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CLINICAL REVIEW

ENCEPHALITOZOON CUNICULI INFECTION IN CATS

European guidelines from the ABCD on prevention and management

Diane D Addie, Séverine Tasker, Corine Boucraut-Baralon, Sándor Belák, Herman Egberink, Tadeusz Frymus, Katrin Hartmann, Regina Hofmann-Lehmann, Fulvio Marsilio, Albert Lloret, Maria Grazia Pennisi, Etienne Thiry, Uwe Truyen, Margaret J Hosie and Karin Möstl

Overview: *Encephalitozoon cuniculi* is a common obligate intracellular microsporidian parasite of rabbits 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.

European Advisory Board on Cat Diseases

The European Advisory Board on Cat Diseases (ABCD) is a body of experts in immunology, vaccinology and clinical feline medicine that issues guidelines on prevention and management of feline infectious diseases in Europe, for the benefit of the health and welfare of cats. The guidelines are based on current scientific knowledge of the diseases and available vaccines concerned.

Any future update of the *Encephalitozoon cuniculi* infection in cats guidelines will be available at www.abcdcatsvets.org

Introduction

Encephalitozoon cuniculi is a common obligate intracellular microsporidian 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.[1]

In rabbits, *E cuniculi* can infect all organs, but specifically causes chronic kidney and central nervous system disease,[2–8] as well as cataract formation[9] with lens capsule rupture and phacoclastic uveitis.[3,5,10–13] Infected rabbits shed spores in urine[3,7,14,15] and faeces.[8]

The susceptibility of cats to *E cuniculi* infection was first reported in 1985, in an experimental infection of feline leukaemia virus-infected kittens.[16]

Epidemiology

Stray or feral cats are more likely to be exposed to *Encephalitozoon cuniculi* than pet cats.

Kvac et al detected *E cuniculi* spores in the faeces of one pet cat and eight strays among 255 cats sampled in central Europe,[17] and Piekarska et al found spores in the faeces of one of 44 Polish cats.[18] No *E cuniculi* spores were detected in the faeces of 40 and 26 cats in two studies in Iran, although *Enterocytozoon bieneusi* spores were found in the faeces of 3/40 and 3/26 cats, respectively.[19,20] No *E cuniculi* spores were found in the faeces of 10 Spanish cats tested.[21]

Halánová et al found antibodies to *E cuniculi* in 17/72 cats in eastern Slovakia using an indirect immunofluorescence antibody test (IFAT).[22] 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).[22]

European Advisory Board on Cat Diseases
www.abcdcatsvets.org
Corresponding author: Diane Addie
Email: draddie@catvirus.com

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Stray[17] and feral[23] cats are more likely to be exposed or infected than pet cats.

A summary of prevalence data is shown in Table 1.

Table 1 Prevalence of *Encephalitozoon cuniculi* infection in feline populations in various countries/regions

Country/region	What was detected	Number of cats	Prevalence (%)	Reference
Austria	Antibodies	100	2.0	24
Central Europe	Spores	255	3.5	17
Iran	Spores Spores	40 26	0 0	19 20
Japan	Antibodies	295	6.1	23
Poland	Spores	44	2.3	18
Slovakia (Eastern)	Antibodies	72	23.6	22
Spain	Spores	10	0	21
UK	Antibodies	27	0	25
USA (Virginia)	Antibodies	232	6.5	26

Transmission

Cats, like humans, are most likely to become infected by ingestion of water or food contaminated with infective spores.[27] Oral and nasal transmission has been described in rabbits,[4] 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.[24] In utero infection is seen in rabbits,[28] but it is unknown if transmission by this route occurs in the cat.[24] Rebel-Bauder et al reported a case of generalised encephalitozoonosis in a kitten with cerebellar hypoplasia, which could have been related to in utero infection.[29]

Clinical signs

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

Anterior uveitis and cataracts

Benz et al reported a study of 19 eyes from 11 European Shorthair cats (median age 3.5 years) in Austria.[24] 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*.[24] *E cuniculi* DNA was detected by PCR[30] and sequencing in 18/19 lenses and in 10/19 aqueous humour samples.[24]

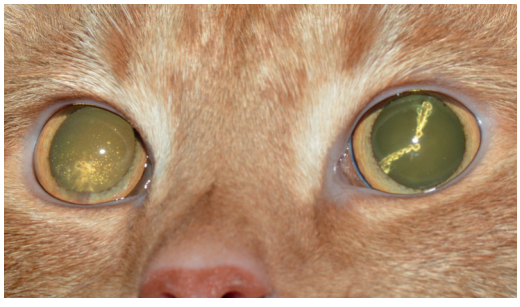

Figure 1 **Ocular signs in a cat with a confirmed *Encephalitozoon cuniculi* infection: the right eye shows signs of keratic precipitates, which is evidence of anterior uveitis; in the cat's left eye, a cataract is visible.** *Courtesy of Barbara Nell*

Conditions not associated with *E cuniculi* infection

There are study findings to suggest no specific link between *E cuniculi* infection 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.[26]
- **Meningoencephalitis (ME):** Künzel et al[31] 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 were positive.

