Parasitol Res (2010) 106:299–309
DOI 10.1007/s00436-009-1679-3

REVIEW

Encephalitozoonosis in rabbits

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Received: 21 October 2009 / Accepted: 28 October 2009 / Published online: 17 November 2009

Abstract *Encephalitozoon cuniculi* is an obligatory intracellular microsporidian parasite that can infect a wide range of mammals, including rodents, rabbits, horses, carnivores and humans, in which the organism is known as an opportunistic pathogen of immunocompromised individuals. Nevertheless, the main host for *E. cuniculi* is the rabbit and infections usually have a sub-clinical course. However, severe disease is recognised in pet rabbits more frequently within the last years. As the central nervous system, the kidney and the eye are predilection organs for the organism, predominant histopathological alterations comprise granulomatous meningoencephalitis, chronical interstitial nephritis and phacoclastic uveitis. A definitive diagnosis of encephalitozoonosis in vivo is difficult, but it is important for specific treatment and the determination of possible zoonotic risks. This review article covers epidemiology, pathology, pathophysiology, immunology, clinical signs, differential diagnosis, diagnosis and treatment of encephalitozoonosis in rabbits.

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Introduction

Encephalitozoonosis was first reported in laboratory rabbits with paralysis by Wright and Craighead (1922). Since that time, many studies have been performed on *Encephalitozoon cuniculi* infections in rabbits as well as humans, where the organism is known to cause opportunistic infections in patients with HIV infections. In laboratory rabbits, encephalitozoonoses used to be a frequent problem, affecting the health status of the animals and interfering with experiments (Petri 1969; Shadduck and Pakes 1971). Therefore, as a part of regular health monitoring of rabbits that are used in research, animals are routinely tested by serological methods for antibodies to *E. cunculi* before they are used for laboratory procedures.

In veterinary practice, encephalitozoonosis is a common cause of neurological disease in pet rabbits. However, a definitive diagnosis in the living rabbit is difficult and treatment protocols for the disease are still nonuniform, since only few controlled studies have been performed to asses therapy options for the use in diseased animals (Neuschl et al. 1999; Suter et al. 2001).

This study gives an overview on studies that have been performed referring to encephalitozoonosis in rabbits with a special focus on clinical signs, diagnosis and treatment options.

General features of *E. cuniculi* and the microsporidia

Encephalitozoonosis is a common infectious disease in rabbits. Its causative agent, *E. cuniculi*, an obligately intracellular parasite, is a member of the microsporidia (Wasson and Peper 2000). Within the genus *Encephalito-*

zoon, two other species, *Encephalitozoon hellem* and *Encephalitozoon intestinalis*, are also known to infect mammals (Wasson and Peper 2000; Mathis et al. 2005).

Although microsporidia are eukaryotic organisms, they show some atypical characteristics; even though they lack mitochondria, enzymes with mitochondrial functions have been detected (Katinka et al. 2001). Their Golgi apparatus is atypical (Weber et al. 1996; Méteniér and Vivarès 2001) and the ribosomal RNA resembles that of pro- rather than eukaryotes (Müller 1998). Based on biochemical and molecular analyses, they are currently thought to be more closely related to fungi than to protozoa (Mathis 2000; Méteniér and Vivarès 2001).

Microsporidia are unusual organisms in many respects. They form unicellular spores as environmental stages which infect cells via a unique invasion apparatus, comprising a polar tube which discharges the sporoplasm into the new host cell after activation (Franzen 2004; Xu and Weiss 2005). After asexual multiplication (merogony) spores develop within the host cell (sporogony) and, in the case of *Encephalitozoon*, inside a parasitophorous vacuole which separates the parasite from the host cell plasma and mostly consists of host cell-derived lipids (Franzen 2004; Rönnebäumer et al. 2008). When the cell is destroyed, spores can be released into the environment. These spores, which are 1–10 µm depending on the species, consist of a polar tube coiled around the sporoblast which contains the nucleus, the posterior vacuole and the ribosomes, and which is surrounded by a plasma membrane. For environmental resistance, these structures are enclosed in an endo- and an exospore (Franzen 2004; Xu and Weiss 2005).

Another unique feature of the microsporidia is their extremely small genome, which only comprises 2.9 Mbp for *E. cuniculi* (Biderre et al. 1995; Katinka et al. 2001). The reduction in genome size is due to tight packing of the genetic information within the chromosomes (with virtual absence of introns, a low number of repetitive sequences and many single-copy genes), a shortening of genes as well as of non-coding sequences and a loss of genetic information regarding certain metabolic pathways that seem to have become irrelevant for the parasitic life style of the microsporidia (Mathis 2000; Katinka et al. 2001).

