Real time PCR assay for the detection of *Encephalitozoon* spp. in rabbit faecal samples

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In the UK, *E. cuniculi* has been reported in both wild and domesticated rabbits (pet, farmed and laboratory), with a seroprevalence of 52% in clinical healthy pet rabbits (Keeble and Shaw 2006). However, little is known of the prevalence of other *Encephalitozoon* species that could be involved in diseases of domestic rabbits. Microsporidial infection can be diagnosed using light microscopic examination, molecular techniques such as PCR or an *E. cuniculi*-specific serological

test. (Mathis *et al.* 2005). A previous study was able to detect microsporidial infection in human stool specimens using SYBR Green real-time PCR (Polley *et al.* 2011). However, this technique has never been used on faecal samples of rabbits in order to detect *E. cuniculi*, *E. intestinalis*, and *E. hellem*. The aim of this project was to adapt the real time PCR assay to detect *Encephalitozoon* spp. in rabbit faecal samples. Modified QIAmp DNA mini kit (QIAGEN®) for tissue was used to extract DNA from faecal samples of rabbits spiked with spores of *Encephalitozoon intestinalis*. SYBR Green Real Time PCR was then used to amplify the extracted DNA. The threshold cycle values obtained were compared against the positive and negative control reactions. Sensitivity was tested with serial dilution and the lower limit of detection was 10^3 spores/ml. The test was also able to amplify *E. hellem* and *E. cuniculi* DNA. This is the first SYBR Green real-time PCR method used to detect *Encephalitozoon* spp. in rabbit faecal samples.

Clinicopathological features of cats with mild, moderate or severe hyperthyroidism at the time of radioiodine treatment

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Many hyperthyroid cats are initially managed with anti-thyroid drugs or dietary therapy. Radioiodine, despite being considered the gold standard therapy, is often pursued after these treatments fail.

The aim of this study was to document the clinicopathological features, at the time of referral for radioiodine treatment, of cats classified as mildly (total thyroxine (TT4) 60–124 nmol/L), moderately (TT4 125–250 nmol/L) or severely (TT4 ≥ 251 nmol/L) hyperthyroid. Retrospective data were collected for cats undergoing radioiodine treatment at the Feline Centre, Langford Veterinary Services between January 2009 and May 2013. Data were analysed descriptively and using Chi-square analysis to test for differences between groups.

There were 79 female and 61 male cats; age range 6.2–18 yrs. 84% of cases were non-pedigree. At referral, 46 cats were mildly (TT4 median 98.9 nmol/L, interquartile range [IQR] 80.8–117.1 nmol/L), 63 moderately (TT4 median 198 nmol/L, IQR 165.2–230.9 nmol/L) and 31 severely (TT4

median 388.3 nmol/L, IQR 272.3–504.3 nmol/L) hyperthyroid. Severely hyperthyroid cats were diagnosed at a younger age (mean 10.7 yrs, standard deviation [SD] 2.3 yrs) than those with mild (mean 12.1 yrs, SD 2.5 yrs) or moderate (11.4 yrs, SD 2.1 yrs) disease. The interval between initial diagnosis and referral was longer in severely hyperthyroid cats (median 16 mths, IQR 4.5–27.5 versus mild median 4 mths, IQR 3–5 or moderate median 4 mths, IQR 1.5–6.5).

At referral, 55% of cats with mild, 67% with moderate and 79% with severe hyperthyroidism were unstable despite medication or dietary management. As the severity of hyperthyroidism increased (mild, moderate, severe), the number of cases with weight loss (87%, 89%, 100%), hyperactivity (26%, 43%, 48%), polyuria/polydipsia (39%, 43%, 77%), diarrhoea (9%, 14%, 19%) and lethargy (13%, 17%, 19%) increased. Chi-square analysis of major comorbidities, with the critical value adjusted for multiple testing ($P=0.017$), revealed that severely hyperthyroid cats were significantly more likely to suffer from cardiac disease when compared to mildly and moderately hyperthyroid cats (mild 37%, moderate 51%, severe 71%, $P=0.009$). Mildly hyperthyroid cats were significantly less likely to be dyspnoeic (mild 0%, moderate 11%, severe 16%, $P=0.012$). No significant difference in the likelihood of renal disease existed between groups (mild 33%, moderate 31%, severe 39%, $P=0.708$).

Cats with severe hyperthyroidism were more likely to have cardiac disease and be chronically hyperthyroid with a history of poor stabilisation history, consistent with the progressive nature of the disease. Radioiodine treatment is recommended before severe hyperthyroidism develops due to reduced comorbidity, lower dose requirements and shorter hospitalisation times.