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LABORATORY DIAGNOSIS OF ENCEPHALITOZOONOSIS IN PET RABBITS

ncephalitozoonosis is caused by a spore-forming, single-celled, obligate intracellular protozoa parasite belonging to the phylum Microspora, genus Microsporidia. Encephalitozoon cuniculi lacks many typical organelles, such as mitochondria and Golgi apparatus, and it may be more closely related to fungi than to protozoa.

Infection has been reported in a wide range of animals (humans, rodents, foxes, monkeys, cats, dogs, sheep, horses, cattle, goats, pigs, llamas, snakes and birds), but clinical signs are most severe in rabbits, dogs, monkeys and guinea pigs. Three strains of E. cuniculi have been identified: strain I (rabbits and humans), strain II (rodents) and

strain III (dogs and humans). A direct zoonotic connection has never been identified between pet rabbits and human cases, but E. cuniculi strains of human origin can infect rabbits and are immunologically and molecularly identical to those isolated from rabbits. It is therefore likely that rabbits infected with E. cuniculi pose a zoonotic risk to immune compromised humans (e.g. HIV infection, immunosuppressive medications or undergoing organ transplantation). E. cuniculi is frequently encountered in farm and laboratory rabbits, but it is rare in wild rabbits in the UK. It is a newly emerging pathogen in pet rabbits with a seroprevalence of 52% reported in healthy pet rabbits in the UK.

The following text refers to pet rabbits only.

Not Risk Factors

- Husbandry
- Diet
- Breed
- Sex
- Body weight
- Vaccination status
- · Health status
- Contact with E. cuniculi positive rabbits
- · Preventive medicine routine

Potential Risk Factors

- Contact with urine
- Immune suppression
- Juvenile animals and neonates with immature immune systems and vertical transmission in utero

PATHOPHYSIOLOGY

Spores are excreted in the urine from 1 to 3 months post-infection when shedding stops The parasite is ingested and carried The spore is the infective form of the in macrophages via blood to target parasite- resistant to environmental tissues (especially kidneys and CNS, changes and able to survive up to but liver, lungs and heart may also be four weeks at 22C in dry conditions involved; eyes are a predilected site easily destroyed by disinfectants for transplacental transmission) The Life Cycle lasts 3 to 5 weeks Transmission - oral ingestion of spores from infected urine or by contaminated food items and Multiplication and maturation water - vertical transmission during pregnancy within the host cells rupture of the (spores cross into the anterior lens capsule, later cell with release of the spores which they multiply and erupt with a resultant can then infect other cells inflammatory response leading to cataract granulomatous inflammation formation and lens rupture associated with phacoclastic uveitis) inhalation (experimentally)

Disease forms range from no clinical signs to life-threatening.



DISEASE FORMS

Subclinical Asymptomatic infection / latent carrier (could relapse at a later stage)

Acute infection (30 days following infection) primarily affects kidneys, lungs and

Chronic infection (100 days after infection) primarily affects the heart and the brain

Dependant on the host / parasite relationship (e.g immune status, route of infection and the species and strain of parasite)

DIAGNOSIS

differential diagnoses

- · Spinal fracture secondary to trauma
- Bacterial abscess and otitis media/ interna (e.g. Pasterurella infection)
- Splay leg
- Lead toxicity
- **Toxoplasmosis**
- Listeriosis
- Heatstroke
- Viral infection (herpes simplex I)
- Degenerative CNS disease
- Neoplasia
- Parasites (Baylisascaris species causing cerebrospinal nematodiasis in the USA), Borna disease virus and rabies in rabbits that have recently travelled

Initial Database

- Full neurological examination
- Complete blood count (Anemia, Leucocytosis)
- Serum biochemistry (Azotemia, Hyperkalemia, Hyponatremia, increased AST & GLDH activities)
- Urinalysis (including cytology and culture)
- Radiography of the skull (rule in/ out otitis media/interna)

Further Laboratory Testing

- Antibody assay tests
- PCR
- · CSF cytology and protein concentration
- · Intradermal skin tests
- Cultures of spores
- Special stains on urine samples
- · Urinary protein: creatinine ratio
- Postmortem

No one laboratory test is able to provide a definitive diagnosis given the variable immune response resulting in differing antibody levels and spore excretion rates between individual rabbits. Often, a range of tests is required to rule out major differential diagnoses and to provide positive information that parasite infection is the likely cause. A presumptive rather than a definitive diagnosis may have to be made with prophylactic treatment being instituted. It is extremely important to keep in mind that concurrent disease is very common.

Antibody assay tests

Despite many diagnostic methods, serological examination remains the most important tool for antemortem diagnosis of encephalitozoonosis.

Available techniques include immufluorescence (e.g. IFA), ELISA assays and carbon immunoassays. The laboratories of the CVS

group offer semi-quantitative measurement of serum IgG (IFA) and IgG and IgM against E. cuniculi (IgG/IgM). IgG antibodies are a marker of exposure and can also support chronic infection. IgM antibodies reflect acute or reactivated infection or re-infection. The presence of the latter antibodies makes it a requirement to institute proper antimicrosporidial therapy.

