Halánová et al., 2003).

Stray (Kvac et al., 2017) or feral (Tsukada et al., 2016) cats are more likely to be exposed to, or infected with, *Encelphalitozoon cuniculi* than pet cats.

A summary of prevalence data is shown in Table 1.

Table 1. Antibody / spores prevalence of *E. cuniculi* infection in the feline populations in various countries

Show entries
Search:

| Country/region | What detected | Number of cats | Prevalence % | Reference |
|---|---|---|---|---|
| Austria | Antibodies | 100 | 2.0 | Benz et al., 2011 |
| Central Europe | Spores | 255 | 3.5 | Kvac et al., 2017 |
| Iran | Spores<br>Spores | 40<br>26 | 0<br>0 | Jamshidi et al., 2012<br>Askari et al., 2015 |
| Japan | Antibodies | 295 | 6.1 | Tsukada et al., 2016 |
| Poland | Spores | 44 | 2.3 | Piekarska et al., 2017 |
| Slovakia (Eastern) | Antibodies | 72 | 23.6 | Halánová et al., 2003 |
| Spain | Spores | 10 | 0 | Lores et al., 2002 |
| UK | Antibodies | 27 | 0 | Meredith et al., 2015 |
| USA (Virginia) | Antibodies | 232 | 6.5 | Hsu et al., 2011 |

# Transmission

Mice appear to be the major reservoir of infection for cats (Benz et al, 2011). Cats are most likely to become infected by ingestion of mice rather than rabbits, since of 11 infected cats in Austria, seven were infected with the mouse strain (strain II), and the remaining four were infected with what is now known as the cat strain (strain IV) (Benz et al, 2011).

Cats, like humans, may also be infected by consuming water or food contaminated with infective spores (Wang et al., 2018). Oral and nasal transmission has been described in rabbits (Harcourt-Brown and Holloway, 2003) but it is unknown if direct transmission occurs in cats. Two uninfected cats that had been in direct contact with infected ones tested negative for blood antibodies in one study (Benz et al., 2011).

*In utero* infection is seen in rabbits (Baneux and Pognan, 2003), but it is unknown if transmission by this route occurs in the cat (Benz et al., 2011). Rebel-Bauder et al. (2011) reported a case of generalised encephalitozoonosis in a kitten with cerebellar hypoplasia, which could have been related to *in utero* infection. Benz et al (2011) speculate that vertical transmission is necessary for cataract formation in the cat because vertical transmission is presumed to play an important role in the mechanisms by which the microsporidium enters the lens in rabbits and mink: the presumption is that the organism would be unable to infect a lens which already had a fully formed capsule.

# Clinical signs

In cats, ocular signs have been associated with *E. cuniculi* infection (Fig. 1).

Figure 1

## Anterior uveitis and cataracts

Benz et al. (2011) reported a study of 19 eyes from 11 European shorthair cats (median age 3.5 years) in Austria. Nine of these cats had bilateral cataracts, with 12/19 eyes having focal anterior cortical cataracts and 7/19 eyes having mature cataracts. In 14/19 eyes anterior uveitis was present. All cats had antibody titres in the blood (titre 1:80–1:10,000) for *E. cuniculi* (Benz et al., 2011). *E.*

*cuniculi* DNA was detected by PCR (Csokai et al., 2010) and sequencing in 18/19 lenses and in 10/19 aqueous humour samples (Benz et al., 2011).

## Conditions not associated with *E. cuniculi* infection

There are study findings to suggest no specific link between *E. cuniculi* and the following conditions in cats:

- Chronic kidney disease (CKD): 4/36 cats with CKD tested positive for *E. cuniculi* antibodies in blood but this prevalence was not significantly different (P>0.05) from cats without CKD (Hsu et al., 2011).
- Meningoencephalitis (ME): Künzel et al. (2017) concluded that *E. cuniculi* was unlikely to be directly associated with (non-suppurative and/or granulomatous) ME in cats in Austria: none of 30 affected cats examined by immunohistochemistry was positive.

# Diagnosis

## Serology

Detection of antibodies in blood by western blot or immunofluorescent antibody test (IFAT) remains the major means of pre-mortem clinical diagnosis in animals. Since the IFAT is quick and easy to perform, it is recommended for routine use in the diagnosis of feline encephalitozoonosis (Künzel et al., 2014). However, antibodies have been detected in cats that appeared to be clinically healthy, which has to be borne in mind when interpreting positive results; a positive result supports a diagnosis of encephalitozoonosis, but is not confirmatory.

## PCR

As discussed above, *Encephalitozoon cuniculi* DNA was detected by PCR (Csokai et al., 2010) and sequencing in 18/19 lenses (liquefied lens material) and in 10/19 aqueous humour samples from 11 cats with cataracts (Benz et al., 2011).

