LUNG INFECTIONS IN RED SQUIRRELS ON THE IOW

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Since 2000, any red squirrel bodies, in good condition, retrieved by the general public have been submitted to a post mortem examination. This article is a shortened version of a paper published in The Veterinary Record (Simpson et al. 2006). It describes a Hepatozoon species found in the lungs of red squirrels and considers its possible role as a pathogen.

Through advertising in Wight Squirrel Project newsletter and the local media, the general public has been asked to retrieve dead red squirrels for post mortem examination. The initial examination was carried out by Helen Butler and, if pathology was suspected, samples were taken and fixed in 10 per cent buffered formol saline. These samples were then sent to Vic Simpson at the Wildlife Veterinary Investigation Centre in Cornwall.

Red squirrels are the only squirrel native to the British Isles. They are disappearing from the Mainland fast and are being replaced by the introduced American grey squirrel (*Sciurus carolinensis*). It's believed the grey squirrel outcompetes the native red squirrel for food and nesting sites. Grey squirrels also carry a squirrel poxvirus, which is fatal to reds.

The Isle of Wight is an important stronghold as the Solent provides a barrier against grey squirrel infiltration. The Isle of Wight has around 3,500ha of woodland which should carry approximately 3000 red squirrels. Numbers fluctuate annually according to the success or failure of the autumn seed crop. They also fluctuate seasonally when young are born.

Between June 2000 and December 2006, 307 post mortem examinations have been carried out on the Island's red squirrels. Between 2002 and 2004, 51 of these animals showed signs of pathology and samples were taken for histological examination. Some were natural deaths but the majority had been killed by road traffic or some other form of trauma. The squirrels were categorised into juvenile, subadult or adult on the basis of weight, dentition, body dimensions and observer experience. Unfortunately, 2 sets of tissue samples were autolysed and had to be discarded, leaving 49 samples for the laboratory. Various histological techniques were used in order to discover the cause of observed tissue damage.

The lung sections from the 49 samples revealed many lesions consistent with death due to trauma, including congestion, emphysema, atelectasis*, thickened alveolar*** walls, thrombosed blood vessels and haemorrhage. Large protozoal-type schizonts** (groups of cells. See Fig 1) were present in the lungs of 18 squirrels (37 per cent), sometimes in very large numbers. The schizonts were ovoid and in various stages of development. The majority were in the alveolar walls and many within the capillaries. Generally, there appeared to be minimal inflammatory response to the schizonts, although in several cases the alveolar walls were thickened and there was evidence of oedema and fibrosis.

DNA extracted from the lungs containing schizonts was amplified and nucleotide sequencing showed the parasite to be a species of *Hepatozoon****.* This was confirmed by immunohistochemistry.

The prevalence of infection with *Hepatozoon* species was different in squirrels of different ages. It was noticeable that none of the 5 juveniles examined carried the infection but there were schizonts in 7 of the 11 subadults and 11 of the 33 adults. There was a significantly higher prevalence of

infection in the 12 squirrels found dead in gardens, 9 of which were infected (75%). Of the 27 road traffic accidents, only 7 were infected (26%). The weight of infection varied greatly and was particularly heavy in some cases.

Hepatozoon infections have been found in a range of vertebrate hosts including reptiles, amphibians, birds and mammals. Their lifecycle involves an invertebrate vector, often an arthropod, and the vertebrate host becomes infected by ingesting the vector. Schizogony occurs in various tissues of the vertebrate host, and gamonts**** are released and infect cells of the haemolymphatic system. The life cycle is completed when arthropods feed on an infected vertebrate host (Smith 1996).

Hepatozoon species infection in grey squirrels was described in the USA by Herman and Price in 1954. The organism was identified by them as *Hepatozoon sciuri* but later renamed by Clark in 1958 as *Hepatozoon griseisciuri.* Clark described gametocytes in blood samples from 22 of 24 apparently healthy wild-caught adults and in 19 of 21 young squirrels aged 36 hours to four weeks. No schizonts were seen in smears and sections of liver, spleen and bone marrow except in the 36 hour old animal where they were present in all three tissues. Lung samples were not examined. Clark (1958) also tried to determine the vector and concluded that a gamasid mite commonly found in dreys was the natural vector. Recent studies have shown that a *Hepatozoon* species, presumably *H griseiscuiri*, is a common parasite of grey squirrels in UK (Jawdat 1975, cited in Watkins and Nowell 1991, Britt and Molyneux 1979, Watkins and Nowell 2003).