Therefore, simultaneous testing of IgM and IgG could provide a better indication of the infective status of the affected rabbit.

Presense of IgM antibodies only - early (acute) infection

Simultaneous detection of IgM and IgG - active infection

Presense of IgG antibodies only - chronic / latent infection (but some chronic infections may show increases of both





When a rabbit is first infected, IgG levels start to rise 2 weeks post-infection, peaking between 6 and 10 weeks, and at least 4 weeks before histopathological changes are visible in the kidney. Histopathological changes in the brain are generally seen much later, usually more than 8 weeks after antibodies are detectable. During the course of infection, IgM titres increase rapidly and then decrease, while IgG antibody levels remain elevated, sometimes for long periods. In some rabbits, however, both IgG and IgM remain elevated over the long term, which makes interpretation difficult.

There is wide variation in antibody response (from persistently high

antibody IgG levels for years even in the absence of clinical signs and others becoming seronegative soon after initial infection). The antibody response appears to depend on the level of exposure to spores. Furthermore, the severity of clinical signs does not appear to be related to how high antibody levels are in the blood.

Antibodies are passively transmitted from an infected dam to the offspring which can show antibodies until they are four weeks old. After maternal antibodies wane, they become susceptible to natural infection and, if infected, after an initial seronegative period, seroconversion occurs at between eight and ten weeks

IgG

Negative Results + clinical sign compatible with E. cuniculi infection



Definately rules out E. cuniculi infection as a cause of clinical disease. Negative Result in a healthy animal



- Possible result in a very young animal or immunosuppressed
- Does not rule out early infection with the parasite as antibodies rise 2 weeks after initial infection and clinical signs are uncommon at this stage of the disease (repeat test 4 weeks later to rule out the possibility of early infection before seroconversion, especially if there is a history of the rabbit being in contact with another positive rabbit)

Positive Result in a rabbit showing signs associated with E. cuniculi infection is suggestive of an active infection.



Rule out differential diagnoses

2 separate serum antibody tests should be carried out at least 4 weeks apart to show a rising antibody titre.

Positive result in a healthly rabbit



The rabbit has been exposed to the parasite

- Has been previously infected and subsequently cleared the parasite and recovered
- Early infection prior to the development of clinical signs
- Chronically infected with no clinical signs (latent carrier status), a change in the rabbit's immune status could precipitate disease at a later date.

PCR

This test is now commercially available in the UK for the detection of E. cuniculi DNA in rabbit cerebrospinal fluid and urine. The laboratories of the CVS group offer this test option too. The results of PCR testing are not necessarily correlated with the severity of

disease. This is an excellent diagnostic tool for the detection of parasite DNA in cases of phacoclastic uveitis (liquefied lens material or the intraocular mass) due to the higher spore concentration in lens material.

POSITIVE RESULT

- Confirms early infection
- More likely in cases presenting with renal disease (40% of cases have a positive result), it depends on whether the animal is excreting spores at the time of sampling from 3 to 5 weeks post-seroconversion and only sporadically (collection of urine over a three-day period increases the likelihood of spore excretion)
- Can occur in both symptomatic and asymptomatic rabbits - PCR cannot be used as a valid method for diagnosing clinical encephalitozoonosis

NEGATIVE RESULTS

- Does not rule out E. cuniculi infection
- More likely in chronic cases with neurological signs (10% of cases have a positive result)



CSF cytology and protein concentration

Non-specific changes such as increased concentration of protein and lymphomonocytic pleocytosis have been shown in rabbits with neurological disorders caused by *E. cuniculi* infection. While this could support a clinical diagnosis of encephalitozoonosis, any other viral, immune-mediated or protozoan encephalitis and CNS lymphoma may induce the same cytological changes.

Intradermal skin tests

These are no longer used.

- Culture of spores from urine or tissue samples on rabbit fibroblast monolayers.
- Urine samples centrifuged and examined for spores using modified trichrome stains, Gram stain, Giemsa or immunofluorescence.

These techniques are cheaper and more rapid than urine PCR, but they are less sensitive and they do not differentiate Microsporidial species

Urinary protein:creatinine ratio

It cannot be used as a diagnostic test because it has been found not to vary between E. cuniculi positive and negative rabbits and seroconversion is not associated with azotemia or proteinuria in healthy pet rabbits.

Renal biopsy collected via laparoscopy

It should provide a definitive diagnosis in cases presenting with renal disease from 9 weeks following infection.

Post-mortem

Unfortunately, in most cases, a definitive diagnosis can only be reached at post-mortem because histopathology is required to conclusively identify the parasite or its spores. Lesions in the kidney and brain are usually found about four and eight weeks, respectively, after initial seroconversion. The parasite is often eliminated from the kidneys, but it persists for longer in the brain. Histopathology is the most sensitive and the only definitive test for Encephalitozoon cuniculi. This parasite appears as a Gram-positive, rod-shaped, refractile, intracytoplasmic structure.

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