Epidemiology and host spectrum

The main host for *E. cuniculi* is the rabbit, and seroprevalence rates are usually high in pet rabbit populations with 37% to 68% of the population (Ewringmann and Göbel 1999; Harcourt-Brown and Holloway 2003; Ebrecht and Müller 2004). In wild rabbit populations, the parasite is less prevalent, probably due to the lower animal density (Wilson, 1979; Cox and Ross 1980; Chalupsky et al. 1990). In experimental animal breeding facilities, the parasite used to be prevalent but with good hygiene standards, and health surveys infections can be controlled (Mathis et al. 2005; Künzel et al. 2008).

E. cuniculi is capable of infecting other mammalian hosts including rodents, horses, carnivores, non-human primates and humans (reviewed by Canning and Lom 1986; Mathis et al. 2005). Three different strains (I = "rabbit", II = "mouse", III = "dog") have been determined according to the number of short repeats in the ribosomal internal transcribed spacer region (Didier et al. 1995a, b). While the geographic distribution of these strains has not been investigated systematically, rabbits have so far only been reported to be infected with the "rabbit" strain under natural conditions, although they are susceptible to the other two strains as well (Mathis 2000; Mathis et al. 2005). Confirmed human cases (only "dog" and "rabbit" strains) have been reported since 1994; earlier, species determination was not done in cases of human microsporidial infections. All cases of confirmed infections were described in immunocompromised patients, mostly HIV-positive cases (Mathis et al. 2005), ranking encephalitozoonosis among the rare opportunistic diseases.

Infected rabbits excrete the small spores of *E. cuniculi* (approximately 1.5×2.5 µm; Wasson and Peper 2000) intermittently, and the infection is transmitted horizontally by direct contact or environmental contamination (Harcourt-Brown 2004; Mathis et al. 2005). Once ingested, the parasite reaches the internal organs via the blood flow and is disseminated into the kidneys, liver, lungs and heart, with kidneys and (subsequently) brain being predilection sites (Cox et al. 1979). During intrauterine transmission *E. cuniculi* spores can also be trapped in the anterior lens capsule (Wolfer et al. 1993). Although intrauterine transmission has been demonstrated by Baneux and Pognan (2003), this route of infection only seems to play a role for the intraocular development of the parasite (Scharmann et al. 1986; Wolfer et al. 1993).

In experimental studies, intravenous, intraperitoneal, intracerebral and intrarectal infections could also be established (Cox et al. 1979; Shadduck et al. 1979; Wicher et al. 1991; Horváth et al. 1998). Spores are subsequently excreted by the urine for several weeks (Cox and Gallichio 1978; Scharmann et al. 1986).

Pathology and pathophysiology

E. cuniculi infection in rabbits usually follow a chronic course, taking weeks to months to develop a significant parasite burden which may or may not lead to clinical symptoms. First tissue alterations are known to be present in the kidney, liver and lung while the brain is not affected at the

beginning, but after about 3 months, the most significant alterations are seen in the kidneys and the brain. At that time, lesions wane in lung and liver, and parasites are usually absent form these organs. The heart can also be involved, although usually to a minor extent (Cox et al. 1979).

Cerebral tissue alterations demonstrate perivascular infiltrations ranging from discrete changes to non-suppurative, focal or multifocal granulomatous (meningo-) encephalitis (Cox and Gallichio 1978; Wicher et al. 1991; Eröksüz et al. 1999; Csokai et al. 2009a). Granulomas contain lymphocytes, plasma cells and glia cells and are often necrotic in the centre (Eröksüz et al. 1999; Harcourt-Brown 2002). The most frequently affected area is the cerebrum (cortex and medulla), but inflammatory lesions can also be found in the brain stem and the spinal cord, to a lesser extent in the cerebellum. The leptomeninges are almost always affected (Scharmann et al. 1986; Csokai et al. 2009a). Occasionally, focal spinal radiculitis is observed (Nast et al. 1996), which might be responsible for atypical neurological symptoms. In a survey on 71 naturally infected rabbits, the vestibular cores were only affected in 37.5% of the cases (Csokai et al. 2009a).

