GUIDELINE for Encephalitozoon cuniculi in cats

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Overview / Abstract

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

Objectives: to review the literature on *E. cuniculi* in cats and provide recommendations for feline clinical conditions in which *E. cuniculi* should be considered as a possible differential diagnosis.

Conclusions: *E. cuniculi* should be considered 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.

Serological tests for antibody detection in 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 more sensitive than histopathology for diagnosis, and is more sensitive when performed on cataractous lenses compared to 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.

***E. cuniculi* is a potential zoonotic infection**, posing a special risk to immunocompromised humans from infected rabbits. Spore shedding has been infrequently identified in cats, so care should be taken around infected cats.

Introduction

Encephalitozoon cuniculi is a common obligate intracellular microsporidian parasite of rabbits (*Oryctolagus cuniculus*), 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., 2009a, 2009b; 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., 2009b; Morsy et al., 2020). Infected rabbits shed spores in urine (Cox et al., 1980; Csokai et al., 2009b; 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 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 *Enterocytozoon 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) and feral (Tsukada et al., 2016) cats are more likely to be exposed or infected 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

COUNTRY/REGION	WHAT WAS 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 Spores	40 26	0 0	Jamshidi et al., 2012 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).

Fig. 1: Ocular signs in a cat with confirmed E. cuniculi infection: keratic precipitates, which is evidence of anterior uveitis, in the right eye; cataract in the left eye. © Barbara Nell, University of Veterinary Medicine Vienna, Austria

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

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 were positive.

Diagnosis

Serology

Detection of antibodies in blood by Western blot or IFAT remains the major means of pre-mortem clinical diagnosis in animals. As 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

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 q24 h 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 reported 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 min or by microwaving at 750 W, for 20 s (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 min (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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