## Histopathology and cytology

Histopathology and cytology are aided by immunohistochemistry. Five tentative positive results were achieved by cytological examination of material removed from cataractous lenses (Benz et al., 2011). Spores were detected in 15 of 19 samples of cataractous lens material with immunohistochemical staining (Benz et al., 2011).

*E. cuniculi* spores are difficult to observe when the samples are stained with haematoxylin and eosin, particularly when there is an inflammatory reaction and tissue damage. The spores are easily mistaken for other microorganisms, such as fungi (yeasts), protozoa and bacteria. Modified trichrome stain (MTS) and Gram stain, detected by light microscopy, and calcofluor white stain, detected by ultraviolet light microscopy, are the best stains for detecting spores of *E. cuniculi* in paraffin-embedded tissues. These stains were superior to Warthin–Starry, Ziehl–Neelsen, Giemsa and periodic acid–Schiff reaction for identifying spores without background ‘noise’ or monochromatic interference. In addition, these stains allow individual spores to be discerned in paraffin-embedded tissues. MTS allows observation of the polar tube, polaroplast and posterior vacuole, the most distinctive parts of the spore (Rodriguez-Tovar et al., 2017).
Leipig et al. (2013) recommended that confirmation of pathogenic *E. cuniculi* infection in rabbits should include standard histology of the predilection sites in combination with a specific aetiological assay, preferably real-time PCR. Presumably the same is true for diagnosis of *E. cuniculi* infection in cats.

# Treatment

Fenbendazole is used to treat *E. cuniculi* infection in cats at a dose of 20 mg/kg q 24h for three weeks (Benz et al., 2011). Cataracts can be successfully treated by phacoemulsification alongside medical treatment for *E. cuniculi* and symptomatic therapy for uveitis (e.g. ointment or drops containing dexamethasone), as shown by Benz et al. (2011).

# Prevention

There is no commercially available vaccine to prevent *E. cuniculi* infection in rabbits or cats. It is noteworthy, however, that an experimental vaccine containing inactivated spores was shown to induce a long-lasting antibody response in rabbits (Sobottka et al., 2001). However, it is unknown whether antibodies are protective in this infection.

Where cats and rabbits are kept together, the main method of prevention of infection is by maintenance of excellent hygiene. Heat or steam cleaning will be the most effective means of eliminating *E. cuniculi* spores. Rabbits suspected to be infected should be tested and treated.

The safest option for individuals - both cats and humans – that consume rabbit meat is for *E. cuniculi*-free sources to be used. However, the prevalence of *E. cuniculi* is extremely high in rabbits kept for meat: 100% of 13 rabbit farms in Italy contained seropositive rabbits (Lonardi et al., 2013), and active *E. cuniculi* infections were determined in 85.9% and 56.3% of rabbits in commercial and household farms, respectively, in the Czech and Slovak Republics (Neumayerová et al., 2014). Where rabbit meat is prepared for feline or human consumption, it should be well cooked. Microsporidian spores in fish were shown to be inactivated by heating to 60°C for 10 mins or by microwaving at 750 W, for 20s (Leiro et al., 2012); similar treatment is likely to be effective for rabbit meat. However, Graczyk et al. (2007) found microwaving to be ineffective against the spores of *E. bieneusi* and *E. intestinalis* in sewage sludge, and pasteurisation failed to inactivate spores in milk (Kváč et al., 2016) so more work is needed to determine appropriate conditions to inactivate *E. cuniculi* spores. Microsporidian spores in fish were also inactivated by freezing at -20°C for more than 48 hours (Leiro et al., 2012).

Any area used to prepare rabbit meat should be thoroughly cleaned then disinfected with sodium hypochlorite (household bleach), ensuring a contact time of at least 16 minutes (Wolk et al., 2000), followed by rinsing with boiling water or steam cleaning. Similar precautions are recommended for pig meat, which Sak et al. (2019) recently reported can also contain *E. cuniculi* spores. Exposure to 70% ethanol for 15 min inactivated microsporidian spores in fish (Leiro et al., 2012), and so is likely to be effective for cleaning hands and utensils following the preparation of rabbit or pig meat (although use of disposable gloves would be more practical in the case of hands).

The ABCD recommends that immunosuppressed cats should not have any contact with infected rabbits or their urine and faeces.

# Zoonotic risk

Since *E. cuniculi* is a zoonotic infection (Mathis et al., 2005), veterinary surgeons and nurses should wear gloves when dealing with infected cats or rabbits. Calcium oxide (quicklime, burnt lime) was 100% effective in inactivating microsporidian spores in landfill leachate and sewage sludge (Graczyk et al., 2007), so could be used in the disposal of infected cadavers.

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