Lesions in renal tissue include focal granulomatous nephritis, primarily with mononuclear cells. Severe grades of chronic interstitial nephritis with macroscopic scar tissue formation due to fibrosis, and multifocal granulomas are frequently documented (Flatt and Jackson, 1970; Cox and Gallichio 1978; Eröksüz et al. 1999; Csokai et al. 2009a). Spores multiply in the tubular epithelium; from there, they are excreted by the urine. As infection proceeds spore detection in the tissue becomes less frequent (Flatt and Jackson, 1970; Csokai et al. 2009a). Phacoclastic uveitis, the consequence of intrauterine infection, is characterised by the infiltration of the eye lens by various inflammatory cells (granulocytes, macrophages, giant cells) leading to a rupture of the lens capsule. Iris and ciliary body are infiltrated by plasma cells and lymphocytes. Parasites are only found in the lens (Giordano et al. 2005).

Inflammations in other organs are rare during the chronic stage of infection. Their affection seems to vary with the route of experimental infection (Von Kücken et al. 1987; Wicher et al. 1991; Nast et al. 1996; Eröksüz et al. 1999) and might be due to a general inflammatory reaction rather than to a specific parasite invasion (Scharmann et al. 1986). Immunosuppression intensifies the development of lesions in experimental-infected animals (Horváth et al. 1998; Levkut et al. 1998).

Immunology

Antibodies against *E. cuniculi* are produced and persist for long-term periods due to the chronic nature of the infection. However, humoral immune responses do not appear to be protective, in contrast to cellular mechanisms which are essential for the control of the parasite and the survival of the host (Khan et al. 2001). Immunosuppressed (athymic or severe combined immunodeficiency) mice succumb to the infection (Schmidt and Shadduck 1983; Koudela et al. 1993). Especially $CD8^+$ lymphocytes (and possibly $\gamma\delta$-T lymphocytes which regulate them) are important for the host's survival while $CD4^+$ cells do not seem to play a significant role (Khan and Moretto 1999; Khan et al. 1999). As observed in other intracellular parasitic diseases in mice lack of interferon-γ or interleukin-12 is associated with a high mortality (Khan and Moretto 1999). Immunosuppressive co-infections such as bovine leukaemia virus (Levkut et al. 1998) or treatment with cytostatic drugs (Horváth et al. 1998) can exacerbate the pathology of encephalitozoonosis in rabbits.

Clinical symptoms

As lesions are caused within the central nervous system, kidney or eye, rabbits suffering from encephalitozoonosis may demonstrate neurological symptoms, signs of kidney failure or phacoclastic uveitis. The three forms of this disease can occur individually or in combination. Neurological signs are clearly dominating in clinical manifest *E. cuniculi* infections. In many cases, the onset of clinical signs is sudden and often follows a stressful event in the rabbit's life (Meyer-Breckwoldt 1996). The most frequently observed neurological sign in rabbits with encephalitozoonosis is vestibular disease (Kunstýř and Naumann 1985; Harcourt-Brown and Holloway 2003; Jass et al. 2008). In addition to head tilt and ataxia, signs of vestibular disease include circling, nystagmus and rotational movements around the body length axis. Usually, the general condition and food intake, even in rabbits with severe head tilt, is not impaired. Rabbits with increased head tilt exhibit rolling clearly more often, occasionally resulting in lateral recumbency with the animal being unable to sit up for a long time. A period of immobilisation following an acute vestibular disorder can limit the recovery of vestibular dysfunction (Thomas 2000).

Other neurological symptoms that are observed in the course of encephalitozoonosis are seizures, paresis, head tremors and swaying or nodding at rest. Occasionally, rabbits also demonstrate behavioural changes (aggression, running or jumping against cage fences, automutilation) and cranial nerve deficits (Ewringmann and Göbel 1999; Harcourt-Brown and Holloway 2003; Jordan et al. 2006).

Phacoclastic uveitis has been reported in many domestic animal species, but the rabbit is relatively unique in that capsule rupture is induced without a prior traumatic insult

(Wolfer et al. 1993). The majority of rabbits suffering from phacoclastic uveitis are young individuals (Grahn et al. 1991; Wolfer et al. 1993; Künzel et al. 2008). Ocular signs of encephalitozoonosis commonly occur unilateral, bilateral lesions are only occasionally detected (Ashton et al. 1976; Harcourt-Brown and Holloway 2003). In many cases, rabbit owners report a visible white mass in the eye (Ewringmann and Göbel 1999; Giordano et al. 2005). Besides uveitis, also cataracts of various degrees can be diagnosed within the ophthalmological examination. Usually, physical examination reveals no abnormalities. Normally, concerned rabbits do not show considerable loss of visual function (Fechle and Sigler 2002).