In England and North America, most authors have not commented on *H griseiscuiri* species as a possible pathogen. Others, (Clark 1958, Hendricks 1975, Watkins and Nowell 1991, 2003) concluded there was no evidence that it was pathogenic. On the other hand, Davidson and Calpin (1976) described *H griseiscuiri* infection in association with thickening of the alveolar walls, pulmonary congestion and other changes similar to those seen in the worst affected cases in the present study. Looking at these two reports together suggests that *Hepatozoon* species may be pathogenic to red and grey squirrels.

Of the 18 Isle of Wight squirrels with *Hepatozoon* species in their lungs, 13 had lesions in other organs, including heart, liver, kidney or spleen. Although many of these lesions did not appear to be directly related to the presence of *Hepatozoon* species, it is possible that the infection may have compromised the squirrels' immune response and allowed other infections such as *Toxoplasma* and *Bordetella* species to occur. In grey squirrels, Davidson and Calpin (1976) suggested that the presence of *Hepatozoon* species may increase the likelihood of their becoming infected by other respiratory diseases and Keymer (1983) reported lesions of pneumonia together with concurrent *Hepatozoon* infection in 3 grey squirrels found dead in Norfolk. A similar situation may exist with reds.

The only previous record of what was probably a *Hepatozoon* species in a red squirrel was made by Coles in 1914. Two red squirrels had been kept in a cage with two grey squirrels. The two red squirrels died and samples of heart, lung and liver from one were examined. The cause of death was severe toxoplasmosis with concurrent infection by what appears to have been *Hepatozoon*. It is possible that the *Hepatozoon* infection was contracted from the grey squirrels.

Although *Hepatozoon* species infection is common in grey squirrels in both England and North America, it has not been proved that the same *Hepatozoon* species infects populations in both countries. Given that grey squirrels are not present on the Isle of Wight, it is possible that the *Hepatozoon* species found in this population of red squirrels is native to them. Further samples are

required in order to establish whether the parasite is present in other red squirrel populations, how it is transmitted and if it contributes to their decline.

Reference:

Simpson, V. R., Birtles, R. J., Bown, K. J., Panciera, R. J., Butler, H., Davison, N. (2006) *Hepatozoon* species infection in wild red squirrels (*Sciurus vulgaris*) on the Isle of Wight. Veterinary Record **159** 202-205

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*Atelectasis: A term used to describe partial or complete collapse of the lung.

** Schizogony: The division of cells, especially of protozoans, in nonsexual stages of the life history of the organism.

***Alveolar: Of the alveolus, the final subdivision of the lung where gas exchange takes place.

****Hepatozoon: A genus of coccidian protozoal parasites, in which schizogony occurs in the visceral organs.

*****Gametocyte: A cell capable of dividing to produce gametes, e.g.,sperm or ova. Synonym: gamont.

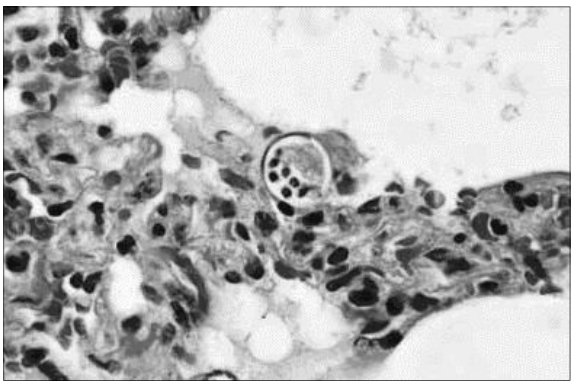

Fig 1. Large Hepatozoon species schizont with 7 visible nuclei. The host cell's nuclear material is displaced to the margin of thecell in this photomicrograph of a section of the lung of a Red Squirrel.
Photograph courtesy of The Veterinary Record.

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