A positive immunofluorescence antibody test result supports a diagnosis of encephalitozoonosis, but is not confirmatory.

Diagnosis

Serology

Detection of antibodies in blood by Western blot or IFAT remains the major means of premortem 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.[32] 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, *E cuniculi* DNA was detected by PCR[30] and sequencing in 18/19 lenses (liquefied lens material) and in 10/19 aqueous humour samples from 11 cats with cataracts.[24]

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.[24] Spores were detected in 15/19 samples of cataractous lens material with immunohistochemical staining.[24]

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.[7]

Leipig et al[33] 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 q24h for 3 weeks.[24] Cataracts can be successfully treated by phacoemulsification alongside medical treatment for *E cuniculi* and symptomatic therapy for uveitis (eg, ointment or drops containing dexamethasone), as reported by Benz et al.[24]

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.[34] 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,[35] 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.[36] 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 20 s;[37] similar treatment is likely to be effective for rabbit meat. However, Graczyk et al[38] found microwaving to be ineffective against the spores of *E bieneusi* and *Encephalitozoon intestinalis* in sewage sludge, and pasteurisation failed to inactivate spores in milk[39] 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 h.[37]

When rabbit meat is prepared for feline or human consumption, it should be well cooked and the food preparation area thoroughly disinfected.

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 mins,[40] followed by rinsing with boiling water or steam cleaning. Similar precautions are recommended for pig meat, which Sak et al recently reported can also contain *E cuniculi* spores.[41] Exposure to 70% ethanol for 15 mins inactivated microsporidian spores in fish,[37] 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,[42] 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,[38] so could be used in the disposal of infected cadavers.

KEY POINTS

- *E cuniculi* is a common obligate intracellular microsporidian parasite of rabbits that is increasingly recognised as a pathogen of cats and other mammals.
- Stray and feral cats are more likely to be exposed to *E cuniculi* or to become infected than pet cats.
- Cats, like humans, are most likely to become infected by ingestion of water or food contaminated with infective spores.
- Western blot or IFAT remains the main tool for clinical diagnosis, although PCR as well as histopathology and cytology have also produced diagnostic results.
- Ocular signs, such as anterior uveitis and cataracts, have been associated with *E cuniculi* infection in cats. CKD and ME are not conditions associated with feline *E cuniculi* infection, however.
- Fenbendazole is used to treat *E cuniculi* infections in cats. Cataracts can be successfully treated by phacoemulsification alongside medical treatment for *E cuniculi* and symptomatic therapy for uveitis.
- Where cats and rabbits are kept together, the principal means of preventing infection is through the maintenance of excellent hygiene. Immunosuppressed cats should not have any contact with infected rabbits or their urine and faeces.

Three Special Issues of JFMS containing ABCD guidelines on feline infectious diseases – some well recognised, others lesser known – have been published:

- Infectious diseases, Prevention and Management: Volume 11, July 2009
- Infectious diseases, Part 2: Volume 15, July 2013
- Infectious diseases, Part 3: Volume 17, July 2015

The special issues are available at: cpsi.jfms.com. In addition, the following individual guidelines articles have been published:

- *Anaplasma, Ehrlichia* and *Rickettsia* species infections in cats: Volume 19, May 2017
- Haemoplasmosis in cats: Volume 20, March 2018
- Dirofilarioses in cats: Volume 22, May 2020

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Conflict of interest

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ABCD SARS-CoV-2 guidelines

The ABCD has produced, and is regularly updating, guidelines on 'SARS-Coronavirus (CoV)-2 and cats'. These are available at: abcdcatsvets.org/sars-coronavirus-2-and-cats/

Ethical approval

This work did not involve the use of animals and therefore ethical approval was not necessarily required.

Informed consent

This work did not involve the use of animals and therefore informed consent was not required. No animals or humans are identifiable within this publication, and therefore additional informed consent for publication was not required.

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