The majority of cases with chronic interstitial nephritis due to an infection with *E. cuniculi* are sub-clinical. Therefore, azotaemia is commonly detected accidentally in rabbits. Significant renal impairment is rarely observed and concerned rabbits show non-specific signs such as inappetence, weight loss, lethargy and dehydration (Ewringmann and Göbel 1999; Harcourt-Brown and Holloway 2003). Chronic renal failure in rabbits can also cause anaemia or even osteodystrophia resulting in pathologic fractures of the long bones (Ewringmann and Göbel 1999). The evidence of signs of polyuria and polydipsia in rabbits with confirmed azotaemia are controversially discussed in the literature (Dipineto et al. 2008; Künzel et al. 2008).

Differential diagnosis

Besides encephalitozoonosis, otitis media/interna represents the main differential diagnoses for vestibular disorders in rabbits. In the majority of the cases *Pasteurella multocida* can be isolated from empyema of the tympanic bullae of diseased rabbits and most commonly both ears are affected (Kunstýř and Naumann 1985). In contrast to rabbits with vestibular disease due to an *E. cuniculi* infection, otitis media/interna is usually associated with signs of upper respiratory infection (sneezing, nasal discharge, stridor) and occasionally pneumonia. While encephalitozoonosis causes central vestibular disease, otitis media/interna typically causes peripheral vestibular disease. However, only in a few cases, a differentiation between central and peripheral vestibular disease can be made based on neurological examination alone. Radiography of the tympanic bullae can help to diagnose otitis media, but changes can be also found accidentally in rabbits with sub-clinical disease (Snyder et al. 1973).

Meningoencephalitis due to bacterial infections is also detected frequently as a cause of neurological disease in rabbits (Murray et al. 1985; Gruber et al. 2009). Infrequently viral infections of the central nervous system (CNS), including herpes simplex virus, Borna disease virus and rabies virus can induce neurological disease in rabbits (Metzler et al. 1978; Weissenböck et al. 1997; Karp 1999; Grest et al. 2002; Müller et al. 2009).

Parasitic infections causing neurological sings in rabbits include *cerebral larva migrans* of nematodes and toxoplasmosis (Dubey et al. 1992; Furuoka et al. 2003). *Baylisascaris* infections are not uncommon in rabbits in the USA (Deeb and Di Giacomo 1994). Signs of infection can mimic those of encephalitozoonosis. Typically, an affected rabbit shows intermittent improvement, followed by more severe signs (Deeb and Carpenter 2004). Toxoplasmosis is an uncommon cause of neurological disease in rabbits, and infections are usually sub-clinical. In contrast to encephalitozoonosis, rabbits with clinical toxoplasmosis mostly show unspecific signs such as lethargy, inappetence and fever (Dubey et al. 1992). *Toxoplasma gondii* may induce granulomatous menigoencephalitis similar to encephalitozoonosis but can be differentiated by serolocical testing, tissue morphology and immunohistochemical labelling (Dubey et al. 1992; Leland et al. 1992). Occasionally, otitis externa due to *Psoroptes cuniculi* infection is associated with a transient head tilt, but it is obvious that ear mites play no susbstantial role in this condition (Kunstýř and Naumann 1985).

In rabbits, neoplastic lesions, most frequently lymphomas, are also documented to involve the central nervous system (Gruber et al. 2009).

Most often, paresis or paralysis in pet rabbits is caused by traumatic insults resulting in fractures or (sub)luxations of the spinal cord. Head trauma is probably not common, but traumatic episodes such as a head blow or fall can cause brain damage followed by neurological disease.

Cardiovascular lesions, metabolic–toxic causes and degenerative lesions of the CNS should be considered as differential diagnosis to neurological disorders in rabbits as well (Gruber et al. 2009).

Apart from encephalitozoonosis, nephrolithiasis represents the main differential diagnoses for renal failure in rabbits. The condition can be detected by radiographic or ultrasonsographic examination and, therefore, easily differentiated from azotemia caused by an *E. cuniculi* infection (Harcourt-Brown 2002).

The most important differential diagnoses for phacoclastic uveitis in rabbits is bacterial uveitis, usually caused by *P. multocida* infection due to haematogenous spread (Williams 1999). Uveitis can be also secondary to severe keratitis or caused by trauma or penetrating foreign bodies (Harcourt-Brown 2002).

Diagnosic procedures

The definitive diagnosis of encephalitozoonosis in vivo is difficult, as signs of neurological or renal disease do not

preclude other diseases. A tentative clinical diagnosis is usually obtained by a combination of clinical, neurological and ophthalmological examinations, serological tests and by the exlusion of differential diagnosis. A negative antibody titer usually excludes an infection, although rarely spores can be found in the absence of specific antibodies, especially in the early stage of infection (but these cases are usually asymptomatic; Csokai et al. 2009b), during infections at a very early age or when immunosuppressive primary diseases are present (Lyngset 1980; Müller 1998; Csokai et al. 2009b). Detection of specific antibodies only confirms previous contact with the pathogen; many clinically healthy rabbits show moderate to high titers (Harcourt-Brown 2004; Künzel et al. 2008; Csokai et al. 2009b). Post-mortem diagnosis demonstrates spores in a large number of animals without symptoms of encephalitozoonosis (Csokai et al. 2009a, b); the infection seems to develop and spread slowly and symptoms do not develop in the majority of cases (Ewringmann and Göbel 1999; Csokai et al. 2009a, b).

Radiography of the skull can help in the diagnosis of otitis media/interna, which represents the main differential diagnosis for vestibular disease in rabbits, but it may lead to false positive or negative results (see differential diagnosis). A recent study documented that the analysis of cerebrospinal fluid (CSF) can support a clinical diagnosis of encephalitozoonosis in rabbits (Jass et al. 2008). It was concluded that lymphomonocytic pleocytosis and increased concentrations of protein in CSF are characteristic signs of encephalitozoonosis in rabbits. However, any other viral, protozoan or immune-mediated encephalitis can induce a similar result (Jass et al. 2008).

Serology

Serological detection of antibodies is the most sensitive diagnostic method during the early stage of infection (Cox and Gallichio 1978; Csokai et al. 2009b). In laboratory rabbits, serum antibodies against *E. cuniculi* develop within 3 weeks post-infection (Cox et al. 1979). Seroconversion can be demonstrated at least 2 weeks before intracellular organisms are detected and 4 weeks before histopathological lesions in the kidney or organisms in the urine can be found. Cerebral lesions are observed only around 8 weeks after initiation of serum antibody response (Cox and Gallichio 1978). Subsequently, antibody titres remain high over several months after exposure to *E. cuniculi*, then decrease slightly and can persist for years with fluctuating levels (Waller et al. 1978; Scharmann et al. 1986). It is not known whether animals excrete spores during episodes of increasing antibody levels (Scharmann et al. 1986).

Passive immunity is transferred from infected dams to their offspring and maternal antibodies are detectable up to the age of 4 weeks. After a seronegative period, seroconversion in response to an active infection develops in young rabbits at an age of 8 to 10 weeks (Lyngset 1980).

Several studies demonstrated a good to excellent correlation between serological results and pathohistological lesions, although regularly a few seronegative rabbits show minimal to moderate levels of interstitial nephritis (Waller 1977; Cox and Gallichio 1978; Cox et al. 1979; Scharmann et al. 1986; Eröksüz et al. 1999; Csokai et al. 2009b).

Worldwide surveys have shown high rates of infection in rabbits with neurological signs as well as in asymptomatic animals (Table 1).

Serology is the most important diagnostic tool for diagnosis of *E. cuniculi* infection in the living animal. Various diagnostic tests have been developed in order to detect antibodies to *E. cuniculi* and all are suitable for the use in rabbits. Indirect fluorescent antibody technique (IFAT) and ELISA are the most common tests and correlate well with each other (Boot et al. 2000; Jordan et al. 2006). Serological screening is used by laboratories to identify and cull potentially infected animals to reduce interference with experimental results (Pakes and Gerrity 1994). Nevertheless, seroconversion only indicates chronic *E. cuniculi* infection but does not confirm the organism to be responsible for disease symptoms, because of the high rate of sub-clinically infected animals. However, a negative serological result rules out *E. cuniculi* as a cause of an apparent disease. Therefore, serological diagnosis can be used to distinguish between the two main possible aetiological agents causative for vestibular disorders in rabbits. If the animals are found to be seronegative to *E. cuniculi*, there is a high probability that *P. multocida* is responsible for clinical signs (Kunstýř and Naumann 1985).

Spore detection in tissue

The pathohistological changes (mostly non-suppurative, often granulomatous, encephalitis and nephritis; see "Pathology and pathophysiology") are indicative of encephalitozoonosis in rabbits. Intracellular spores can be detected post-mortem in sections primarily of the kidneys and the brain and are not strictly associated with pathohistological changes (Shadduck et al. 1979). The infection seems to spread from the urinary tract to the neurological organs; therefore, spores are first detected in the kidneys 4 weeks after seroconversion, followed by the brain 8 weeks after seroconversion (Cox and Gallichio 1978). In naturally infected rabbits, spore can be detected slightly more frequently in the kidneys than in the brain (Csokai et al. 2009a, b). Due to the small size of the spores the detection with routine staining methods, e.g. hematoxylin–eosin, is difficult; specific methods such as chromotropic staining

Table 1 Prevalences of *E. cuniculi* infections in rabbits determined by serology

Country	Author(s)	Method	Husbandry/health status/clinical signs	Total	Seropositive	
					N	%
Australia	Cox and Pye (1975)	IFAT	Laboratory rabbits	191	98	51.3[a]
Sweden	Waller (1977)	CIA	Laboratory rabbits	200	43	21.5[b]
Czech Republic	Chalupský et al. (1979)	IFAT	Laboratory and breeding rabbits	>500	–	0–95[c]
Australia	Cox et al. (1980)	IFAT	Wild rabbits	823	0	0
UK	Cox and Ross (1980)	IFAT	Wild rabbits	175	0	0
Norway	Lyngset (1980)	CIA	Breeding rabbits	66	48	73.0
Germany	Lev (1982)	CIA, IFAT	Laboratory and breeding rabbits	276	17	6.2
Germany	Neuwirt (1988)	IFAT	Pet rabbits	42	17	40.5
			Laboratory, breeding and farming rabbits	142	5	3.5
			Wild rabbits	155	28	18.1
France	Chalupsky et al. (1990)	IFAT	Wild rabbits	204	8	3.9
Germany	Meyer-Breckwoldt (1996)	CIA	Pet rabbits	200	84	42
			Wild rabbits	100	0	0
Australia	Thomas et al. (1997)	IFAT	Wild rabbits	81	20	24.7
Switzerland	Müller (1998)	ELISA, IFAT	Asymptomatic breeding and farming rabbits	292	22	7.5
			Suspected pet rabbits	72	61	84.7
Germany	Ewringmann and Göbel (1999)	CIA	Pet rabbits[d]	277	125	45.1
Turkey	Eröksüz et al. (1999)	CIA	Laboratory/breeding rabbits[e]	150	98	65.3
Slovakia	Halánová et al. (2003)	IFAT	–	571	238	41.7
UK	Harcourt-Brown and Holloway (2003)	ELISA	Asymptomatic pet rabbits	38	14	36.8
			Suspected pet rabbits	87	60	69.0
			Neurological signs	53	38	71.7
			Vestibular disease	23	21	91.0
			Ocular lesions	9	9	100
UK	Keeble and Shaw (2006)	ELISA	Asymptomatic pet rabbits	97	50	52
Austria	Künzel et al. (2008)	IFAT	Asymptomatic pet rabbits	54	20	37.0
			Suspected pet rabbits	184	144	78.3
			Neurological signs	140	108	77.1
			Vestibular disease	104	95	91.3
			Ocular lesions	25	21	84.0
Italy	Dipineto et al. (2008)	CIA, ELISA	Asymptomatic pet rabbits	47	32	68,1
			Suspected pet rabbits	78	52	66,7
			Neurological signs	42	32	76.2
Japan	Igarashi et al. (2008)	ELISA	Healthy pet rabbits	195	113	57.9
			Pet rabbits with neurological signs	105	85	81.0
			Pet rabbits with other clinical signs	37	16	43.2
Nigeria	Okewole (2008)	IFAT	Asymptomatic rabbits	237	39	16.5
Austria	Csokai et al. (2009a, b)	IFAT	Pet rabbits with other diseases	38	19	50.0
			Suspected pet rabbits	33	23	69.7
Italy	Santaniello et al. (2009)	CIA, ELISA	Farm rabbits	1600	505	31.6

CIA carbon immunoassay, *IFAT* indirect fluorescent antibody technique, *ELISA* enzyme-linked immunosorbent assay

[a] Sera from four different laboratories of Australia with an incidence ranging from 25% to 75%

[b] Sera from six institutes; seropositive rabbits were found from all institutes, with an incidence ranging from 9.1% to 88,9%

[c] Rabbits from 12 laboratory colonies and seven breeding farms were included in the study. The number of seropositve animals fluctuated in different colonies between 0% and 95%. Two of the breeding farms were located in Cuba

[d] Pet rabbits either with suspected encephalitozoonosis or with other clinical signs

[e] Rabbits from 15 different colonies (amateur breeders or department facilities)

(e.g. acid-fast trichrome or Ziehl–Neelsen), immunofluorescence or chemofluorescence are more sensitive (Weber et al. 1999). Electron microscopy is considered the "gold standard" for spore detection (Didier et al. 1995a, b); however, it is laborious to perform, and as with chemofluorescence or chromotropic staining, it cannot differentiate between *E. cuniculi* and *E. hellem* (Didier et al. 1991), which is important for the species diagnosis in human samples.

Spore detection in body fluids

Excretion of spores with the urine can be considered the primary mode of dissemination for *E. cuniculi*. The first spores in urine can be detected 3 to 5 weeks after seroconversion (Cox and Gallichio 1978; Scharmann et al. 1986), but excretion declines over the following months (Cox and Pye 1975; Cox et al. 1979). As with histopathology, specific staining methods are more sensitive than standard techniques (Didier et al. 1995a, b). Since the excretion of spores with the urine takes place sporadically, a negative result does not exclude infection (Cox et al. 1979; Csokai et al. 2009a). In urine as well as in cerebrospinal fluid, DNA can be detected by polymerase chain reaction (PCR) if spores are excreted (see below). Excretion via faeces is discussed but could not be demonstrated regularly (Von Kücken et al. 1987; Harcourt-Brown and Holloway 2003).

PCR-based detection and differentiation

For human samples, DNA-based differentiation and genotyping (see "Epidemiology and host spectrum") is mandatory since infections with *E. cuniculi* and other microsporidia are usually of zoonotic origin (Deplazes et al. 1996). Electron microscopy allows differentiation of most but not all species of microsporidia and usually requires multiplication of spores in cell culture (De Groote et al. 1995; Deplazes et al. 1996; Weber et al. 1999). For clinical applications, direct PCR amplification either with species-specific primers (Weber et al. 1999) or with conserved primers in combination with sequencing or restriction fragment length-polymorphism analysis is applied (Katzwinkel-Wladarsch et al. 1997). Alternatively, nested PCR is carried out to increase the sensitivity of detection (Katzwinkel-Wladarsch et al. 1996; Kock et al. 1997).

In rabbits, the use of (nested) PCR is less common. The rationale for applying this technique is the increased sensitivity. DNA of *E. cuniculi* can be detected in tissue (Baneux and Pognan 2003; Csokai et al. 2009b) and by nested PCR in the urine and liquor of infected rabbits (Jass et al. 2006).

However, PCR of body fluids seems to be an unreliable method, since the presence of DNA is not correlated with disease, and even in clinically ill animals, DNA detection can be negative (Jass et al. 2006; Csokai et al. 2009b). In tissue samples, DNA amplification by conventional or nested PCR is less sensitive than histopathological spore detection after specific staining (Csokai et al. 2009b). On the other hand, PCR from liquefied lens material after phacoemulsification is highly suitable for the detection of *E. cuniculi* in rabbits with phacoclastic uveitis (Künzel et al. 2008; Csokai et al. 2009b).

Therapy and control

In rabbits with encephalitozoonosis clinical signs are not only associated with the presence of the organism in the different tissues but can be also a result of the inflammatory process that follows after the parasite has been eliminated. In addition, therapeutic and control measures depend on the clinical manifestation of the infection in the central nervous system, the urinary tract or the eye or a combination of these. Consequently, no uniform treatment protocol exists for rabbits with presumptive encephalitozoonosis. Treatment regime for rabbits with neurological disease should include causative therapy (limitation of the spread of the organisms in the CNS), suppression of accompanied inflammatory reaction, control of potential concurrent disease (e.g. bacterial infection) and control of seizures and/or rolling (Deeb and Carpenter 2004).

Until now, no drugs have been approved for the treatment of *E. cuniculi* infection in rabbits. In some case studies of pet rabbits showing vestibular disease, treatment with glucocorticoids in combination with either enrofloxacin or oxytetracycline or fenbendazole resulted in a 50% initial recovery rate, but it is difficult to evaluate the efficacy of those treatment protocols (Ewringmann and Göbel 1999; Harcourt-Brown and Holloway 2003; Künzel et al. 2008).

Several in vitro studies have been carried out to evaluate the drug susceptibility of *E. cuniculi*. Antibiotics, such as fumagillin, sparfloxacin, oxytetracycline and anthelmintics, including albendazole, oxibendazole and thiabendazole, were the most effective (Waller 1979; Shadduck 1980; Beauvais et al. 1994; Franssen et al. 1995).

According to Beauvais et al. (1994), albendazole is regarded as the most effective drug against microsporidioses in humans. The results of Neuschl et al. (1999) concerning the influence of albendazole on the development of encephalitozoonosis in rabbits indicate its inhibitory effect on the development of the disease and suggest that it is well tolerated. However, albendazole is known to be embryotoxic and teratogenic in rabbits. Fenbendazole,

another benzimidazole, has also been shown to prevent and treat *E. cunciuli* infections in rabbits (Suter et al. 2001). Rabbits that were infected experimentally during their oral treatment with fenbedazole (20 mg/kg/day) did not seroconvert, and spores could not be isolated from their brain tissue. In the therapeutic part of the study of Suter et al. (2001) naturally infected rabbits were orally treated with fenbendazole for 4 weeks. Attempts to isolate parasite stages from the brain tissue after therapy failed. Due to this study, the drug of choice for the causative treatment of encephalitozoonosis in rabbits is considered fenbendazole (20 mg/kg orally, once a day over a period of 28 days).

Neurological signs associated with *E. cuniculi* infections are due to the inflammatory reaction caused by the rupture of brain cells by the multiplying organisms, rather than to the organisms themselves (Feaga 1997). Therefore, corticosteroids are used to reduce the inflammatory reaction, as it is done in other animal species to treat granulomatous meningoencephalitis. Nevertheless, anti-inflammatory treatment with corticosteroids is discussed controversially, because of their immunosuppressive effects. *E. cuniculi* infection is controlled by the regulatory function of the T lymphocytes. Therefore, immunosuppressive doses of corticosteroids may affect T-cell populations and the production of T-cell-derived cytokines, which is believed to be the main defence mechanism against *E. cuniculi*. In accordance with these theses, Horváth et al. (1998) described that low and repeated dosage of cyclophosphamide can modify the pathomorphological response to *E. cuniculi*. The use of glucocorticoids in order to improve clinical treatment of *E. cuniculi* induced central nervous encephalitis has to be further evaluated in controlled studies.

As otitis media/interna is the main differential diagnosis for rabbits showing vestibular signs, some authors recommend, besides causative and anti-inflammatory treatment, additional antibiotic therapy (Kunstýř and Naumann 1985).

In rabbits showing seizures or rolling sedatives such as diazepam or midazolam are recommended.

Recently, supportive treatment options such as stress reduction (noise) and physiotherapy have become more important in the therapy of pet rabbits with vestibular signs.

Mostly, rabbits with signs of renal failure due to encephalitozoonosis are euthanized because of the poor prognosis.

The successful conservative treatment of phacoclastic uveitis in rabbits is only reported by Ewringmann and Göbel (1999). These authors describe a treatment regime with systemic-administered dexamethasone and oxytetracycline in conjunction with eye ointments containing dexamethasone and tetracycline. In most of the pet rabbits, signs of reactive uveitis permanently disappeared, but white granulomas and cataracts were still persisting after conventional therapy. Furthermore, several other authors reported poor response to topical therapy (Wolfer et al. 1993; Stiles et al. 1997). Surgical removal of the lens by phacoemulsification has been shown to be a successful causative treatment modality for rabbits with phacoclastic uveitis, together with medical management (Stiles et al. 1997; Fechle and Sigler 2002). Therefore, treatment of choice for this condition is the immediate removal of the lens. The sooner surgery is performed, the better the clinical outcome. In chronic cases of endophthalmitis, enucleation of the affected eye is recommended.

In order to prevent the spread of infection within (laboratory) rabbit colonies, seropositive rabbits should be removed, as they may excrete spores. However, a complete eradication programme must include disinfection of the animal facilities, cages and equipment (Waller 1979). A study to investigate the sensitivity of spores to various temperatures and common disinfectants was performed by Waller (1979). Boiling for 5 min or autoclaving at 120°C for 10 min killed all spores. Of the 11 disinfectants studied, nine (among them ethanol 70%, formaldehyde 0.3%, hydrogen peroxide 1%, sodium hydroxide 1%) inactivated all *E. cuniculi* spores exposed for 30 min at recommended working concentrations.

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